

Mechanisms of Coexistence: Implications for Biodiversity-Ecosystem Functioning  
Relationships in a Changing World

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## **Dedication**

*Dedicated to my family.*

## **Abstract**

Recent advances in the understanding of the effects of biodiversity on ecosystem processes have left unanswered questions on the mechanistic basis of this relationship and how it may change in the face of global climate change. There is a strong theoretical basis connecting species coexistence with the positive effect of biodiversity on productivity (overyielding) and my research program examines two such mechanisms of coexistence and overyielding in grassland communities: belowground spatial resource partitioning by plant species, and plant-soil feedbacks. In chapter 1, I first assessed how plant diversity and warming will directly and interactively affect plant communities in a long-term warming and diversity field experiment. I found that warming and diversity have positive direct effects on plant biomass, and the two factors have positive interactive effects on aboveground biomass while the effect of warming on belowground biomass trends negative at the highest diversity level, and this response may be related to shifting community structure under warmed conditions. In chapter 2, I examined the belowground biomass dynamics and nitrogen supply rates under warming and diversity treatments and the implications of these for plant productivity. My results hint at a potential for a positive feedback between diversity-enhanced deep-soil nitrogen mineralization and increased fine rooting depth that may drive positive effects of warming on plant productivity. In chapter 3, I address whether feedbacks between plants and associated plant species-specific soil biotic communities contribute to the positive effect of diversity on plant productivity. I conclude that while present, plant-soil feedbacks are not the dominant driver of overyielding in our grassland system. By furthering the understanding

of drivers of diversity-productivity relationships and how these may be altered under climate change, we allow a more complete, mechanistic understanding of community dynamics, enabling stronger prediction of the consequences of human dominance of the earth.

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## **Chapter 1**

### **Introduction**

It is well documented that the loss of biodiversity in an ecosystem has negative repercussions for a whole suite of ecosystem processes. Biodiversity loss is linked to, among other things, increased invasibility, increased nutrient leaching, decreased productivity and loss of ecosystem stability (Tilman et al. 2014). The implications of biodiversity loss on the functioning of ecosystems are of great ecological and economic concern, as human well-being is dependent on the highly valued ecosystem services provided by these properties and processes. Alongside the loss of biodiversity, human actions are leading to a suite of climatic changes, including global climate warming. Understanding the mechanistic bases for the provision of biodiversity-based ecosystem services and how these may shift with climate change will be invaluable for predicting the future implications of human activities on the sustainability of the planet.

There is a strong theoretical link between the positive effect of biodiversity on productivity (overyielding) and the mechanisms that allow species to coexist. While classic coexistence theory posits that only one species may persist at equilibrium per limiting resource, the high biodiversity in areas such as grasslands that have very few limiting resources prompted researchers and theoreticians to highlight ways that species can side-step this inevitable competitive exclusion. Simply put, species must be distinct in some facet of their ecological niche such that heterospecific competitors inhibit a species' growth and survival less so than conspecific competitors. Species then use resources in a complementary manner, more completely using available resources and

thus leading to overyielding. Vandermeer (1981), Lehman and Tilman (2000) and Loreau (2004) have demonstrated, using descriptive models, that the conditions for overyielding are the same as those for stable species coexistence (also see Beckage & Gross 2006, Hector 2006, Isbell et al. 2009, Wilsey et al. 2009, Carroll et al. 2011).

However, few studies have extended this theory to explicitly examine the ecological mechanisms underlying the link between coexistence mechanisms and overyielding. Classically, treatment of this question has been to separate biodiversity effects into selection and complementarity effects (Loreau & Hector 2001). The selection effect is caused by the covariance of diverse community productivity with monoculture biomass of the constituent species, simply that there is a higher likelihood of including a species with a high-yielding monoculture. Complementarity effects are a broad grouping of any niche partitioning or facilitation among coexisting species. Studies commonly invoke biological mechanisms of resource partitioning and/or facilitation, however very few studies provide direct empirical evidence to support these claims (Cardinale et al. 2011). Thus, the understanding of the effects of biodiversity on ecosystem processes has largely remained on a strictly phenomenological level. Ultimately, There is a need therefore for more explicit connections between the complementarity effects and underlying mechanisms in order to understand the provisioning of biomass-based ecosystem services.

There has been a recent resurgence of papers focused on filling in this gap (Fornara & Tilman 2009, Ashton et al. 2010, Maron et al. 2011, Schnitzer et al. 2011, de Kroon et al. 2012, Mueller et al. 2013). Many of the studies focus on mechanisms also linked to the coexistence of species and maintenance of diversity. My dissertation work focuses on

two coexistence mechanisms that have previously been shown to play large roles in structuring grassland ecosystems – belowground niche partitioning and species-specific plant-soil biotic interactions (McKane et al. 1990, Fargione & Tilman 2005, Klironomos 2002, van der Heijden et al. 2008, Petermann et al. 2008, von Felten et al. 2009, Dornbush & Wilsey 2010).

The first such mechanism is belowground niche partitioning. McKane et al. (1990) and Fargione & Tilman (2005) have shown, within our study system, the Cedar Creek Ecosystem Science Reserve (MN), that species that place roots and retrieve nutrients from a different depth than a dominant competitor are better able to coexist with the dominant species than those with more overlap in rooting and nutrient acquisition depth. Further studies have shown that diverse communities can have larger and deeper rooting systems than low diversity systems and suggest that this is indicative of vertical niche partitioning (de Kroon et al. 2012, Mueller et al. 2013). By increasing rooting depth, diverse communities increase the volume of soil that is exploited for nutrients, known to be an important determinant ofoveryielding (Dimitrikopoulos & Schmid 2004).

Understanding the role of rooting depth shifts in biodiversity-productivity relationships is especially important in the face of climate warming. While warming experiments overall have showed a positive effect of temperature on aboveground productivity (Rustad et al. 2001, Lu et al. 2013), warming also can negatively affect plant growth through indirect effects such as soil drying (Dieleman et al. 2012). The effects of warming are likely to be largest at the soil surface, due to the buffering capacity of the soil, thus any roots closer to the soil surface will be more strongly affected by warming. Examining how this mechanism ofoveryielding and coexistence interacts with warming will likely provide

valuable insight for projecting the effects of these two important global change drivers on the functioning of ecosystems. My first chapter examines how biodiversity and warming independently and interactively affect aboveground productivity and community structure, setting the stage for chapter two, explicitly examining this proposed belowground mechanism.

The second mechanism, and the focus of my third chapter, is species-specific plant-soil biota feedbacks (PSFs). PSFs describe the accumulation of plant species-specific biotic communities that affect growth and competitive ability of the plant species. Negative PSFs have long been considered to be an important mechanism by which species coexist (Janzen-Connell hypothesis; e.g. Petermann et al. 2008). PSFs act as a mechanism of coexistence in the same manner as resource niche partitioning – a species inhibits conspecifics more than heterospecific neighbors through the accumulation of pathogens and other enemies preferential to that species. Recent studies have linked biodiversity-productivity specifically to effects of soil biota, showing that in the absence of soil microbes the diversity effect was much more modest (Schnitzer et al. 2011, Maron et al. 2011). My work builds off of these studies by incorporating soil from a long-term biodiversity experiment and specifically controlling for nutrient differences in the soil in a conservative experimental design.

By understanding the processes underlying coexistence and the diversity-productivity relationship, we will be able to increase the predictive power of current models and further gain a more accurate understanding of how different drivers of environmental change will affect ecosystem processes and the subsequent provisioning of valued services.

## Chapter 2

### **Shifting plant community structure drives positive interactive effects of warming and diversity on aboveground net primary productivity**

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## Abstract

Ecosystems worldwide are increasingly impacted by multiple drivers of environmental change, including climate warming and loss of biodiversity. We show, using a long-term factorial experiment, that plant diversity loss alters effects of warming on productivity. Aboveground primary productivity was increased by both high plant diversity and warming, and, in concert, warming ( $\approx 1.5^\circ\text{C}$  average above and belowground warming over the growing season) and diversity caused a greater than additive increase in aboveground productivity. The aboveground biodiversity effects increased over time, particularly at higher levels of diversity, perhaps because of warming-induced increases in legume and C4 bunch grass abundances, and facilitative feedbacks of these species on productivity. Moreover, higher plant diversity was associated with the amelioration of harsher warming-caused environmental conditions, such as decreased humidity and surface soil moisture and increased surface soil temperature. Root biomass (0-30 cm) was likewise greater with warming in monocultures and at intermediate diversity, but an effect of warming on root mass was absent (though trended negative) in the high diversity plots. This may be because warming increased the abundance of legumes, which have lower root:shoot ratios than the other types of plants. In addition, legumes increase soil nitrogen supply, which would make N less limiting to other species and potentially decrease their investment in roots. The negative warming x diversity interaction on root mass led to an overall negative interactive effect of these two global change factors on the sum of above and belowground biomass, and thus likely on total plant carbon stores. In total, plant diversity increased the effect of warming on aboveground net productivity and moderated the effect on root mass. These divergent effects suggest that warming and



changes in plant diversity are likely to have both interactive and divergent impacts on various aspects of ecosystem functioning.

## **Introduction**

The functioning of ecosystems, and their ability to supply ecosystem services, will depend on ecosystem responses to the direct and interactive effects of two major drivers of global change, biodiversity loss and warming. Global temperature is projected to increase 2-4.5°C above pre-industrial levels by 2100 (Rodelj et al. 2012). Overall, warming tends to have a positive effect on plant growth and other ecosystem processes such as nutrient cycling (Rustad et al. 2001, Lin et al. 2010, Wu et al. 2011). Such effects tend to be strongest towards the poles and in temperate climates, while tropical systems can have negative effects of warming (Corlett 2011). Additionally, the effects of warming can be highly context specific and indirect negative effects of warming on abiotic properties such as soil moisture can shift the overall response to be negative (Dieleman et al. 2012). The effect of warming also depends on specific traits of the plant species. More drought tolerant species (eg. warm season C4-grasses) can be less limited by the drying effects of warming (Ward et al. 1999) than less drought tolerant cool season C3-grasses). Other groups, such as legumes, may experience temperature-limited nitrogen fixation (Whittington et al. 2012), and thus warming could increase available nitrogen, therefore increasing biomass production.

Simultaneously, human activities are leading to species losses via habitat loss and fragmentation, simplification, and nutrient eutrophication, and to shifts in community composition and species dominance (Vitousek et al. 1997). Recent research has questioned the universality of global biodiversity loss at small spatial scales (Dorneles et

al. 2014), however as most North American prairies have been converted from high diversity natural communities to monoculture crops or low diversity pasture systems, the overall question of how local biodiversity can affect ecosystems is still important. The effects of this loss on ecosystem properties have been well studied in many long-term biodiversity experiments. Overall, increasing species richness has a positive effect on plant productivity (Cardinale et al. 2011, Hooper et al. 2012, Tilman et al. 2012, Balvanera et al. 2006, Tilman et al. 2014) and these effects tend to escalate over time (Reich et al. 2012).

Although both warming and loss of diversity are known to affect ecosystem functioning, their potential interactive effects are poorly understood. Because warming can decrease species richness over time (Klein et al. 2004), experiments that only examine warming are unable to parse the direct effects of warming (likely positive if water is sufficient) from the indirect effect through the loss of diversity (likely negative). Our experiment is a full-factorial combination of 3 levels of plant diversity with 3 levels of warming that is designed to determine how and why these global change factors directly and interactively affect ecosystems. As such, it is the first long-term field experiment to examine the independent and interactive effects of warming and diversity loss on the functioning of an ecosystem.

## **Methods**

Our Biodiversity and Climate experiment (“BAC”) is nested within the existing long-term biodiversity experiment at Cedar Creek Ecosystem Science Reserve in Minnesota, USA described in detail in previous papers (Tilman et al. 2001, Tilman et al. 2006). To

summarize, 168 plots, 9 m x 9 m each, were seeded at equal densities in 1994 with random combinations of prairie perennial species at differing diversity levels, with compositions maintained by hand weeding. Weeding occurs when non-focal species are small in order to minimize disturbance and nutrient loss through weeding. The subset of 32 plots used in the BAC experiment consists of 14 monoculture plots, 10 4-species plots, and 8 16-species plots. The species in the 4-species and 16-species plots are randomly selected from all 4 functional categories (C3 grasses, C4 grasses, non-leguminous forbs, and legumes; each containing 4 species (Tilman et al. 2001).

Low warming (600 W) and high warming (1200 W) treatments were imposed on 2.5 m x 3 m subplots within each of the 32 plots by using infrared heaters (Kimball et al. 2007) suspended from metal frames at a height of 1.8 m above the ground. Control subplots in each of the 32 plots had a metal shade the same size, shape and height as heaters. Heaters were in continuous operation, 24 h/d, March through November, focusing on the bulk of the growing season, since 2009. The diversity and warming treatments were fully factorial, with a high, a low, and a control warming treatment nested within each of the 32 diversity plots (Whittington et al. 2013).

No experimental warming treatment to date has been found to precisely replicate the various environmental conditions expected under global climate change (Aronson & McNulty 2009, Amthor et al. 2010, Rich et al. 2015). While bare ground experimental trials at our field site indicated our treatments increased soil temperature at 1 cm depth by approximately 1.5°C and 3°C above ambient in the low and high warming treatments, the actual warming is expected to depend on the wind conditions (Kimball et al. 2012), time of day (Wall et al. 2011, Kimball et al. 2012), plot productivity (Luo et al. 2010), soil

moisture (Wall et al. 2013, McDaniel et al. 2014), and soil depth (Luo et al. 2010). Thus we directly measured various abiotic conditions to quantify the realized effect of warming on each subplot.

To quantify the efficacy and abiotic effects of our treatments, soil temperature, soil moisture and air humidity were measured throughout the growing season. Soil temperature was measured via iButton data loggers (Maxim, Sunnyvale, CA, USA) at 1 cm depth in all years, with multiple depths measured in 2012 (1, 10 cm), 2013(1, 10 cm), and 2014 (1, 10, 20, and 30 cm). Ibuttons deeper than 1 cm were affixed to a wooden stake inserted into the soil. Relative air humidity and air temperature were measured hourly from 2012-2014 in each subplot using an iButton data logger sheltered from direct sunlight and rainfall under a white plastic cup elevated 10 and 25 cm above the soil surface to assess microclimate conditions at various points in the canopy. Soil moisture was measured in the top 6 cm every two weeks each growing season at six points per subplot using Thetaprobe ML2 and SM150 soil moisture sensors (Dynamax, Houston, TX, USA). Plots were burned each spring, removing essentially all aboveground biomass and as such, biomass harvested late in the growing season measures annual aboveground net primary productivity (minus any productivity removed by small mammal and insect herbivory).

For 2009-2014, aboveground biomass was annually harvested in one 0.1 x 3 meter (2009), two 0.1 x 1.5 meter (2010-2011), two 0.1x2 meter (2012), or two 0.1x1 meter (2013-2014) clip strips in early August, sorted to species, dried, and weighed. Clip strips varied in size and location in order to avoid year-to-year overlap in sampling location. Belowground biomass was sampled immediately afterwards in 2009, 2010, and 2012

using pooled, replicate 5 cm diameter x 30 cm deep root cores. Each root sample was gently rinsed on a 1.5 mm mesh screen to remove all soil, dried, and weighed.

We analyzed data using linear mixed effect models in the nlme package in R 2.14.1. All models included subplot and plot (nested random intercept effects) and experiment year (fixed effect; categorical variable except where explicitly testing for time by treatment interactions) along with variables discussed below. Warming (coded as 0, 1 and 2 for control, low and high warming, respectively) and  $\log_e[\text{diversity treatment}]$  were included as continuous fixed effects. Models of species proportional abundance only include plots planted with  $>1$  species. All response variables were transformed to meet normality assumptions. As such, all responses were log transformed except aboveground biomass (square root transformation), C4 grass absolute biomass (square root transformed), C4 grass proportional biomass (square root transformed), surface soil moisture (no transformation), and relative humidity (no transformation). Wald chi-squares are presented for all results.

## **Results**

The effects of the warming treatments were dependent on the time of the season and diversity of the plots (Fig. 2.1, Fig. 2.S1). The measured temperature increase with warming was the greatest at 1 cm below the surface throughout the season. Across the entire growing season (May-Aug) and across all heights and soil depths (from 25 cm aboveground to 30 cm belowground), the average warming effect measured was  $\approx 1.5^\circ\text{C}$  above ambient in the high warming treatment and  $\approx 0.75^\circ\text{C}$  in the low treatment, relative to controls. The observed effect of warming treatment was greatest in at the beginning of

the growing season in May (High warming  $\approx 2^\circ\text{C}$  above control) and smallest at peak biomass in July ( $\approx 1^\circ\text{C}$  increase in High warming treatment). Diversity strongly decreased the measured effect of warming treatment on temperatures (Fig 2.1; negative warming x diversity interaction, average of all depths across entire season:  $\chi_{1,62}=13.7$ ,  $p=0.0002$ ), especially below the canopy and in the middle of the growing season. (Fig 2.S1).

Across all years, net aboveground primary productivity, as estimated by total aboveground living biomass, responded positively and significantly to both warming and planted diversity, and had a significant, positive warming by diversity interaction (Fig. 2.2, Fig. 2.3; Diversity:  $\chi_{1,30}=79.5$ ,  $P<0.0001$ ; Warming:  $\chi_{1,62}=53.6$ ,  $P<0.0001$ , Interaction:  $\chi_{1,62}=5.42$ ,  $P=0.0199$ ). The main effect of warming increased significantly over time (Fig. 2.2; Year x Warming interaction:  $\chi_{1,477}=4.20$ ,  $P=0.0405$ ).

Functional groups responded differentially to warming. Legumes increased in abundance in the warmed treatments relative to controls, both absolutely (Fig. 2.5; Warming:  $\chi_{1,37}=49.6$ ,  $P<0.0001$ ) and as a proportion of total aboveground biomass (Fig. 2.5; Warming:  $\chi_{1,29}=18.1$ ,  $P<0.0001$ ). C4 grasses, dominant in our study system (the tallgrass prairie), increased in absolute abundance with warming (Fig. 2.5; Warming:  $\chi_{1,37}=10.3$ ,  $P=0.0013$ ) but not in proportional abundance (Fig. 2.5; Warming:  $\chi_{1,29}=1.51$ ,  $P=0.219$  NS) because of the much larger response of legumes to warming. Forbs did not respond to warming in absolute abundance (Fig. 2.5; Warming:  $\chi_{1,35}=1.62$ ,  $P=0.204$  NS) or in proportional abundance (Fig. 2.5; Warming:  $\chi_{1,27}=2.02$ ,  $P=0.155$ ). C3 (cool season) grasses decreased in both absolute (Fig. 2.5; Warming:  $\chi_{1,37}=8.890$ ,  $P=0.0029$ ) and proportional aboveground abundance with warming (Fig. 2.5; Warming:  $\chi_{1,33}=32.6$ ,  $P<0.0001$ ).

Root mass (belowground biomass) also increased with warming and diversity in our experiment, but there was a significant negative interaction between the two factors, because the effect of warming on belowground biomass trended towards negative at high diversity (Fig. 2.4, Fig. 2.S2; Diversity:  $\chi_{1,30}=50.6$ ,  $P<0.0001$ , Warming:  $\chi_{1,62}=4.04$ ,  $P=0.0443$ , Interaction:  $\chi_{1,62}=4.82$ ,  $P=0.0281$ ). Combined with the positive interaction on aboveground biomass, this led to a marginal decrease in root to shoot ratio in the high diversity-high warming plots (Interaction:  $F_{1,62}=3.36$ ,  $P=0.0715$ ). Further, because of the high proportion of biomass that is belowground in our system, the results for the sum of aboveground plus belowground biomass to 30 cm (total biomass) in 2009, 2010, and 2012 mirrored the belowground results. Total biomass increased with diversity ( $\chi_{1,30}=61.0$ ,  $P<0.0001$ ) and warming ( $\chi_{1,62}=7.99$ ,  $P=0.0047$ ) and the effect of warming on total biomass marginally decreased with increasing diversity ( $\chi_{1,62}=3.43$ ,  $P=0.0639$ ).

Our treatments also impacted the abiotic properties of the system. Surface soil moisture increased with diversity (Fig. 2.6a;  $\chi_{1,30}=14.0$ ,  $P=0.0002$ ) and decreased with warming (Fig. 2.6a;  $\chi_{1,62}=89.9$ ,  $P<0.0001$ ), but there was no significant interactive effect of warming and diversity on surface soil moisture (Fig. 2.6a;  $\chi_{1,62}=1.01$ ,  $P=0.315$  NS).

Diversity increased relative humidity 10 cm and 25 cm above the soil surface (Fig. 2.6b; 10 cm  $\chi_{1,30}=34.4$ ,  $P<0.0001$ , 25 cm:  $\chi_{1,30}=13.03$ ,  $P=0.0003$ ) while warming decreased relative humidity (Fig. 2.6b; 10 cm  $\chi_{1,62}=172$ ,  $P<0.0001$ , 25 cm  $\chi_{1,62}=220$ ,  $P<0.0001$ ), but the interaction between warming and diversity was not significant for either height. Mean soil moisture throughout the soil profile did not show significant effects of warming, even though the temperature effect reached the deepest sensors at 30 cm in our experiment



(Fig. 2.S1), and reached 105 cm in a similarly structured warming experiment in Oklahoma, USA (Sherry et al. 2008).

While mean percent soil moisture across the soil profile (3 to 157 cm) was unrelated to total, aboveground, or belowground biomass, surface soil moisture was significantly positively correlated with belowground and total biomass, both in a simple model just containing year and surface moisture as predictors and when also including the warming, diversity, and warming x diversity treatments as predictors (Total Biomass: alone:  $\chi_{1,188}=8.28$ ,  $P=0.0040$ ; in full model:  $\chi_{1,188}=9.52$ ,  $P=0.0020$ ; Belowground Biomass: alone:  $\chi_{1,188}=6.682$ ,  $P=0.0097$ ; in full model:  $\chi_{1,188}=7.04$ ,  $P=0.0080$ ). Surface soil moisture was also positively related to aboveground biomass, but this effect was only marginal and only present in the full model with warming, diversity, and the interaction between warming and diversity (Aboveground Biomass: in full model:  $\chi_{1,472}=3.83$ ,  $P=0.0503$ ).

## **Discussion**

### Aboveground Effects

Our study found that warming and diversity had positive individual and interactive effects on aboveground biomass. Moreover, the strength of this warming effect increased from the first through the sixth year of this experiment. This has several important implications. First, in order to maximize aboveground productivity, our results suggest that the restoration and maintenance of high-diversity ecosystems will be increasingly important as climate changes, since the positive effect of biodiversity on surface soil moisture can at least in part counter the negative drying effect of warming (for another

example see Wright et al. 2014). Further, the increasing strength of the warming effect over time, when combined with emerging trends from long-term diversity experiments (Reich et al. 2012), indicates that the effect of both factors may be underestimated in short-term experiments.

Our results finding interactive effects of biodiversity and warming on aboveground net primary productivity and on root mass support an emerging trend for biodiversity to interact with other drivers of global changes in its effects on productivity. Earlier studies have found interactive effects of plant diversity and elevated CO<sub>2</sub> on aboveground productivity (Reich et al. 2001) and of plant diversity and nitrogen addition on aboveground productivity (Reich et al. 2001). In a correlational study, higher plant diversity was associated with smaller declines in productivity when water availability declined during drought (Tilman and Downing 1994, Tilman et al 1996).

Might such interactive effects of diversity with global change factors share some underlying causes? Communities that are more diverse contain species with a greater range of traits, allowing fuller use of limiting resources, e.g., soil nitrogen, soil moisture or atmospheric CO<sub>2</sub> and greater tolerance of novel conditions, which could cause greater aboveground net primary productivity at higher diversity. The same mechanism may apply to the diversity by warming interaction we observed for aboveground productivity. Soil temperature data from 2012 indicate that warming extended the growing season (days with mean temperature >10C) by 14 or 7 days in the high or low warming treatments, respectively, thus the diverse communities containing a variety of species, some that grow preferentially in the early season and some that grow later, would better capitalize most on this increased growing season length. Warming-dependent increases in

legume and C4 grass abundances may contribute to the positive diversity by warming interaction on aboveground productivity, as one factor causing higher productivity at higher diversity in our biodiversity experiment is a positive legume by C4 grass interaction (Fornara & Tilman 2008). Elevated temperature has caused similar compositional shifts in other grassland experiments (Niu et al. 2010, Cantarel et al. 2013, but see Alward et al. 1999 and discussion below). Finally, the positive effects of biodiversity on surface soil moisture and the positive correlation between surface soil moisture and aboveground net primary productivity may be an additional reason for the diversity by warming interaction.

Although aboveground biomass production increased with warming in our study and others (Rustad et al. 2001, Lin et al. 2010, Wu et al. 2011), the effects of warming may be mediated by water availability, nutrient availability, humidity and growing season length (Dieleman et al. 2012), possibly with an overall larger response of productivity to warming in cooler, wetter ecosystems (Rustad et al. 2001) and a smaller response if warming causes marked soil drying (Bai et al. 2013, Zhou et al. 2012). Thus, warming can affect biomass production positively, as we saw, or negatively, depending on environmental context.

### Belowground Effects

Both warming and diversity increased standing root biomass, but the effect size for diversity is markedly greater than that for warming (Fig 4b). Increases in root biomass with increased species richness have been well documented in biodiversity experiments (Mueller et al. 2013, Ravenek et al. 2014). Like our aboveground effects, the positive

effect of warming belowground was the reverse of other warming studies (De Boeck et al. 2007, Zhou et al. 2012) and may indicate many warming results are situational. Many of the species in this study are located towards the northern end of their geographic ranges, and thus temperature increases and longer growing seasons likely have positive effects on biomass compared with species at the southern edge of their range (Reich et al. 2015). This may explain differences between our study and that of Zhou et al. (2012), conducted with similar species composition but at the southern edge of the species range limits. Further, the smaller (and positive) changes we observed in root biomass may be due to a lagged response (Zhou et al. 2012) of the long-lived roots of established mature prairie plants and might show a more negative effect of warming over time over time.

Species composition may play a role in study-specific effects of warming on communities. The use of C<sub>3</sub> grasses in a warming and diversity greenhouse study (De Boeck et al. 2007) might account for the difference in results between our study and theirs, since our and other studies report C<sub>4</sub> grasses responding positively and C<sub>3</sub> grasses responding negatively to warming (Cantarel et al. 2013, Sherry et al. 2008). However, Alward and colleagues (1999) found that the dominant native C<sub>4</sub> grass decreased with warming and an exotic C<sub>3</sub> grass became more dominant in a 4-season nighttime warming experiment in the shortgrass steppe. Their hypothesis, that the reversal is caused by early season growth of C<sub>3</sub> grasses preempting the reduced soil moisture supply for the C<sub>4</sub> grasses in the spring, could be a reason for the opposite effects seen in the two studies. The warming treatments in this experiment do melt snow in March and April, but May and June are the two wettest months of the growing season, so any effects of early spring C<sub>3</sub> grass growth on soil moisture would likely not impact C<sub>4</sub> grasses, which begin

growing in mid-May when soils are frequently brought to full water holding capacity by numerous rains.

Though small relative to the positive effect of diversity on root mass (Fig 4), we observed a negative interactive effect of warming and diversity on root biomass that may be due to the greater legume abundance and/or higher availability of soil nitrogen at higher diversity and warmer temperatures (Fornara and Tilman 2008, Dijkstra et al. 2010). Legumes have smaller root:shoot ratios than other functional groups in our system (Craine et al. 2002), and decomposition of their N rich roots would increase soil available N (Fornara et al. 2008). Increased N availability would be expected to favor lower allocation to roots. in non-legume plant species. If warming were to consistently lower root allocation over a long period, soil carbon stores might decrease. Soil carbon stores could also decline if, as has been reported (Rustad et al. 2001), warming increases decomposition rates. Since belowground biomass and soil organic carbon are a large proportion of the carbon in grassland systems, these possibilities merit further attention.

### Conclusions

Biodiversity is known to be a major determinant of both ecosystem productivity and the stability of productivity when climate extremes occur. Our work shows that biodiversity is also an important determinant of ecosystems response to climate warming. The positive interactive effect of warming and diversity on aboveground productivity was not mirrored in belowground or total biomass, however, indicating that while high-diverse ecosystems may have a higher ability to respond positively aboveground to warming in our system, bolstering aboveground ecosystem services, such as forage quantity, positive

effects of warming on belowground biomass, linked to such ecosystem services as carbon storage, may be smaller than average, even trending negative in diverse communities. However, the positive effects of diversity on belowground biomass are much greater than this negative interactive effect, indicating that preserving biodiversity is still essential to promoting both aboveground and belowground ecosystem services in grassland ecosystems.

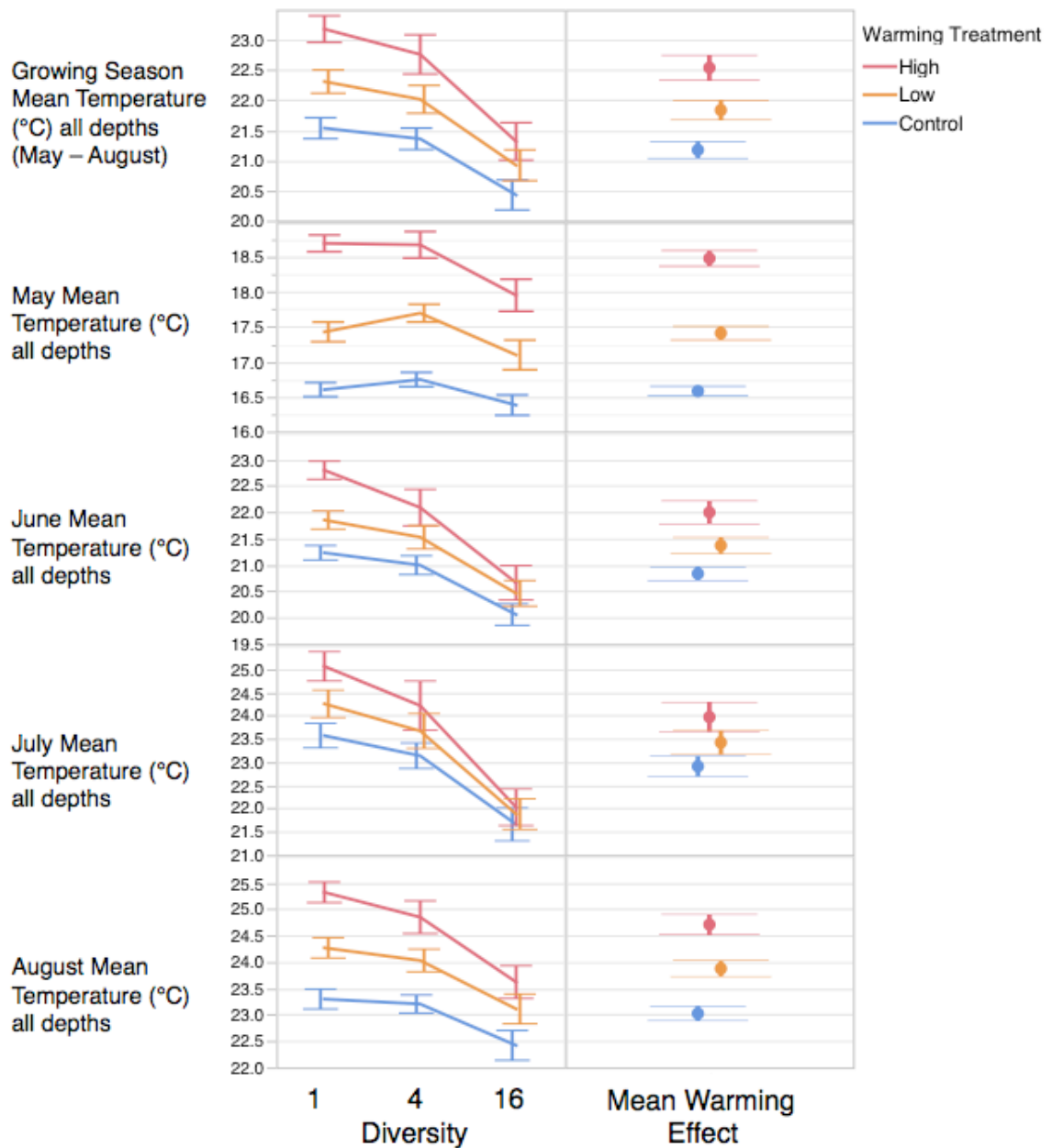


Figure 2.1. Observed effects of warming and diversity treatments on temperature averaged across all depths/heights (25, 10 cm above surface, 1, 10, 20, 30 cm below surface) in 2014 (left) and the mean effect of warming across all diversity levels and depths/heights (right). Measurements were recorded via iButton data loggers from 10

May 2014 – 31 August 2014. Aerial ibuttons (25 and 10 cm above surface) were affixed to a wooden stake and sheltered from wind and direct sunlight by an inverted lightweight, white plastic cup. Belowground ibuttons were placed either straight into the ground (1 cm depth) or inserted into the ground affixed to a wooden stake. The top row is the average across all seasons, and subsequent rows are monthly averaged for May, June, July and August respectively. Error bars are standard error of the mean.



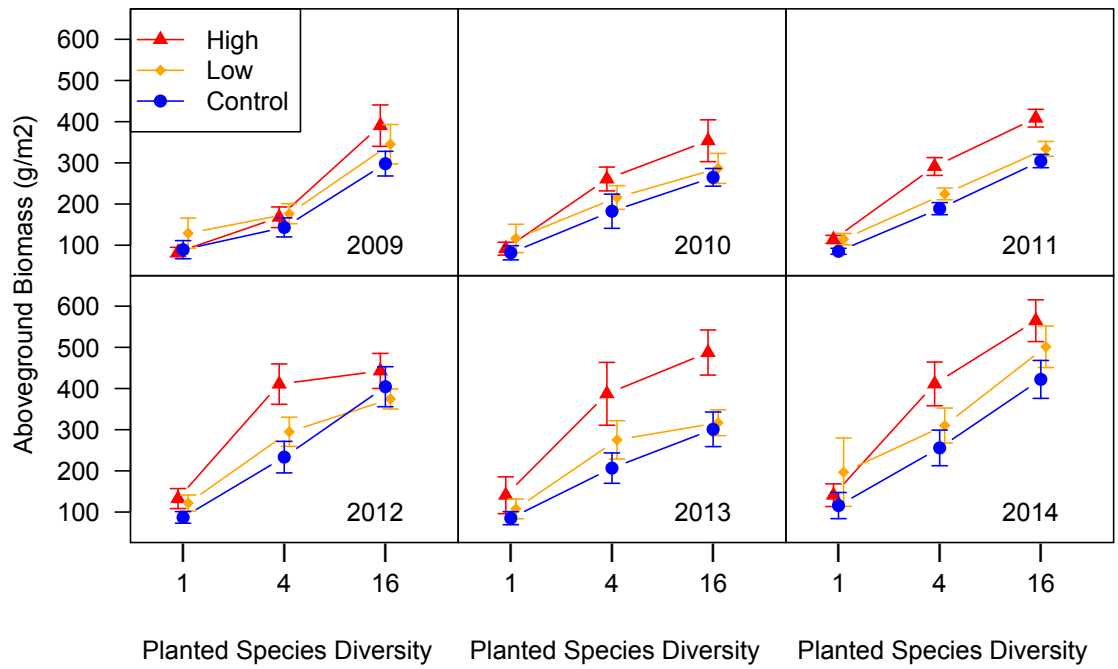


Figure 2.2. The effect of planted diversity and warming on aboveground biomass production for the six years of the experiment. Error bars depict standard error of the mean.

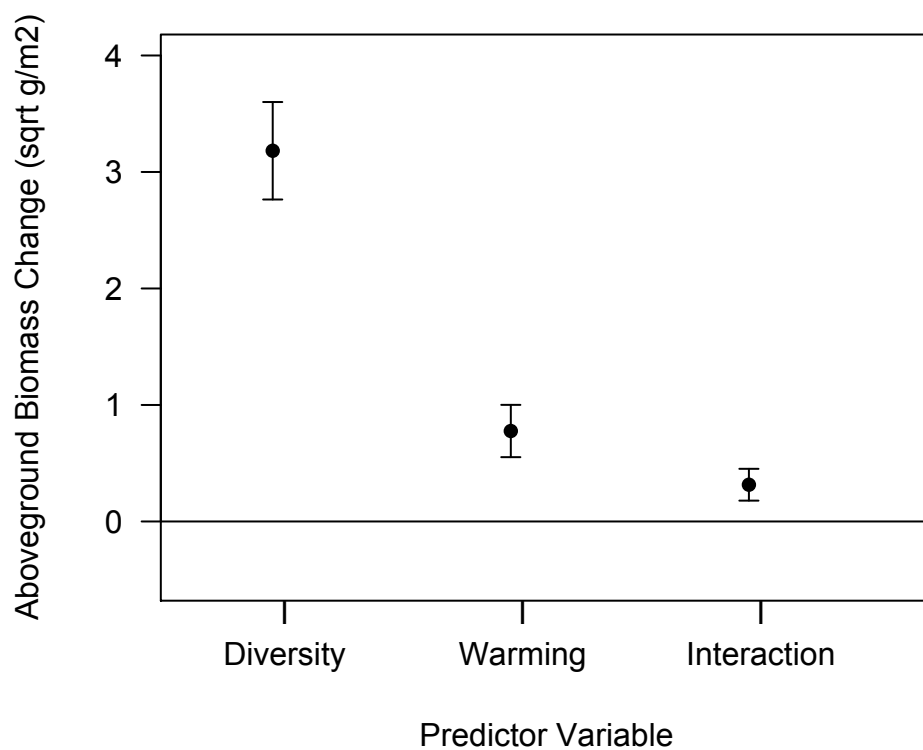


Figure 2.3. Mixed-effects model parameter estimates for the effects of diversity ( $\log_e$  species number), warming (0, 1, or 2 for control, low and high warming), and the interaction of the two on aboveground biomass (square root) across all years of the experiment. Error bars depict standard errors of the estimates.

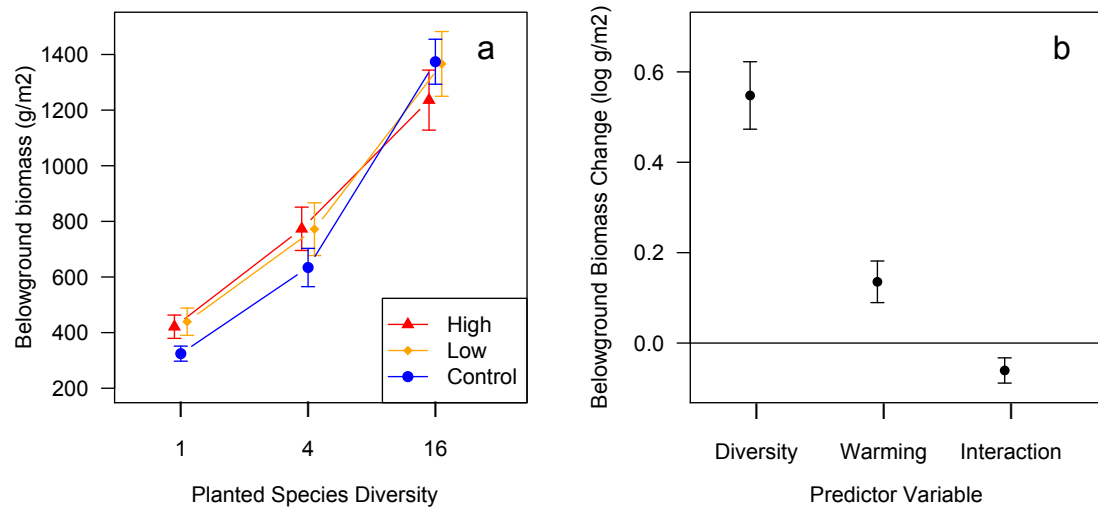


Figure 2.4. (a) The overall effect on belowground biomass (0-30 cm) across all years sampled (2009, 2010, 2012). Error bars depict standard error of the means. (b) Mixed-effects model parameter estimates for effects of diversity ( $\log_e$  species number), warming (nominal degrees C of warming), and the interaction of the two on belowground biomass. Error bars depict standard errors of the estimates.

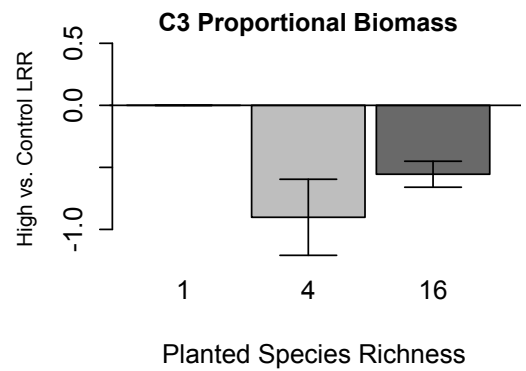
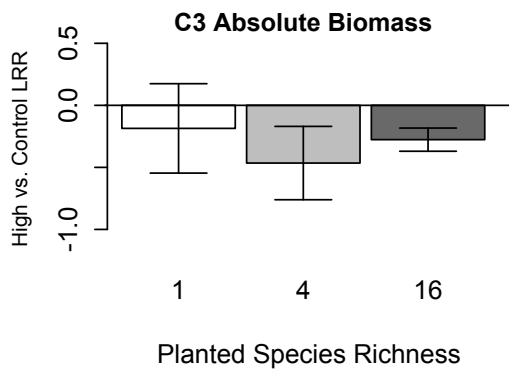
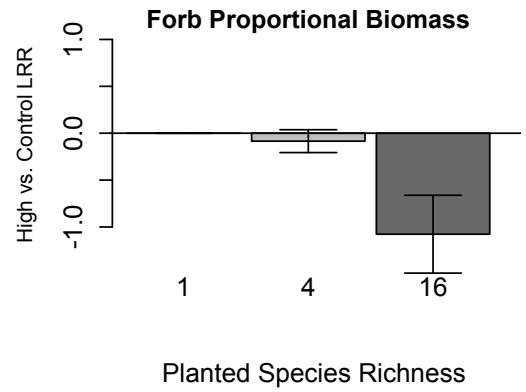
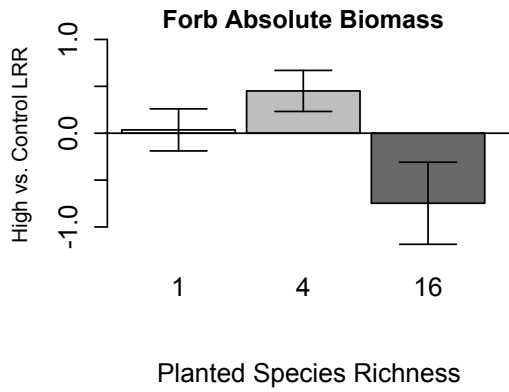
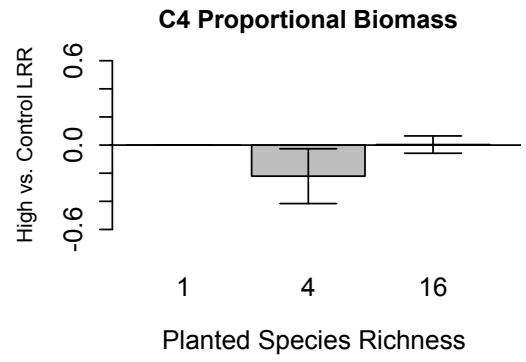
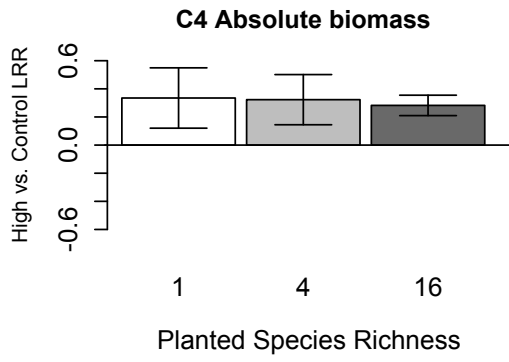
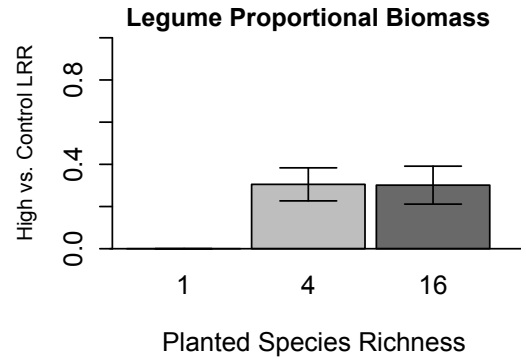
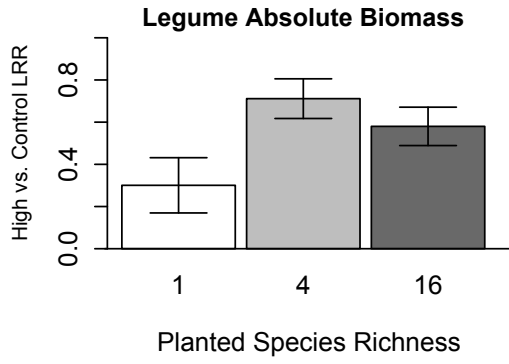


Figure 2.5.  $\text{Log}_e$  ratio (log response ratio; LRR) of aboveground biomass (left) or proportional biomass (right) in high warming to control for legumes, C4 grasses, Forbs, and C3 grass functional groups across all years. Low warming had an overall intermediate effect and is not shown. A positive LRR indicates a positive effect of warming, and a negative LRR indicates warming reduced biomass relative to controls. Error bars depict standard error of the mean.

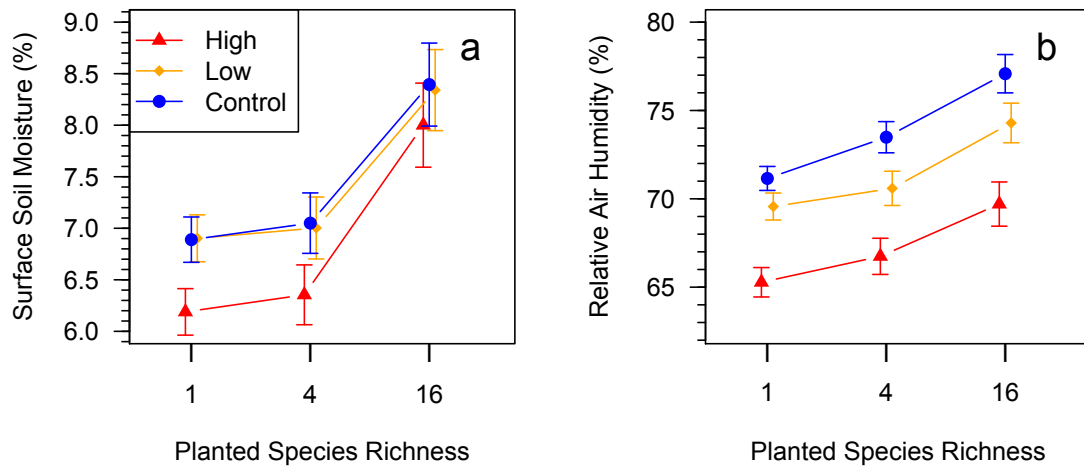


Figure 2.6. Planted diversity moderated the impacts of warming on soil moisture to 6 cm depth (a) and relative air humidity (%) 10 cm above soil surface (b). Error bars depict standard error of the mean.

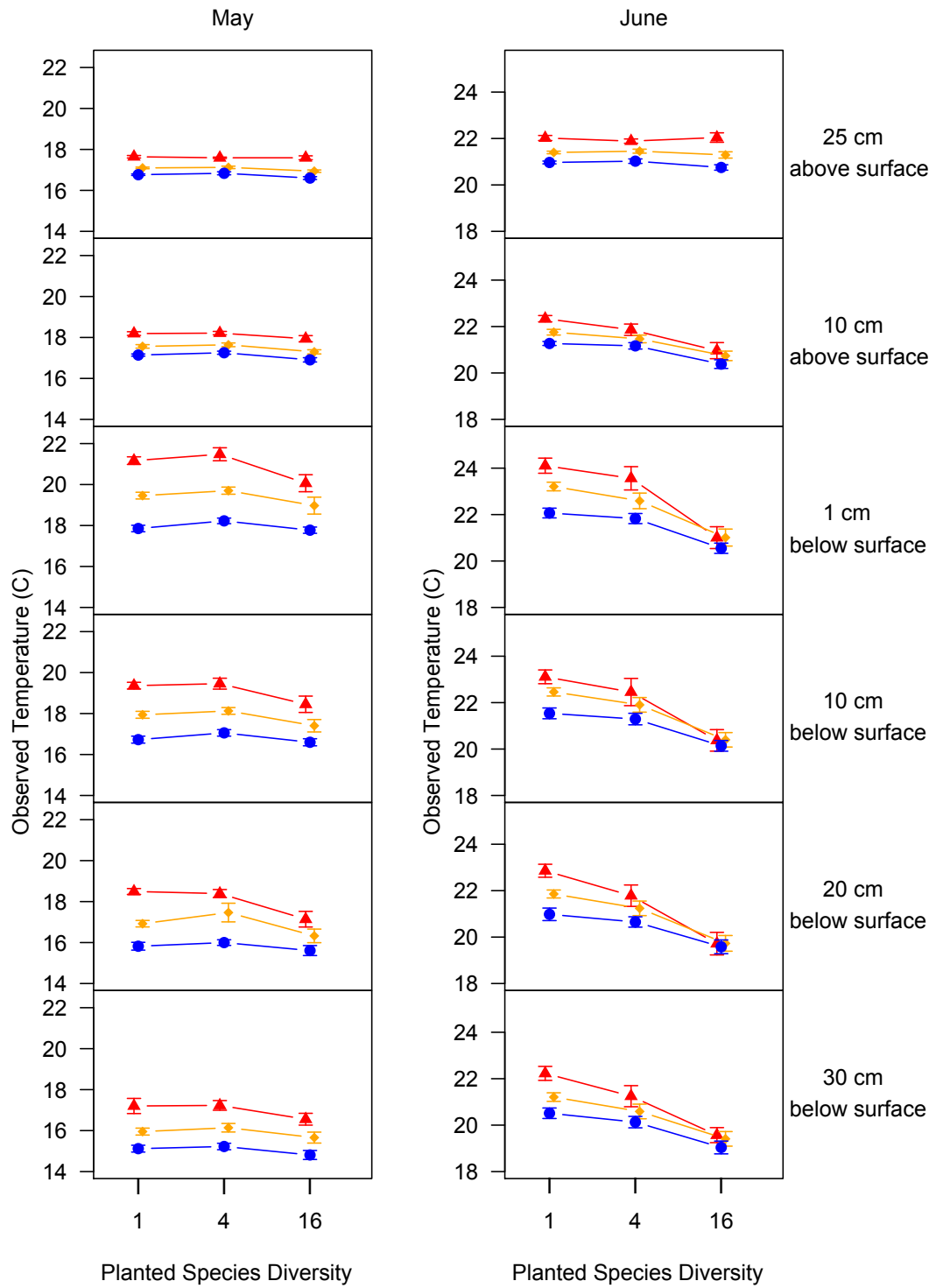


Figure 2.S1. (a) The effect of warming and diversity on air and soil temperature in May (left) and June (right) 2014. Error bars depict standard error of the mean.

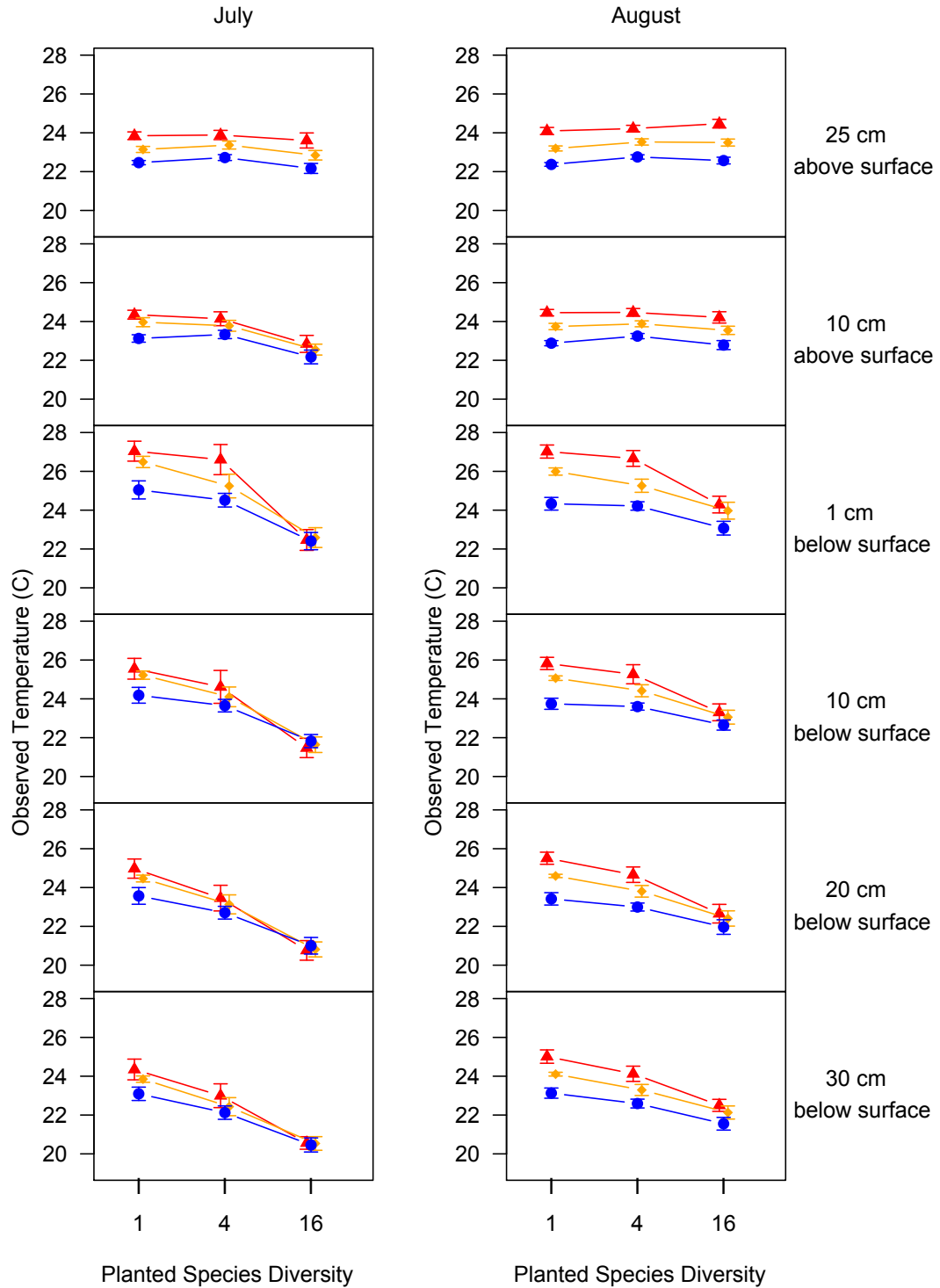


Figure 2.S1. (b) The effect of warming and diversity on air and soil temperature in July (left) and August (right) 2014. Error bars depict standard error of the mean.



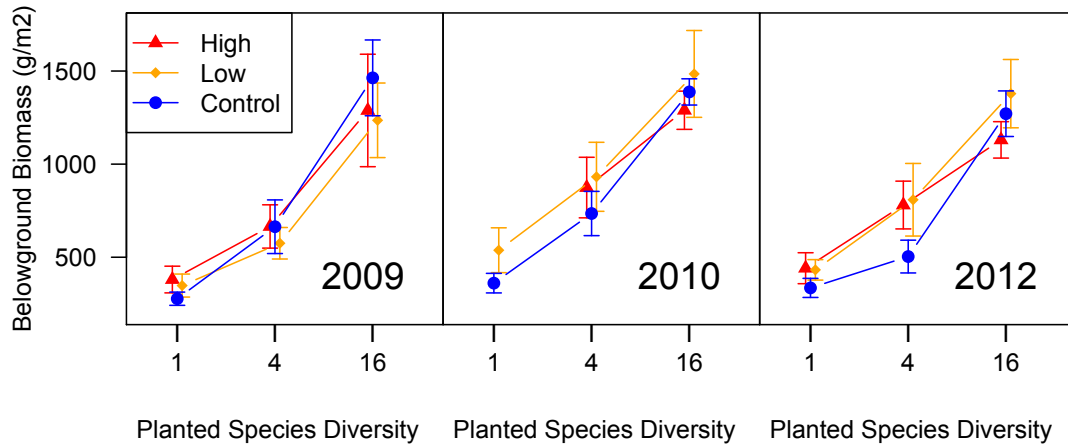


Figure 2.S2. Effects of warming and diversity on belowground biomass (0-30 cm) in 2009, 2010, and 2012 separated by year. Error bars depict standard error of the mean.

## **Chapter 3**

### **Deep soil net nitrogen mineralization, rooting depth, and aboveground productivity in a grassland biodiversity and warming experiment**

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## ABSTRACT

The effects of plant biodiversity and warming on the functioning of ecosystems may depend on their impacts on both above and belowground processes. Moreover, belowground processes may in part drive aboveground productivity, which in turn may impact belowground processes. Here we explore the roles that such potentially interactive effects may play in determining how diversity and warming impact primary productivity, carbon storage, and nutrient dynamics. We do so using a factorial biodiversity and warming field experiment to examine the drivers of rooting depths and biomass allocation, and in turn how belowground processes affect aboveground biomass. Both diversity and warming increased total and fine root biomass to 90 cm. There was a negative interactive effect of warming and the highest level of diversity on fine root biomass, suggesting that species compositional shifts or increased light competition shifted allocation aboveground in the warmed, high diversity treatments, where we saw a positive interactive effect of the two global change drivers on aboveground production. Average rooting depth of total and fine roots was increased in the diverse plots relative to low-diversity monocultures, while fine roots were also deeper in the warmed treatments. Shifts to larger and deeper root systems were correlated with increased nitrogen mineralization rates, which increased with diversity and warming. An important driver of above and belowground productivity may be diversity-dependent increases in deeper soils of both nitrogen mineralization rates, indicative of increased fertility and carbon storage at depth, and root mass, which may be a diversity-dependent positive feedback loop. Future studies of belowground trends especially deeper in the soil profile will be

essential in cataloguing the sustainability of ecosystem functioning in the face of climate change.

## INTRODUCTION

Experimental manipulations of plant species richness have shown that decreases in species richness lead to decreased productivity in the majority of terrestrial ecosystems (Cardinale et al. 2011, Tilman et al. 2014), regardless of whether the species loss occurs randomly or non-randomly (Zavaleta & Hulvey 2004) and is not driven by the presence of certain functional groups (van Ruijven et al. 2003, Reich et al. 2004). These effects tend to increase with increasing experiment duration (Tilman et al 2006; Cardinale et al. 2007; Reich et al. 2012), indicating that short-term results do not fully measure the implications of biodiversity shifts. Although the qualitative cause of greater productivity at higher diversity was initially open to question, studies showed that the selection effect -- that diversity effects are driven by the increased likelihood of including productive species with higher diversity -- diminished as experiment duration increased and that the importance of niche complementarity predominated (Fargione et al. 2007, Hille Ris Lambers et al. 2004; Loreau & Hector 2001). However, the underlying drivers of such complementarity effects have not been well established, though mechanisms such as soil fertility feedbacks (Dybzinski et al. 2008, Reich et al. 2012), plant-soil biotic feedbacks (Schnitzer et al. 2011, Maron et al. 2011, van der Putten et al. 2013), niche differentiation (Fargione & Tilman 2005, Mommer et al. 2010, de Kroon et al 2012, Mueller et al. 2013), lower disease incidence at high diversity (Mitchell et al. 2002), and food web shifts (Haddad et al. 2009), among others, have been posited as important mechanisms in grassland ecosystems.

Like biodiversity shifts, warming may affect the functioning of ecosystems through a variety of direct and indirect processes. On average across experimental warming studies, warming has a positive effect on aboveground biomass productivity, with the strongest effects seen towards the poles (Rustad et al. 2001, Lu et al. 2013). However, warming can have indirect effects through altered abiotic conditions, such as soil drying, which can lead to an overall negative effect of warming (Dieleman et al. 2012). These abiotic effects can be mediated by the species richness of the plant community, leading to an interactive effect between warming and plant species richness (Cowles et al., in review). Warming can lead to shifts in plant species richness (Klein et al 2004) and composition, which in turn could alter the effects of warming on ecosystem functioning over time.

Warming effects may vary through the soil profile and be dependent on aboveground biomass. Experimental aboveground warming can have detectable effects on soil temperature at a soil depth of 1 m (Sherry et al. 2008). However, because of the thermal mass of soil, soil temperatures are much less variable than air temperatures, and experimental warming effects are likely greater and more variable in the upper soil horizons (Luo et al. 2010, Rich et al. 2015), with effects damping out and stabilizing deeper into the soil profile. The differences in strength and variation of experimental warming at depth, the differences in the root masses of different species with depth (Craine et al. 2002), and the effects of aboveground warming on water demand and thus soil moisture depletion might combine to cause warming and species richness to interactively affect root biomass differentially across the soil profile, leading to shifts in rooting depth, and thus alter responsiveness to future climate change and nutrient capture.

Here we report the results of a long-term grassland field experiment factorially manipulating both species richness (henceforth diversity) and temperature. We examined the independent and interactive effects of diversity and warming on belowground biomass, vertical allocation of roots, nitrogen cycling and soil moisture, and examined correlations among these processes and with aboveground and total biomass production to ascertain if information on these belowground processes can provide insight into the mechanisms by which diversity and warming affect net primary productivity.

We expected our warming treatment to have a smaller proportional effect on root systems in the high diversity plots, as diverse plots have more biomass deeper in the soil profile (Mueller et al. 2013), where the warming effects on mean temperature and temperature variability are more moderate (Luo et al. 2010, Rich et al. 2015). We hypothesize that decreased surface soil moisture with warming could lead to an increase in rooting depth, as the plants would preferentially forage in moist areas and warmer and perhaps drier surface soils could lead to increased fine root mortality at the surface. We further hypothesize that increased root biomass at depth could be linked to increased nitrogen mineralization rates deeper in the soil profile, and this relationship may be indicative of a positive feedback loop. Increased root biomass deeper in the profile and root turnover can increase nitrogen mineralization (Fornara et al. 2008), especially in conjunction with the increased legume abundance seen in previous studies (Cowles et al. in review). If this is the case and nitrogen mineralization deeper in the soil increases, this could become a positive feedback loop, where roots preferentially grow deeper to the higher nitrogen availability as it leaches easily through the sandy soil. Lastly we expect that deeper rooting systems and increased nitrogen mineralization would lead to increased

aboveground net primary productivity, as more resources would be available to support greater aboveground biomass andoveryielding in diverse (Dimitrikopolou & Schmid 2004) and warmed communities.

## **MATERIALS AND METHODS**

### *Experimental Design*

The biodiversity experiment at Cedar Creek Ecosystem Science Reserve, MN, USA (CDR) and the subset of plots utilized in the Biodiversity and Climate experiment (BAC) have been described in details in many papers (eg. Tilman et al. 2001, Whittington et al. 2013, Steinauer et al. 2014, Cowles et al. in review). Briefly, 168 9m x 9m plots were planted in 1994 with random combinations of 1, 2, 4, 8, or 16 native or naturalized grassland species grouped into 4 functional categories. The species richness treatments have been maintained via handweeding and plots are burned each spring to mimic a potential natural prairie fire regime.

Warming treatments have been implemented from March-November since 2009 via infrared heaters elevated above the plots. Each of 32 1, 4 or 16 species plots was subdivided into 3 2.5m x 3m subplots receiving a warming treatment via infrared heater (Kimball et al. 2007) with either a 1200W element (High warming, aiming for ~3C above ambient), a 600W element (Low warming, aiming for ~1.5C above ambient, or no element (Control), with just a metal shade identical in size and shape to the infrared heaters. Our experiment is thus fully factorial, with each of 3 species richness levels receiving each of three warming treatments.



## *Sampling*

We collected a suite of biotic and abiotic measurements at multiple depths in the soil profile (Table 3.1). We measured surface soil temperature at 1 cm depth using iButton data loggers (Maxim, CA) during the growing seasons each year of the experiment.

Additionally, in 2014 each subplot had 1 or 2 iButtons placed at 10, 20, and 30 cm depths below the soil surface and two iButtons at 10 cm and 25 cm above soil surface, covered in a white plastic cup and affixed to a wooden stake. Although these temperature profiles were not contemporaneous with the root biomass sampling, they should be indicative of soil temperature gradients established by the warming treatments.

To understand the drivers of rooting depth, we measured variables relating to root growth and nutrient capture. Surface soil moisture measurements were taken in the top 6 cm every two weeks each growing season at six points per subplot using Thetaprobe ML2 and SM150 soil moisture sensors (Dynamax, Houston, TX, USA). Subsurface soil moisture measurements have been collected every two weeks during each growing season since the warming treatments were instated. In 2009, PVC tubes were installed in each plot reaching down to a depth of 175 cm. Every other week, a time-domain reflectometer (TDR) probe took measurements at 4 depths (3-20cm, 20-37cm, and 80-97cm) in these tubes to document the soil moisture across the depth profile. Tubes were capped between measurements to avoid accumulation of moisture. Before measuring TDR, residual moisture was removed from each tube by wiping. Soil moisture data from the growing seasons from 2009-2012 were included in analyses.

Because mineral nitrogen limits plant growth at Cedar creek (Tilman 1987), net nitrogen mineralization was measured via in-lab incubations in June 2013. Soil was taken in 4 replicate cores in each subplot, keeping soil from 0-20 cm, 20-40 cm, and 40-60 cm separated. Soil was immediately brought back to lab and subsamples from each depth x subplot combination were taken for gravimetric soil moisture, initial inorganic nitrogen ( $\text{NO}_3^-$  and  $\text{NH}_4^+$ ) extractions (1M KCl extractions), and one-month lab incubations. After gravimetric soil moisture was ascertained for each sample and every subsequent week, incubation vials were brought up to field capacity (9% gravimetric moisture; Fornara & Tilman 2009) to eliminate confounding factors in nitrogen mineralization. All samples were incubated at the same, constant temperature (22°C). After 4 weeks, soil was sampled again for extractable inorganic nitrogen. Extracts were analysed for  $\text{NO}_3^-$  and  $\text{NH}_4^+$  concentrations using a microplate spectroscopy method (Hood-Nowotny et al. 2010).

In August 2012, root biomass was measured in each subplot for each of five soil depths (0-10cm, 10-20cm, 20-30cm, 30-60cm, 60-90cm) by collecting and then subdividing 2-5 cm diameter soil cores of 90 cm depth per plot. Each sample was washed using a gentle stream of water over a 1.5-mm mesh screen, sorted into fine roots, coarse roots, tubers, root crowns, and any stem material residual after the aboveground biomass harvest, dried and weighed. Since prairie plants regrow from extensive rooting systems each year, these samples measure standing root stock – incorporating roots from the previous years and any new growth. Mean rooting depth was calculated by multiplying the proportion of total biomass in each depth interval by the mean depth of each interval and summing these values. This was calculated for each root category except stem material.

Aboveground biomass was measured via a 10 cm clip strip in mid-august 2012, just prior to root sampling, sorted to species (data not included herein), dried and weighed.

### *Statistical analyses*

Mixed effects models were used to assess the effects of treatments on abiotic properties and belowground biomass measures and were run using the nlme package in R 2.14.1. All models included plot number and subplot number as random effects, to account for non-independence of samples at different depths and the nested design of the experiment, along with the variables discussed below. Warming (0, 1, or 2 for control, low and high, respectively) and log diversity were included as continuous variables. When depth was included in a model, it was treated as continuous, with each depth increment coded as the mean depth of the sample.

To assess correlations between shallow belowground biomass metrics and environmental variables, we combined our root biomass measurements from 0 to 20 cm and regressed them against net nitrogen mineralization (0-20 cm), soil moisture (3-20 cm), and mean temperature (averaged from 1-20 cm). To this model, we then added proportion of legumes in the subplot, in order to assess whether legumes were a more important driving force than the environmental covariates. Lastly, we added our treatment effects and their interactions, to see if the effects of environmental changes and shifting legume abundance are able to explain the measured diversity and warming effects and deem these treatments and their interactions no longer significant.

We then repeated this for deep roots (20-60 cm), deep net nitrogen mineralization (20-60 cm), soil moisture (20-37 cm), and mean soil temperature (averaged from 20-30 cm, the

deepest measurements available)) sequentially adding in proportion of legumes and treatment effects, as above. We ran separate models for total root biomass and fine root biomass. Legumes were included as they are known to strongly affect root biomass and nitrogen supply rates (Fornara et al. 2008, Mueller et al. 2013) and respond strongly to warming (Whittington et al. 2013, Cowles et al. in review).

We further tested whether mean rooting depth for total and fine roots were correlated with deep soil net nitrogen mineralization, and to do this we followed a similar structure to the prior analyses, starting with a model examining solely deep soil net nitrogen mineralization (40-60 cm) as a predictor for total or fine mean rooting depth, then sequentially added in proportion of legume biomass and the treatment effects, as above.

Finally, to link above and belowground processes, we ran a linear mixed effects model examining how total rooting depth or fine rooting depth, shallow (0-20 cm) and deep (20-60 cm) soil net nitrogen mineralization, surface soil moisture (0-X cm?), and temperature averaged across all depths relate to aboveground net primary productivity. Surface soil moisture measurements were used as they had a much stronger correlation with total aboveground biomass than both soil moisture averaged across the depth profile (Pearson's correlation coefficient of 0.342 vs 0.005) and soil moisture from 3-20 cm depth (Pearson's correlation coefficient of 0.342 vs -0.0164). We next ran a model including the above predictors and proportion of legume biomass, followed by the complete model including diversity, warming, and their interaction. Finally, we ran the same models for total biomass (aboveground biomass plus belowground root biomass to 90 cm).

In these previously described models, by adding in proportion of legume biomass and subsequently treatment effects to the model of abiotic (and rooting depth variables in the aboveground model) we were able to ascertain whether the abiotic variables (described in the above paragraphs) themselves are driving the strong correlations. The second and third models fitted for each response variable asked whether the correlations were driven by increased legume abundance in the plots (Cowles et al., in review) or if the predictor variables and response variables are both responding to the experimental treatments. If either of these are the case, given our type III (marginal) ANOVAs, we expected the effects of abiotic and rooting depth variables to strongly decrease with the addition of the legume and treatment terms.

We acknowledge that many relationships in semi-natural systems are likely not simple, one-way causal relationships. Over time, feedback loops among variables make assigning causality a difficult task. However, these feedback loops themselves provide valuable information for understanding dynamic ecosystems. Thus, we discuss the results in terms of these feedback loops, and do not mean to assert the relationships described by correlations are either directly causal or unidirectional.

## **RESULTS**

### **Abiotic measurements**

Observed temperature for the entire growing season and across all heights and depths measured increased with warming, with an approximate gain (above and belowground) of 0.75°C in the low treatment and 1.5°C in the high treatment, relative to control (Fig. 3.1a;  $\chi^2_{1,62}=201$ ,  $p<0.0001$ ). The effect of warming was greatest in May (approx. 2°C warming

in high treatment relative to control) and smallest in July (high warming approx. 1°C above control temperatures). The species richness treatment had a cooling effect on soil temperature (Fig. 3.1a;  $\chi_{1,30}=10.7$ ,  $p<0.0001$ ), and also reduced the warming treatment effect on soil temperature (negative interaction with warming treatment, Fig. 3.1a;  $\chi_{1,62}=13.7$ ,  $p=0.0002$ ). Across the depth profile, the strength of the richness effect on mean temperature did not change (richness x depth interaction;  $\chi_{1,245}=0.390$ ,  $p=0.532$ , but the effect of warming treatment on mean temperature decreased deeper in the soil horizon (Fig. 3.1b; warming x depth interaction;  $\chi_{1,245}=33.1$ ,  $p<0.0001$ ). Adding in total aboveground biomass in addition to or instead of diversity did not significantly improve the model, and thus models containing diversity instead of aboveground biomass are presented here.

Surface soil moisture (0-6 cm) increased with species richness (Fig. 3.2a;  $\chi_{1,30}=3.25$ ,  $p=0.0715$ ) and decreased with warming (Fig. 3.2a;  $\chi_{1,62}=39.0$ ,  $p<0.0001$ ). We were unable to detect significant effects of warming, diversity or depth on soil moisture below the soil surface (Fig. 3.2b; Warming:  $\chi_{1,62}=0.259$ ,  $p=0.611$  NS; Diversity:  $\chi_{1,30}=0.002$ ,  $p=0.965$ ; Depth:  $\chi_{1,188}=2.44$ ,  $p=0.118$ ). There were no significant interactive effects of warming, diversity and depth on soil moisture.

In-lab incubations performed at a single temperature using soil sampled from just the High and Control warming treatments indicated that the soil net nitrogen mineralization rate increased with species richness (Fig. 3.3a;  $\chi_{1,30}=6.03$ ,  $p=0.014$ ) and decreased with soil depth (Fig. 3.3a, Fig 3.3b;  $\chi_{1,124}=43.1$ ,  $p<0.0001$ ), and there was a significant interaction between warming and diversity such that the positive effect of diversity on nitrogen mineralization was greater in the high warming treatment (Fig. 3.3c;  $\chi_{1,30}=4.86$ ,

p=0.0274). This warming x diversity interaction marginally decreased deeper in the soil profile (negative warming x diversity x depth interaction;  $\chi_{1,124}=2.72$ , p=0.0990).

### **Belowground and total biomass**

Total root biomass (all roots totaled from surface to 90 cm depth) increased with species richness (Fig. 3.4a;  $\chi_{1,30}=44.9$ , p<0.0001) and warming (Fig. 3.4a;  $\chi_{1,62}=6.22$ , p=0.0126). Like total root biomass, fine root biomass across the profile likewise increased with species richness (Fig. 3.4b;  $\chi_{1,30}=63.0$ , p<0.0001) and warming (Fig. 3.4b;  $\chi_{1,62}=25.0$ , p<0.0001), though fine roots displayed a negative interaction between species richness and warming (Fig. 3.4b;  $\chi_{1,62}=11.5$ , p=0.0007), trending towards a non-significant overall negative effect of warming at the highest richness level. Plots with higher species richness had a greater mean rooting depth for all roots (Fig. 3.4c;  $\chi_{1,30}=5.93$ , p=0.0149). When examining just fine root biomass, the mean depth of fine root allocation was greater with warming (Fig. 3.4d;  $\chi_{1,62}=4.37$ , p=0.0365) and species richness (Fig. 3.4d;  $\chi_{1,30}=3.46$ , p=0.0631).

Total biomass (the sum of aboveground biomass and total root biomass to 90 cm) was increased by both warming and diversity (Fig 3.S1b; Warming:  $\chi_{1,62}=7.98$ , p=0.00473; Diversity:  $\chi_{1,30}=67.4$ , p<0.0001), with no significant interaction between the two treatments (Fig 3.S1b;  $\chi_{1,62}=0.361$ , p=0.548 NS).

### **Linking resource supply to belowground biomass**

Shallow roots

There were no significant correlations between shallow total root biomass (0-20 cm) and either net nitrogen mineralization (0-20 cm;  $\chi_{1,29}=1.91$ ,  $p=0.167$ ), soil moisture (3-20 cm;  $\chi_{1,29}=1.81$ ,  $p=0.179$ ), or temperature (1-20 cm;  $\chi_{1,29}=0.173$ ,  $p=0.188$ ). Adding proportion of aboveground biomass from legumes (to test whether root biomass patterns were driven by legume abundances rather than the abiotic variables) did not change the model results and was not significant itself ( $\chi_{1,28}=0.007$ ,  $p=0.934$ ). The addition to this multiple regression of treatments and their interaction (warming \* diversity), changed the model such that diversity was positively associated with shallow root biomass (0-20 cm;  $\chi_{1,30}=21.4$ ,  $p<0.0001$ ), as was warming ( $\chi_{1,26}=4.19$ ,  $p=0.041$ ). In this model, we found a significant negative correlation between total root biomass to 20 cm and soil moisture to 20 cm ( $\chi_{1,26}=4.48$ ,  $p=0.0343$ ). There was no significant interaction between diversity and warming in this model ( $\chi_{1,26}=1.09$ ,  $p=0.296$ ).

Shallow fine roots (0-20 cm) were not significantly correlated with net nitrogen mineralization from 0 to 20 cm ( $\chi_{1,29}=0.0244$ ,  $p=0.876$ ), soil moisture ( $\chi_{1,29}=1.60$ ,  $p=0.206$ ), or temperature ( $\chi_{1,29}=0.745$ ,  $p=0.388$ ). Adding in the proportion of legume biomass aboveground, to test for the importance of legumes in addition to or instead of that of the abiotic shifts, significantly changed the model. In this expanded model, proportion of legumes was significantly negatively correlated with shallow fine roots ( $\chi_{1,28}=22.0$ ,  $p<0.0001$ ), soil moisture had a marginal negative correlation with shallow fine roots ( $\chi_{1,28}=3.43$ ,  $p=0.0641$ ), and there was a significantly positive relationship between soil temperature from 1-20 cm and shallow fine roots ( $\chi_{1,28}=7.25$ ,  $p=0.00709$ ). Finally, adding in diversity, warming and the warming x diversity interaction further changed the



model such that diversity had a strong positive effect on shallow fine roots ( $\chi_{1,30}=29.9$ ,  $p<0.0001$ ) and warming had a marginally positive effect ( $\chi_{1,26}=2.78$ ,  $p=0.0952$ ). Further, the interaction between warming and diversity was significantly negative ( $\chi_{1,26}=4.31$ ,  $p=0.0379$ ). Soil moisture still had a marginal negative correlation with shallow fine roots ( $\chi_{1,26}=3.33$ ,  $p=0.0677$ ) and the proportion of legume biomass aboveground maintained a strong, negative correlation with shallow fine roots ( $\chi_{1,26}=10.6$ ,  $p=0.00116$ ).

### Deep roots

Both total and fine roots deeper in the soil (20-60 cm) were strongly correlated with net nitrogen mineralization. In the simple model examining whether net nitrogen mineralization (20-60 cm), soil moisture (20-37 cm), and mean temperature (20-30 cm) correlated significantly with total root biomass (20-60 cm), net nitrogen mineralization had a large positive correlation with total root biomass ( $\chi_{1,28}=22.2$ ,  $p<0.0001$ ), while moisture and temperature exhibited no significant relationship (moisture:  $\chi_{1,28}=0.156$ ,  $p=0.693$ , temperature:  $\chi_{1,28}=1.18$ ,  $p=0.277$ ). Adding proportion of legumes to test if legume abundance aboveground was a strong predictor of deeper soil roots instead of or in addition to the environmental variables above, did not change the results for the three environmental covariates, and proportion of legumes itself had no significant relationship with total roots from 20-60 cm ( $\chi_{1,27}=1.83$ ,  $p=0.176$ ). Adding the treatment effects (to assess whether the results were solely due to all variables responding to treatment effects, or whether the treatment terms explained additional variation in deep root biomass) significantly affected the model, as diversity and warming again had strong positive effects on total deep root biomass (Diversity:  $\chi_{1,30}=34.9$ ,  $p<0.0001$ ; Warming:  $\chi_{1,25}=11.1$ ,  $p=0.000877$ ), and there was a strong negative interaction between diversity and

warming ( $\chi_{1,25}=8.07$ ,  $p=0.00449$ ). The positive correlation with net nitrogen mineralization from 20 to 60 cm remained significant in this model ( $\chi_{1,25}=4.01$ ,  $p=0.0452$ ), and when treatment effects were included in the model, proportion of legumes had a significant positive relationship with deep root biomass ( $\chi_{1,25}=8.41$ ,  $p=0.00372$ ).

Fine root biomass from 20-60 cm depth was significantly positively correlated with net nitrogen mineralization from 20 to 60 cm ( $\chi_{1,28}=14.3$ ,  $p=0.000155$ ) but not with moisture (20-37 cm;  $\chi_{1,28}=0.0814$ ,  $p=0.775$ ) or temperature (20-30 cm;  $\chi_{1,28}=0.349$ ,  $p=0.555$ ).

Adding proportion of legumes did not significantly affect the model and proportion of legumes was not significantly correlated with deep fine root biomass ( $\chi_{1,27}=0.842$ ,  $p=0.359$ ). In the full model including treatment effects, there were significant positive effects of diversity ( $\chi_{1,30}=37.2$ ,  $p<0.0001$ ) and warming ( $\chi_{1,25}=32.8$ ,  $p<0.0001$ ) and a negative interactive effect of diversity and warming ( $\chi_{1,25}=13.1$ ,  $p=0.000299$ ) on deep soil fine roots.

### Rooting depth

Deep soil net nitrogen mineralization was strongly correlated with the mean rooting depth of both total and fine roots, likely due to the strong relationship between deep soil net nitrogen mineralization on deeper soil roots as discussed above. In the simplest model, net nitrogen mineralization from 20-60 cm was positively correlated with total rooting depth ( $\chi_{1,31}=8.40$ ,  $p=0.00376$ ) and fine rooting depth ( $\chi_{1,31}=14.6$ ,  $p=0.000135$ ).

Proportion of legume biomass aboveground also had a significant positive correlation with both total ( $\chi_{1,30}=4.12$ ,  $p=0.0424$ ) and fine ( $\chi_{1,30}=20.2$ ,  $p<0.0001$ ) mean rooting depth; and the relationship with net nitrogen mineralization (20-60 cm) remained

significant (total rooting depth:  $\chi_{1,30}=4.50$ ,  $p=0.0338$ , fine rooting depth:  $\chi_{1,30}=7.04$ ,  $p=0.00799$ ). Finally, when adding in diversity, warming and the diversity x warming interaction, we found that diversity marginally significantly increased total rooting depth ( $\chi_{1,30}=3.84$ ,  $p=0.0501$ ), as did proportion of legumes ( $\chi_{1,28}=6.89$ ,  $p=0.00868$ ), but the relationship with net nitrogen mineralization did not remain detectable in this full model for total roots ( $\chi_{1,28}=9.380$ ,  $p=0.538$ ). For fine roots, however, the only significant term in the full model was the positive correlation with the proportion of legumes ( $\chi_{1,28}=23.3$ ,  $p<0.0001$ ) while diversity ( $\chi_{1,30}=3.26$ ,  $p=0.0707$ ) had a marginal positive effect.

### **Linking resource supply and capture to aboveground biomass**

We found that deep soil net nitrogen mineralization (20-60 cm) was strongly, positively linked to aboveground biomass production (Table 3.2), though when diversity was included in the model the effect was only marginally significant. When mean rooting depth for total roots was included in the model, it was only significant in the full model with treatment effects included. In this model, there were significant positive effects of diversity and warming on aboveground biomass, and the correlation between aboveground biomass and mean rooting depth was negative. In all other models, mean rooting depth trended towards a positive but non-significant effect on aboveground biomass. The inclusion of mean fine rooting depth instead of mean total rooting depth showed the same pattern as for mean total rooting depth but was never a significant variable in the model (Table 3.2).

### **Resource supply and total biomass**

Net nitrogen mineralization (20-60 cm) had a strong, positive relationship with total biomass (without proportion of legumes included:  $\chi_{1,28}=17.7$ ,  $p<0.0001$ ; with proportion of legumes included:  $\chi_{1,27}=18.1$ ,  $p<0.0001$ ), while proportion of legumes had no effect on total biomass ( $\chi_{1,27}=0.419$ ,  $p=0.516$ ). Mean temperature across all depths/heights also was positively correlated with total biomass in both models without treatment effects (without proportion of legumes included:  $\chi_{1,28}=5.60$ ,  $p=0.0180$ ; with proportion of legumes included:  $\chi_{1,27}=5.04$ ,  $p=0.0247$ ). When treatment effects were added to the model, only diversity ( $\chi_{1,30}=35.2$ ,  $p<0.0001$ ), warming ( $\chi_{1,25}=5.47$ ,  $p=0.0194$ ) and net nitrogen mineralization (20-60 cm) ( $\chi_{1,25}=4.20$ ,  $p=0.0404$ ) significantly (positively) affected total biomass.

## **DISCUSSION**

Warming and diversity had strong and interactive effects on root biomass. Diversity increased total root biomass, fine root biomass, and the mean rooting depth for both total and fine roots. Warming also increased total and fine root biomass and mean rooting depth for fine roots. We found a significant, negative interactive effect of warming and diversity on fine root biomass, such that the positive effect of warming on fine roots was greatest at lower levels of diversity. Combined, these results show that these two global change factors influenced belowground dynamics of plant communities throughout the soil profile.

Our experimental warming via infrared heaters likely differs in several ways from the warming that would result from global climate change (Aronson & McNulty 2009, Amthor et al. 2010, Rich et al. 2015) with our warming method warming the surface

more so than deeper soils. While experimental warming treatments via overhead IR lamps may not achieve the temperature increases deeper in the soil expected with global warming (Rich et al. 2015), soil buffering effects (Fierer et al. 2003) may still play a role in damping temperature extremes and variability as global warming progresses (see discussion for further information). Further, the microclimate amelioration effect of diversity (Wright et al. 2013) is likely to be present under actual as well as simulated climate change, due to the buffering effect of increased biomass on harsh climatic conditions.

We found significant effects of our experimental treatments on surface soil moisture. Both a positive effect of diversity (Wright et al. 2013) and a negative, drying effect of warming (Luo et al. 2010) would be expected under simulated and actual climate warming. The lack of a treatment effect on soil moisture below the surface, even with temperature effects found to 30 cm in our experiment and to over a meter in a similarly designed experiment (Sherry et al. 2008), is of note. A possible explanation is that hydraulic redistribution may occur as long as rooting systems span a gradient in soil water (Caldwell et al. 1998), and this would likely occur with (1) greater drying effects near the surface and (2) the relatively shallow water table at our site (Trost 2010). That deeper roots in these communities equilibrate soil moisture below the soil surface is a hypothesis that merits further study.

While net nitrogen mineralization decreased with increasing soil depth, the positive effects of diversity on net nitrogen mineralization were present throughout the depth profile and were strongest in warmed plots. Our examination of net nitrogen mineralization across the depth profile provides novel data to examine nitrogen supply

rates in our system. To our knowledge, few other studies have examined net nitrogen mineralization across the depth profile (Iversen et al. 2011, Iversen et al. 2012). These past studies, conducted in a sweet gum plantation (Oak Ridge, TN), found that increased nitrogen cycling at depth supported increased fine root growth at depth and helped sustain positive ecosystem productivity responses to eCO<sub>2</sub>. Additionally, this increase in fine roots and associated C inputs at depth were not counteracted by increased C mineralization deeper in the soil, and therefore increased soil C storage as depth in their experiment. Thus, it is possible that our increased nitrogen mineralization across the depth profile with increasing diversity, in conjunction with the marginally positive effects of warming on net nitrogen mineralization, could help drive positive responses of biomass production to diversity and warming in our system as well as increase deeper soil carbon storage.

Our reported negative interactive effect of diversity and warming on fine root biomass, such that the effects of warming were greatest at lower levels of species richness, is the reverse of what we observed for aboveground biomass, where the effects of warming were greatest at the highest level of species richness (Cowles et al. in review). There are a few possible explanations for this. First, there was a shift towards dominance of legumes in the warming treatments, which have less extensive rooting systems than prairie grasses (Craine et al. 2002). Second, the plants in diverse, warmed plots may have more intense light competition due to the higher amount of aboveground biomass. Thus, plants may be allocating more energy to aboveground biomass or photosynthetic ability in order to compete for this limiting resource. Third, we found evidence, as did Mueller et al. (2013), that diverse plots have a greater proportion of their root systems in deeper soil profiles

where the effects of our warming treatment on soil temperature and soil moisture were diminished, and thus high-diverse warmed plots may not experience as strongly the effects of warming as their low-diversity counterparts.

We also found that warming increased fine rooting depth. Contrary to expectations, we did not find surface soil drying to be a driver of increased rooting depth (not shown), but we did find strong positive correlations between fine rooting depth and deep soil (40-60 cm) net nitrogen mineralization rates and the proportion of legume biomass. Legumes are known to have deep tap root systems (Craine et al. 2002), and thus could drive rooting depth shifts via increased root growth and decomposition at depth, which could in turn increase nitrogen supplies (Fornara et al. 2008).

Net nitrogen mineralization overall was a strong driver of ecosystem productivity above and belowground, as has been shown many times in our strongly nitrogen limited system (Tilman 1987, Fornara & Tilman 2009). We show here that diversity strongly impacted net nitrogen mineralization throughout the soil profile, and the positive effects of diversity on net nitrogen mineralization were greatest in the warmed plots. We found the correlation with nitrogen mineralization and root biomass to be strongest on deeper soil roots, especially deep soil fine roots.

The link between net nitrogen mineralization and deep root biomass may be indicative of an important positive feedback loop. As diversity and warming induce fine roots to grow deeper in the soil profile and legume abundance increases (Cowles et al. in review), nitrogen mineralization may increase due to the decomposition of the deeper, nitrogen rich legume roots (Craine et al. 2002, Fornara et al. 2008), which may lead to further root

growth deeper in the soil profile. This is a potentially important feedback that could drive the sustainability of positive effects of global change factors on ecosystem functioning. The increased carbon at depth linked to increased rooting depth may also potentially counter some of the increased carbon release due to warming-enhanced decomposition (Rustad et al. 2001), however the relative effects of these processes on net carbon storage needs further examination.

In previous work, we found strong, positive independent and interactive effects of warming and diversity on aboveground biomass. The current study allowed an in depth view of potential mechanisms driving these effects. Both net nitrogen mineralization across the soil profile and abundance of legumes were positively related to aboveground biomass production, which may imply that these positive feedbacks between plant diversity, nitrogen cycling, and warming-induced compositional shifts may be driving the positive effects of diversity, warming and their interaction on aboveground biomass.

Examining belowground processes enables a deeper understanding of ecosystem responses to multiple global change drivers. We found that diversity driven increases in net nitrogen mineralization, especially deeper in the soil profile, were strongly correlated with aboveground and belowground biomass. We found that legumes were also strongly correlated with increased biomass and increased rooting depth. Legumes were strong responders to warming, and as such likely drive the biomass response to warming via their deeper root systems and subsequent increases in nitrogen supply at depth through root decomposition (Fornara et al. 2008). By looking at nutrient and root dynamics deeper in the soil profile, we found strong correlations between root growth, aboveground



primary productivity and nitrogen supply that could indicate a driving positive feedback loop underlying the positive effects of both diversity and warming.

Tables and Figures

Table 3.1. An outline of the timing and depth of each variable measured in the experiment. Shaded cells indicate a measurement was taken in that year, at that depth, of that variable. Text within the blocks give specific information on the sampling location or timing.

Depth	2012						2013						2014	
	Biomass	TDR Moisture	Surface Soil Moisture	Temperature	Biomass	TDR Moisture	Surface Soil Moisture	Temperature	Nitrogen Mineralization	Temperature				Temperature
30	Aboveground				Aboveground									
20	Biomass (August)			25 cm	biomass (August)			25 cm						25 cm
10				10 cm				10 cm						10 cm
0	0-10 cm	3-20 cm	0-6 cm	1 cm				3-20 cm	0-20 cm					1 cm
10	10-20 cm													10 cm
20	20-30 cm	20-37 cm						20-37 cm	20-40 cm					20 cm
30	30-60 cm													30 cm
40									40-60 cm					
50														
60	60-90 cm													
70														
80		80-97 cm						80-97 cm						
90														

Table 3.2. Results of linear mixed effects models examining the effects of measured variables and treatment effects on aboveground biomass. Each column is a different model and contains the parameter estimate for the effect of the variable on aboveground biomass and in parentheses is the significance of the term.

Predictor (Response is aboveground biomass)	Estimate (signif)	Estimate (signif)	Estimate (signif)	Estimate (signif)	Estimate (signif)	Estimate (signif)	Estimate (signif)
Mean rooting depth (total roots)	0.0591 (NS)	0.260 (NS)	-1.36 (*)				
Mean rooting depth (fine roots)				0.617 (NS)	1.36 (NS)	-0.337 (NS)	
Net N mineralization (0-20 cm)	16.8 (NS)	23.7 (.)	-6.61 (NS)	15.2 (NS)	23.6 (.)	-5.04 (NS)	
Net N mineralization (20-60 cm)	74.6 (***)	13.4 (***)	38.6 (*)	71.2 (**)	76.5 (***)	36.0 (.)	
Mean surface soil moisture	0.145 (NS)	-0.223 (NS)	-0.583 (NS)	0.308 (NS)	-0.0282 (NS)	-0.569 (NS)	
Temperature (all depths/heights)	0.506 (NS)	0.386 (NS)	-1.70 (.)	0.521 (NS)	0.342 (NS)	-1.36 (NS)	
Proportion of legume biomass aboveground		-4.23 (NS)	-0.0781 (NS)		-6.00 (*)	-0.868 (NS)	
Diversity			3.35 (***)			3.18 (***)	
Warming			2.43 (**)			2.19 (*)	
Diversity x Warming			-0.218 (NS)			-0.155 (NS)	

.P<0.1 \*P < 0.05; \*\*P < 0.01; \*\*\*P < 0.001

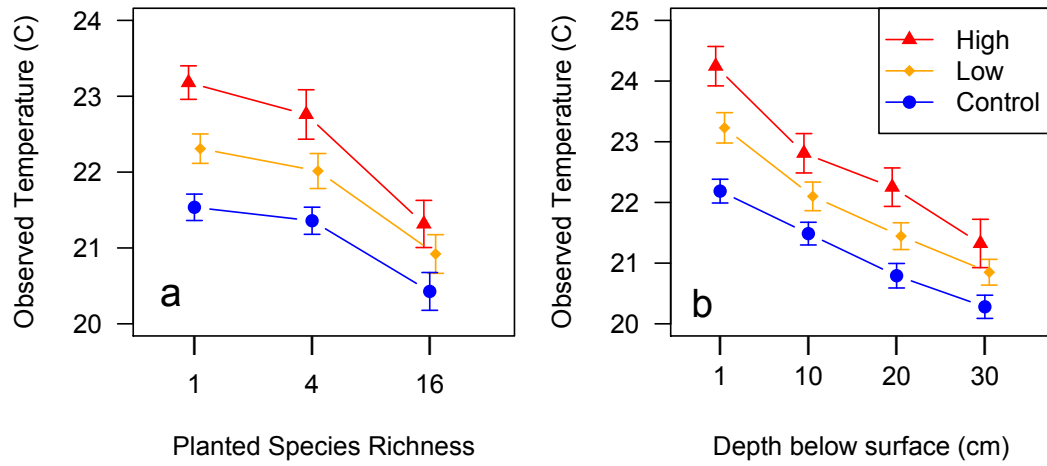


Figure 3.1. Mean temperature (a) by diversity at the soil surface and (b) throughout the depth profile (b) based on 2014 measurements. Lines represent different warming treatments. Error bars are standard error of the mean.

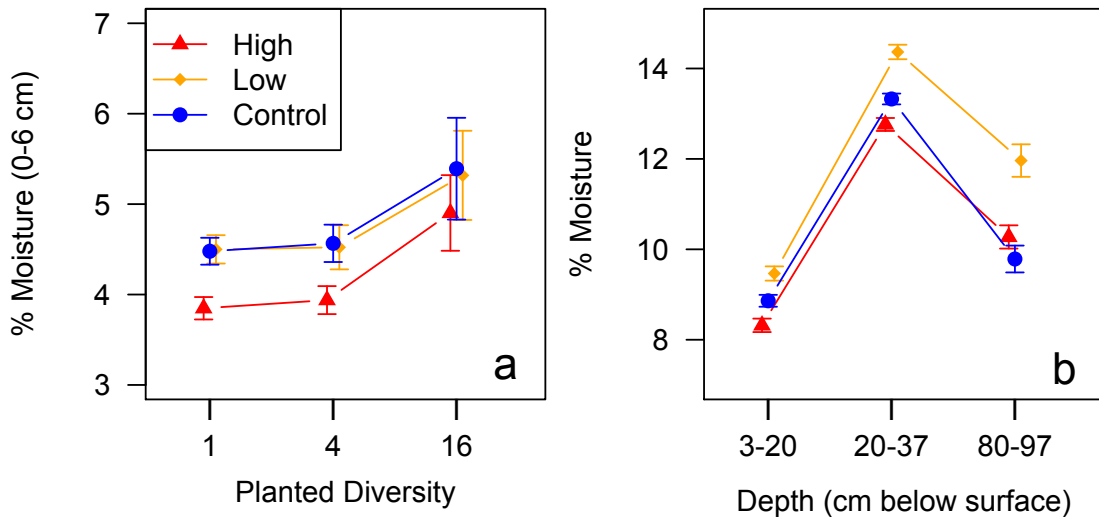


Figure 3.2. The effect of warming and diversity on surface soil moisture (a) and warming effects on deeper soil moisture (b). While the non-linear response of soil moisture deeper in the profile to warming is of note and merits further investigation, the “low” warming treatment was not included in most models. We conducted net nitrogen mineralization assays in the High and Control warming treatments only, thus any model containing net nitrogen mineralization as a covariate did not include the low warming treatment.

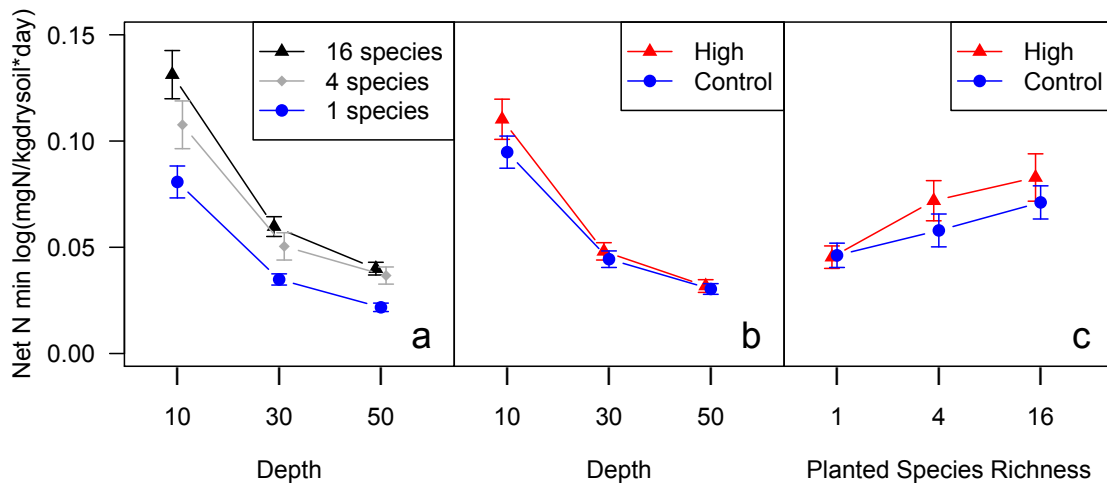


Figure 3.3. Net nitrogen mineralization rate by (a) diversity and depth, (b) warming and depth and (c) warming and diversity. Error bars are standard error of the mean.

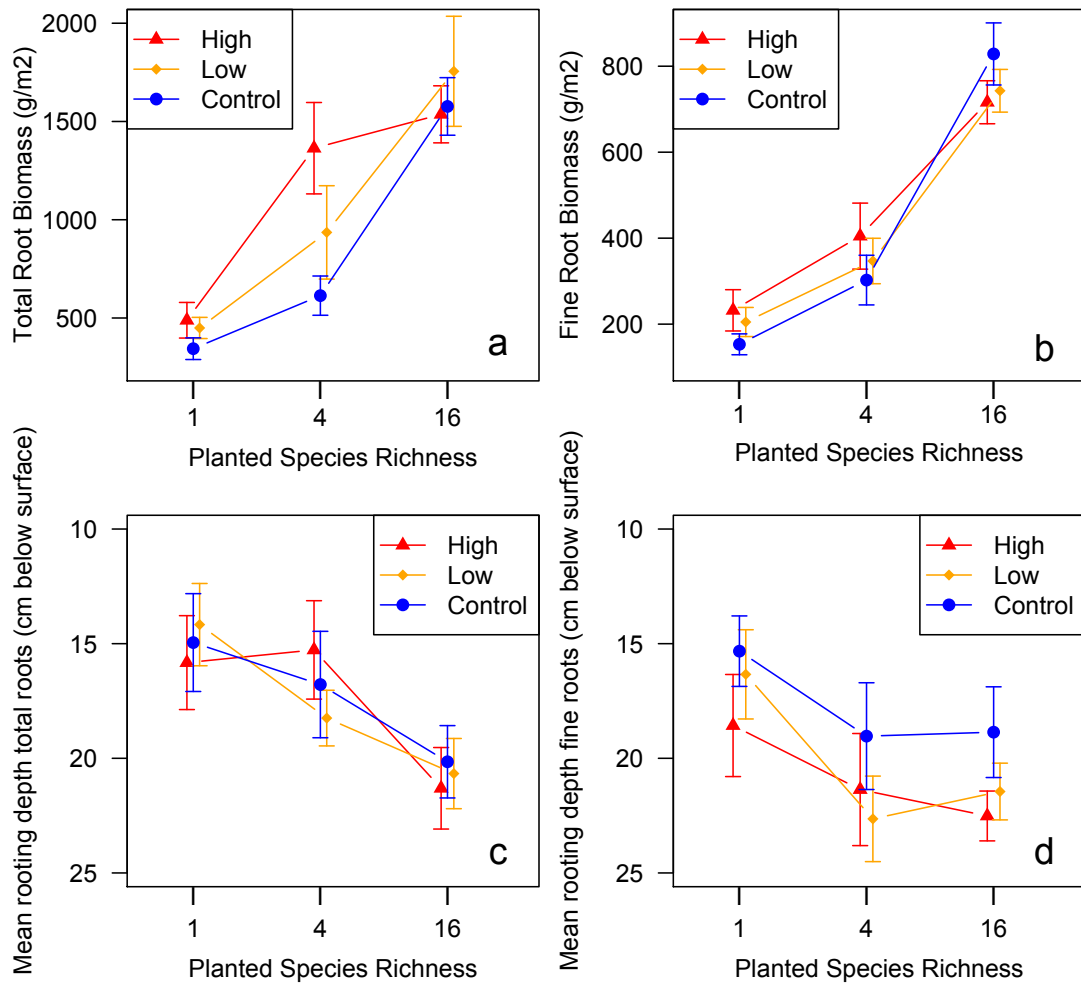


Figure 3.4. The effect of diversity and warming on root biomass and mean rooting depth (see methods for calculation). (a) total root biomass 0-90 cm, (b) fine root biomass 0-90 cm, (c) mean rooting depth for total roots, (d) mean rooting depth for fine roots. Error bars are standard error of the mean.

SUPPLEMENTAL FIGURE

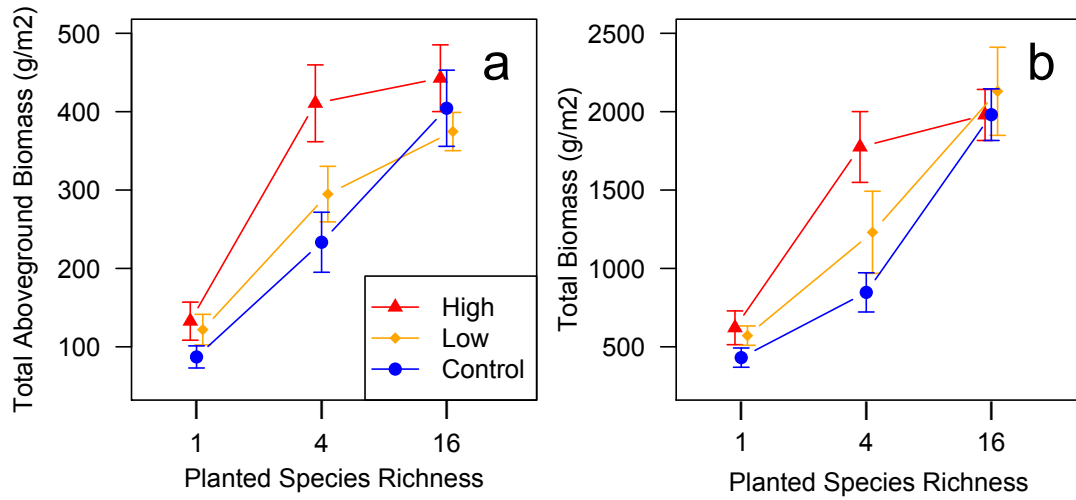


Figure 3.S1. The effect of warming and diversity on (a) total aboveground biomass and (b) total biomass (aboveground biomass and total roots to 90cm) in 2012. Error bars are standard error of the mean. See Cowles et al. (in review) for data over multiple seasons.



## Chapter 4

### **Plant-soil microbe feedbacks are not a major driver of diversity-productivity relationships in a long-term grassland experiment.**

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## **Abstract**

The positive relationship between biodiversity and productivity is well documented experimentally, yet the relative importance of various mechanisms that might drive this relationship is less clear. Some recent studies suggest that feedbacks between plant species and soil microbial communities might be a previously unsuspected mechanism. To further explore this possibility, we conducted a greenhouse experiment that used soil from an 18-year grassland biodiversity experiment at Cedar Creek, Minnesota, USA, to test the following hypotheses: 1) Growth of each species is inhibited more by soil from its own monoculture than by soil from monocultures of other species because of species-specific pathogen accumulation (plant-soil feedbacks (PSF), 2) Inhibitory effects of the monoculture soil of a species on itself (a species PSF) is lower when that species is grown in multi-species communities than by itself, and 3) Soils from high-diversity plots inhibit the growth of species less than soils from the monocultures of those species because of lower densities of species-specific pathogens. We planted 504 plant communities of one or six species in pots inoculated with soil from each of eight monocultures or from one of three high diversity (16 species) plots of the experiment. To minimize chemical soil fertility differences while maintaining distinct microbial communities, we used the soil inoculum method in which each greenhouse pot contained 93% identical, sterilized “base” soil that was then inoculated with 7% live soil from a desired soil source. Contrary to our expectations, the average feedback effect of the soil from a monoculture of each species on that species was not significantly different from

zero for either its biomass production or its germination, thus failing to support the hypothesis that plant-soil feedbacks (PSFs) contributed to greater productivity at higher diversity via diversity-induced dilution of negative PSFs. Four of the eight species displayed significant net-pairwise plant-soil feedbacks in germination, biomass production, or both, with soil from monocultures of two species having positive effects on themselves, and two having negative effects on themselves. Growing in a diverse community in the greenhouse decreased the strength of these biomass plant-soil feedbacks such that both the positive and negative PSFs were less strong. Furthermore, pots inoculated with soil from high diversity plots had higher germination success, but not biomass production, for all species. Combined, these results show that while present, microbially mediated PSFs were both positive and negative, and in total were not a dominant force driving biodiversity-productivity relationships. Rather, based on other analyses of the causes of diversity-productivity relations in the field biodiversity experiment, niche differentiation and diversity-dependent changes in soil fertility and nutrient utilization in the field explain a much larger portion of the diversity-productivity relationship than do PSFs.

## **Introduction**

The positive relationship between biodiversity and ecosystem productivity has been well documented across grassland systems, but the mechanisms driving these patterns are less clear (Cardinale et al. 2011). The positive diversity-productivity relationship (overyielding) increases over time (Tilman et al 2006; Reich et al. 2012) and at least in those studies was associated with positive and growing effects of diversity on soil fertility over time (Tilman 2006; Fornara and Tilman 2009; Reich et al. 2012). Because the diversity effect increases over time, it is likely that some feedback effects play a role in driving the diversity-productivity relationships. Previous research has focused on the role of nutrient cycling and fertility feedbacks (Dybzinski & Tilman 2008; Fornara and Tilman 2009), as it has long been hypothesized that the major factor limiting growth in grassland ecosystems is soil resource availability. Alternatively, pathogens and plant enemies may also play large roles in driving plant species abundances, spatial distributions and coexistence (Janzen-Connell effects). Since coexistence mechanisms are intrinsically linked theoretically to diversity-productivity relationships (Vandermeer 1981, Lehman and Tilman 2000, Loreau 2004), species-specific pathogens are an additional possible driver of diversity-productivity relationships. In particular, in a long-term grassland biodiversity experiment in Germany, belowground biomass of monoculture plots declined much more extremely over time than their high diversity counter-parts (Ravenek et al. 2014), suggesting a potential inhibitory role for pathogen accumulation at high conspecific densities.

Interactions between plants and soil biota have the potential to cause or contribute to positive diversity-productivity relationships (Eisenhauer 2011). For example, arbuscular-mycorrhizal fungi (AMF) diversity often increases with plant diversity, and experimentally manipulated increases in AMF diversity promote plant productivity by increasing resource exploitation (van der Heijden et al. 1998, Klironomos et al. 2000, van der Heijden et al. 2008, but see Antoninka et al. 2011). This trend of more diverse microbial communities with higher plant species richness has also been observed between planted species richness and soil bacterial communities (Bartelt-Ryser et al. 2005). This plant-microbe link suggests a positive plant-soil microbe feedback loop in high diversity communities leading to increased overall productivity.

Conversely, species-specific negative plant-soil feedbacks (PSF) that result from the accumulation of more plant-inhibiting soil organisms in monocultures and low-diversity plots than at higher plant diversity (Bever 1994, Klironomos 2002) could potentially explain why higher-diversity plots are more productive, as conspecific effects on soil microbial communities would be diluted by neighborhood diversity (Schnitzer et al. 2011, Maron et al. 2011). Negative plant-pathogen/predator feedbacks have long been considered a potential mechanism of species coexistence (Janzen-Connell hypothesis; e.g. Petermann et al. 2008) through which individuals of one species more strongly inhibit neighboring conspecifics than heterospecific neighbors, which is a classic requirement for coexistence. Clearly, such soil feedback effects could also be positive (e.g. Mangan et al. 2010, Klironomos 2002) or neutral.

Plant-soil feedbacks have the potential to explain both under- and overyielding in diverse communities. Underyielding may occur where plants experience positive PSF in monocultures that are muted in high diversity communities; overyielding where species are released from negative PSF experienced in soils on which they grow in high densities. Since the preponderance of reported diversity-productivity effects are positive, for plant-soil feedbacks to be a major explanation for observed diversity-productivity relationships, the plant-soil feedbacks would need to have net effects that are strongly negative.

Here we report an experiment designed to address the contribution of plant-soil feedbacks (PSF) to the increased productivity found in diverse plant communities. We employed a variation, described below, on the “home-and-away” PSF experimental technique of Bever (1994), Klironomos (2002), Mangan et al. (2010) and others. This approach examines how well a particular species grows in soil in which that species had grown in monoculture previously (aka “home” soil) and compares it to that species’ growth in soil on which it had not been growing but on which one or more different species had been previously growing (aka “away” soil). In the simplest form, if a species grows faster on “home” soil than “away” soil, this is taken as evidence consistent with a positive PSF. If a species grows more slowly on “home” soil, this is consistent with a negative PSF.

Our experiment is a novel extension of classic home versus away experiments in that we explicitly address how PSFs operate (1) in soil from monoculture and diverse plant communities and (2) when growing with many other species on these soils. The added experimental features in our experiment potentially allow more robust conclusions about how PSFs operate in soils from field monoculture and diverse communities, and when

growing in low and high plant diversity. Further, our approach minimized nutrient differences among soil types in order to isolate the effects of the soil microbial communities from effects of nutrient differences on plant germination and growth.

Our experiment had three distinct goals. The first goal was to determine the strength and sign of PSF among 8 focal species included in the Cedar Creek (CDR) biodiversity experiment (Tilman et al. 2001) via a home and away experimental design. The second goal was to determine if the strength of such home-and-away effects on focal species grown in monoculture soils is impacted by the presence of additional species. This comparison asks whether decreasing the conspecific density and increasing heterospecific density (while holding community density constant) ameliorates the negative PSF or whether competition with heterospecifics compounds with and intensifies the negative PSF on “home” soil. Third, we examined how plant germination and growth was impacted by soil inoculum from the field monoculture plots versus from field high-diversity plant communities.

## **Methods**

Our experiment used soil from CDR’s large biodiversity experiment, where there had been 18 years of growth of controlled species assemblages at multiple diversity levels in a fully randomized and replicated field experiment (Tilman et al. 2001). In brief, 9 meter x 9 meter communities were seeded and have been maintained via hand weeding at set diversity levels (1, 2, 4, 8, or 16 species) since 1994. The species compositions of the

plots were randomly selected from a pool of 18 species (16 herbaceous, 2 woody species) from five functional categories (C3 cool season grasses, C4 warm season grasses, nitrogen fixing legumes, and non-leguminous forbs, and woody species). Woody species did not establish or grow well, and in 2010 the few remaining (and generally very small) individuals were removed from the subset of plots from which we sampled in 2010. See Tilman et al. 2001 for more experimental details.

The soil for our greenhouse study was collected as 8 replicate 2.5 cm diameter x 20 cm depth cores per source plot. Source plots were monocultures of each of the 8 focal species and three of the 16 species mixture plots that contained all 8 of these species. The species included in the experiment were 2 representatives from each of the four herbaceous functional groups included in the biodiversity experiment (C3 grasses, C4 grasses, legumes, non-leguminous forbs; See Table 1). In our PSF greenhouse experiment, plant communities were seeded and grown in pots to assess the strength and importance of plant-soil feedbacks in regulating plant germination and growth. The experimental design is further outlined in Table 1. Each focal species monoculture was planted in 6 replicate pots on home soil (taken from a monoculture plot of that species), 3 replicates on each of the 7 soils from monocultures of other species, and 2 replicates on each soil collected in each of the three 16-species plots. Each 6 species community was replicated 3 times on each monoculture soil and 2 times on each 16 species soil. Eight distinct 6-species communities were utilized as a precaution against having strong effects of one particular species (eg. as seen in Mommer et al. 2010) drive the relationship or mask other potentially important effects.



Whether plant-soil feedback effects are indeed caused by soil organisms instead of by effects of plant nutrient uptake or plant- or diversity- dependent changes in nutrient supply rates has been often contested, because soil fertility and microbial communities commonly co-vary and are thus difficult to disentangle. Researchers have utilized a variety of methods to tease apart such effects, including adding a sterile control for each soil origin or adding soil nutrients (fertilizer) in an attempt to mitigate any among-treatment differences in soil fertility such that any differences in plant performance could then be attributed to differences in the microbial community. We employed the soil inoculum experimental design (e.g. Mangan et al 2010), with each pot containing a well-mixed combination of 93% (by volume) identical, sterile Cedar Creek sandy soil and of 7% soil inoculum collected from a given treatment in the biodiversity experiment. This approach dilutes any initial differences in abiotic properties among inoculum sources. Thus, rather than comparing unsterilized “home” soil versus sterilized “home” soil, our main contrasts were between initially identical background soils that were inoculated with 7% of “home” versus “away” natural soil communities. We feel that focusing on the effects of the different 7% inocula provides a better test of our underlying hypotheses than would a more traditional comparison of a sterile soil versus a natural soil. In a review of experimental approaches, Brinkman et al. (2010) found that home versus away (or own versus foreign) experiments, even without the inoculum method, detected less extreme feedback results than methods comparing live to sterile soils, thus our approach is a conservative test of the role of plant-soil feedbacks in diversity experiments.

**Potting and Seeding** Soil inoculum was thoroughly mixed with the sterilized soil by hand in large buckets and then distributed to individual pots (5”x3.5” Azalea pots; Volume: 0.77 liters). Pots of each soil type were separated into blocks to avoid contamination and the location of the blocks in the greenhouse and pot location within these blocks were randomized biweekly. Pots were watered with deionized water frequently to maintain moist soil (at least 1x per day depending on greenhouse conditions). After one week of watering to leach out any initial nutrient pulse, pots were seeded with the communities described in Table 1. The seeds were spatially segregated into 12 seeding spots and the seed number per spot was based on seed size. In the monoculture pots all 12 spots were seeded equally with that species and in the 6-species pots each species was seeded into 2 spots. Pots were covered with a plastic film to keep in moisture and encourage germination. This film was removed from all blocks before the film could hinder plant growth.

**Sampling** Germinants were censused after 3 weeks of growth and plants were harvested after 15 weeks of growth. Aboveground biomass was clipped at soil surface, sorted to species, dried, and weighed. The belowground biomass was not extricable due to interweaving with gauze at the base of the pots. Soil was sampled at the beginning (day of planting) and end (day of harvest) of the experiment and analyzed for available soil nitrate and ammonium using 0.01M KCl extractions and analyzed via colorimetric analysis. Final nitrate and ammonium levels were below the detection limit of the colorimetric microplate method used for analyses (Hood-Nowotny et al. 2010), and therefore not presented herein.

**Field Nitrogen Sampling** Inorganic nitrogen ( $\text{NH}_4$  and  $\text{NO}_3$ ) was sampled in all field plots included in the experiment eight times throughout the season in the same year as the soil inoculum was sampled. For this field sampling, soil was sampled in the plots using nine replicate 1 cm x 20 cm cores, homogenized and processed similarly to the greenhouse soil samples described above. We compared these measurements to the nitrogen concentration found in our greenhouse pots in order to assess the success of our inoculum approach in minimizing abiotic differences among soil sources.

## **Analyses**

*Statistical Detection of Plant Soil Feedbacks:* We operationally define a plant-soil feedback to occur if a species experiences a larger effect of its “home” soil on growth or germination than do other species (either more negative or positive). We tested for these PSFs using the interaction term between species identity and soil type in a linear model, thus determining if a source soil affects that species in a significantly different manner than the average effect of that source soil on all other species (following Mangan et al. 2010). By doing this we are able to assess the effect of a soil source on that species relative to all other species, which assures that we encompass the species-specificity of the effect (Bever et al. 1994, Mangan et al. 2010), a necessary component of PSFs as a mechanism of coexistence. We tested for two different types of plant-soil feedbacks: feedbacks on germination and survival to 3 weeks and on biomass production at the time of harvest. PSF effects on germination stage have rarely been tested (but see Bartelt-Ryser et al. 2005, Bagchi et al. 2014) but are likely important for plant establishment, while final productivity is indicative of soil effects on competitive ability.

All analyses were conducted in R 2.14.1. Data were first transformed to fit normality assumptions of the model. Multiple linear regression models were conducted using a priori contrasts to assess for species-specific plant soil feedbacks. Where zero-inflation with continuous data was an issue, separate analyses were run for (1) presence or absence of species (logistic regression) and (2) if present, regular linear model on normalized data after excluding zeros. Because results were not different when zeros were included or discarded (aside from then fitting normality assumptions), the presence/absence models were not included in the results. When analyzing effects of soil inoculum diversity on germination and biomass, linear mixed effects models (package nlme) were run with source plot as a random effect to avoid pseudoreplication. Following Mangan et al. (2010), a priori contrasts within the soil source x species identity interaction were used to determine the strength and direction of PSFs. These will henceforth be referred to as net-pairwise feedbacks (Bever et al. 2003, Mangan et al. 2010).

## **Results**

### *1) Net Overall Effects of PSF on Community Responses (hypothesis 1)*

On average across all 8 plant species, we found no significant net overall effect of soil history (home versus away) on germination ( $F_{1,55}=0.0508$ ,  $p=0.8225$  NS) in the monoculture pots, indicating that on average, species did not consistently experience inhibited or enhanced germination and survival to 3 weeks on home soils (Fig. 4.1). Additionally, we found no significant net-effect of home vs. away soil on overall final biomass ( $F_{1,55}=0.0883$ ,  $p=0.7675$  NS) on average across all species (Fig. 4.1).

## 2) *Responses of Individual Species to PSF Treatments (hypothesis 1)*

Two of eight species had significant positive net-pairwise PSFs on germination (*S. scoparium* and *K. cristata*; Fig. 4.1 Table 4.1), indicating their germination and initial survival on their own soil was enhanced relative to other species. This positive effect of *K. cristata* on itself disappeared over time, while *S. scoparium* maintained this positive self-promotion in the final biomass harvests (Fig. 4.1, Fig. 4.2, Table 4.2). One species, *A. millefolium*, exhibited a highly significant negative net-pairwise PSF on final biomass at 15 weeks, but not on germination (Fig. 4.1, Fig. 4.2, Table 4.2). A second species, *D. purpurea*, was also found to have a negative PSF on harvested biomass ( $p=0.054$ ), but not germination ( $p=0.16$ ) (Fig. 4.1, Fig. 4.2, Table 4.2).

## 3) *Modification of PSFs by the Presence of Other Species (hypothesis 2)*

We were unable to accurately identify all small germinants to species in the multispecies pots so were unable to assess whether the two significant PSFs on rates of germination/survival to 3 weeks in monoculture pots were altered by the presence of 5 additional plant species.

For the final biomass harvests, all three species with significant net-pairwise biomass PSFs in single species pots saw a significant decrease in the intensity of the PSF in multispecies pots (marked by a significant soil source x species id x planted diversity interaction; Table 4.3). Even with these significant decreases, *A. millefolium* maintained a significantly negative PSF when growing in a 6 species pot, while *D. purpurea* went from a significantly negative PSF in monoculture to a significantly positive PSF in 6 species

mixtures. *S. scoparium*, which had a significant, positive PSF in monoculture had no significant interaction in the 6 species mixtures, but the sign of the interaction remained positive (Fig. 4.3, Table 4.4).

4) *Does High Plant Diversity Have Lasting Effects on Germination and Biomass via Soil Biotic Changes? (hypothesis 3)*

The total count of all individuals in each pot at 3 weeks increased with inoculum-source plant diversity (Fig. 4.4b,  $F_{(1,9)}=5.556$ ,  $p=0.0428$ ) and the planted diversity in the pots (Fig. 4.4.b,  $F_{(1,163)}=34.996$ ,  $p<0.0001$ ). Total biomass was unaffected by inoculum-source plant diversity (Fig. 4.4a,  $F_{(1,9)}=-0.03241$ ,  $p=0.9749$  NS) but did increase with planted diversity in the pots (Fig. 4.4a,  $F_{(1,163)}=17.095$ ,  $p=0.0001$ ). There was no significant inoculum source plant diversity by planted diversity interaction for either germination or final biomass.

5) *Are these Results Solely Due to Soil Chemical Shifts?*

Inoculum-source plant diversity had no significant effect on soil extractable  $\text{NH}_4$  (Fig. 4.5a,  $F_{(1,9)}=1.7539$ ,  $p=0.219$  NS) or soil extractable  $\text{NO}_3$  in pots (Fig. 4.5b,  $F_{(1,9)}=2.00947$ ,  $p=0.19$  NS). The field nitrogen samples indicated a significant increase in  $\text{NH}_4$  in the high diversity plots relative to monoculture plots (Fig 4.5c,  $F_{(1,9)}=14.60748$ ,  $p=0.0041$ ) and no significant difference in  $\text{NO}_3$  (Fig. 4.5d,  $F_{(1,9)}=0.398454$ ,  $p=0.4436$ ). Though not significant in either, the direction of diversity's effect on  $\text{NO}_3$  trended positive in the greenhouse experiment and negative in the field. Analyses of nitrate across the entire

biodiversity experiment indicate a large, negative relationship between  $\text{NO}_3$  and planted diversity in the field plots (Tilman et al. 1996).

When including soil available  $\text{NH}_4$  or  $\text{NO}_3$  in the pots as a covariate in the models described above (1, 2 and 3), these terms were sometimes significant but never changed the significance or direction of the other terms and therefore these results are not presented here.

## **Discussion**

Our experimental results suggest that plant-soil feedbacks are not a major determinant of the diversity-productivity relationship observed in the Cedar Creek biodiversity experiment. Our use of soil inoculum with an 18-year history of experimentally maintained plant diversity and community composition should have increased the chance of detecting any plant-soil feedbacks that may have existed, while use of a small amount of soil inocula should have decreased the chance of observing the “false positive” effect that would have resulted from the long-term increase in soil fertility that occurred in this experiment (Tilman et al. 2006; Dybzinski et al. 2008; Fornara and Tilman 2009). We note that the soil dilution methodology has revealed plant-soil feedbacks (van der Voorde et al 2012).

1) *What Are the Net Overall Effects of PSF on Community and Species Responses?*  
(*hypothesis 1*)

Overall, we observed no significant effect of home versus away soils across all species, which suggests that PSFs are, in aggregate, not a major factor determining the high productivity (overyielding) observed at higher plant diversity in the biodiversity experiment.

If negative dilution of PSFs in high-diversity mixtures were a dominant driver of positive diversity-productivity relationships, we would expect many species to exhibit soil-based self-inhibition in monocultures, and to overyield in mixtures that have soils that were less inhibitory. In contrast, of the 8 species we studied, we observed significantly ( $P < 0.05$ ) positive soil feedback effects on germination of two species and on biomass production of one species, with the only significantly ( $P < 0.05$ ) negative soil feedback being on biomass production of one species.

The two species with positive and significant ( $P < 0.05$ ) PSFs were the C3 cool season grass, *K. cristata*, and the C4 warm season grass *S. scoparium*. *K. cristata* had a positive PSF only for germination, but *S. scoparium* had positive effects for germination and biomass production. The forb *A. millefolium* had a negative PSF for biomass production, but not germination. The legume *D. purpurea* was significantly less productive ( $P = 0.054$ ) in its own soil relative to other species, but the seeming strength of its PSF mainly came from other species performing better in soil inoculated with *D. purpurea* field soil. Thus, of the 8 species we tested, it seems likely that only *A. millefolium* was actually detectably limited by its own soil. As to *D. purpurea*, many species do well if invading a legume plot, likely because of the elevated nitrate and ammonium levels of its soil (Mueller et al. 2013a). It thus seems possible that soil inoculum from *D. purpurea* had a nitrogen effect,



or microbial differences, that increased growth of other species, thus causing *D. purpurea* to have a lower growth rate relative to these other species.

2) *Are PSFs Modified by the Presence of Other Species? (hypothesis 2)*

We found that higher planted diversity in our greenhouse experiment decreased the strength of both the positive and the negative PSF effects. Since the dilution effect equally lessened both positive and negative effects, this further indicates that this possible mechanism by which plant-microbial interactions could cause overyielding may not be a major driving force in the Cedar Creek biodiversity experiment. If only species with negative PSFs displayed a dilution effect of community diversity, this could indicate that PSFs were a significant driver of overyielding. On average across 2001-2014, the 8 species studied account for 69% of the harvested biomass of the 16 species plots, and 61% of the biomass of all 8-species plots (Tilman et al., *unpublished data*). By studying the more abundant species, our results further suggest that PSF's are not a major cause of the 280% overyielding observed in the experiment during that time period (Tilman et al., *unpublished data*).

While we found reduced PSF strength in multi-species communities, the interplay between competitive interactions and PSFs has been variable across studies. Other studies have found both stronger (Kardol et al. 2007, Shannon et al. 2011) and weaker (Casper & Castelli 2007) PSFs when grown under higher competitive stress, and thus ecological context, such as successional stage (Kardol et al. 2007) or invasiveness (Shannon et al. 2011), might influence the relative effects of competitive stress and dilution of PSFs.

4) *Does Plant Diversity Have Lasting Effects on Germination and Biomass via Soil Biotic Changes? (hypothesis 3)*

We found that pots containing soil inoculum from field plots with diverse plant communities had increased overall germination counts at 3 weeks but did not have greater total biomass at 15 weeks. Further work will be needed to determine how or if the strength of PSF effects changes through time. Our results corroborate previous studies that found positive interactions between diversity and soil biota on the germination of trees (Wurst et al. 2014) and grasses (Bartelt-Ryser et al. 2005). This important trend of diversity effects on germination merits further study and examination of particular mechanisms. While we do not know why diversity increased germination, diversity and species identity both can alter soil communities that can in turn affect germination. For instance, Latz et al. (2015) found that plant species identity and planted species richness both affected the expression of biocontrol compounds that promote microbial suppression of soil pathogens. Increased diversity could thus promote suppression of pathogen species that would affect germination rates.

*Overall Conclusions*

Our results provide an empirical counterpoint to the results of Schnitzer et al. (2011) and Maron et al. (2011). Those authors concluded that their studies showed evidence for pathogen-mediation of the diversity-productivity relationship, however they did not control for nutrient differences between soil sources as we did in our study. In contrast,

by controlling for nutrient differences between soil types by using a small amount of long-term “trained” soil from different communities, we found much smaller PSF effects. This raises the possibility that the driver of their results might be changes in soil chemical fertility associated with higher plant diversity (Fornara and Tilman 2009).

As a part of their experiment (experiment 2), Schnitzer et al. (2011) grew plants on 100% soil from multiple diversity levels to examine the effects on disease and productivity. They did find increased disease prevalence but not decreased productivity, in home versus away soil and found increased productivity and decreased disease in soil from diverse plots relative to monoculture plots. Because plants were grown in pots containing full field-soil (sampled from 0-60 cm), the possible effects of diversity-dependent changes in soil fertility cannot be separated from the possible effects of soil microbes. Indeed, a greenhouse study by Dybzinski et al. (2008) found that seedlings of a plant not included in the Cedar Creek biodiversity experiment grew to have an average of 70% greater biomass in soils from diverse plots than in soils from monocultures, and that this greater growth rate was tightly associated with the greater soil N and N mineralization rate of soils from high diversity plots.

By minimizing the potential effects of diversity-dependent differences in soil fertility, our study provides greater power to infer that observed effects result from microbial drivers. While we suspect this to be the main driver of differences, there are a few alternate reasons our results may be different from the previous studies by Schnitzer et al. (2011) and Maron et al. (2011). First, soil pathogens might be patchily distributed in soils and thus at times missed when sampling soils. We did attempt to control for this by

combining many (8) cores taken throughout each plot into one sample. Further, as is true for earlier studies (Hendriks et al 2013) we have no way to directly determine that our inoculum approach effectively recreated the soil communities of the sampled plots. Future work with molecular analyses in tandem with microbial function assays and specific tests for causality could provide a clearer understanding of mechanisms underlying PSFs (Mills & Bever 1998). Such microbially focused studies are a logical next step.

Our results suggest the possibility that reducing the soil fertility feedbacks present in biodiversity experiments might eliminate the seemingly positive PSF effects that have been attributed to higher plant diversity. Our results also suggest that the strength of non-nutrient linked PSFs may be small in comparison to other previously identified mechanisms, such as niche partitioning and complementary resource use. Multiple studies in the long-term biodiversity experiment that was the source of the inoculum in this study have shown that niche differentiation and diversity-induced shifts in nitrogen cycling, allocation, and use are strong determinants of productivity (Dybzinski et al. 2008, Fornara & Tilman 2009, Mueller et al. 2013b). Fornara & Tilman (2009) noted that while six variables related to soil fertility and nitrogen use explain a large proportion of the diversity-productivity relationship, adding plant diversity as a covariate in the model still improved model fit. This indicates there are still other mechanisms not included in their analyses that may be in place, one of which could be PSFs. Thus, further work is needed to explicitly link PSFs into this framework and ascertain it's relative role in driving observed productivity patterns.

Even if, as our work suggests, microbial plant-soil feedbacks may not be a major driver of overyielding, our results do suggest that soil pathogens and mutualists can have important impacts on the abundances of particular species, and that further and more mechanistic studies of soil microbe – plant interactions could provide novel insights into the functioning of terrestrial plant communities.

## Tables and Figures

Table 4.1. Plant species communities and soil sources used.

Planted Community			Soil inoculum source		
Monoculture communities				Monoculture Plots	
	<i>Achillea millifolium</i> (F)	All communities planted in each soil inoculum (full factorial)		<i>Achillea millifolium</i> (CDR plot 69)	
	<i>Liatris aspera</i> (F)			<i>Liatris aspera</i> (CDR plot 167)	
	<i>Andropogon gerardii</i> (C4)			<i>Andropogon gerardii</i> (CDR plot 109)	
	<i>Schizachyrium scoparium</i> (C4)			<i>Schizachyrium scoparium</i> (CDR plot 280)	
	<i>Poa pratensis</i> (C3)			<i>Poa pratensis</i> (CDR plot 237)	
	<i>Koeleria cristata</i> (C3)			<i>Koeleria cristata</i> (CDR plot 268)	
	<i>Lupinus perennis</i> (L)			<i>Lupinus perennis</i> (CDR plot 83)	
	<i>Dalea purpureum</i> (L)			<i>Dalea purpurea</i> (CDR plot 291)	
6 species communities				High diversity plots	
	All except C3 grasses				16-species 1 (CDR plot 108)
	All except C4 grasses				16-species 2 (CDR plot 253)
	All except forbs				16-species 3 (CDR plot 328)
	All except legumes				
	All except AG and LP				
	All except KC and SS				
	All except SS and LP				
	All except AM and LP				

Table 4.2. Plant soil feedback strength for germination and biomass for all species growing in monoculture in pots containing soil inoculum from monoculture field plots.

Response	Species	Estimate of soil x species interaction	Std. Error	DF	t-value	Pr(> t )
Count of individuals at 3 weeks	<i>K. cristata</i>	0.3334134	0.0824217	146	4.045	<b>8.44e-05</b>
	<i>S. scoparium</i>	0.3283375	0.0819213	146	4.008	<b>9.73e-05</b>
	<i>A. millefolium</i>	0.0962661	0.0814179	146	1.182	0.238981
	<i>L. perennis</i>	0.0093910	0.0829191	146	0.113	0.909984
	<i>L. aspera</i>	-0.0633350	0.0814179	146	-0.778	0.437886
	<i>P. pratensis</i>	-0.0774685	0.0814179	146	-0.951	0.34293
	<i>D. purpurea</i>	-0.1258862	0.0882052	146	-1.427	0.155659
	<i>A. gerardii</i>	-0.1367892	0.0814179	146	-1.680	0.095079
Final biomass (g) of all individuals at 15 weeks	<i>S. scoparium</i>	0.132405	0.040287	149	3.287	<b>0.001265</b>
	<i>L. perennis</i>	0.031796	0.040287	149	0.789	0.431229
	<i>K. cristata</i>	0.015121	0.040039	149	0.378	0.706223
	<i>P. pratensis</i>	-0.002343	0.040039	149	-0.059	0.953411
	<i>L. aspera</i>	-0.020739	0.040039	149	-0.518	0.605252
	<i>A. gerardii</i>	-0.055932	0.040039	149	-1.397	0.164509
	<i>D. purpurea</i>	-0.083519	0.042918	149	-1.946	<b>0.053536</b>
	<i>A. millefolium</i>	-0.156342	0.040039	149	-3.905	<b>0.000143</b>

Table 4.3. Dilution of plant-soil feedback strength with increasing in-pot community diversity. The Soil x Species x Diversity interaction estimates how the strength of the species specific plant-soil feedback for biomass changes between when species are grown in monoculture or in 6-species mixtures on the same monoculture soil comparisons. The estimates in all cases are reverse in sign to the sign of the species significant plant soil feedback displayed in table 2, indicating a dilution of feedback strength.

Species	Estimate of soil x species x diversity interaction	Std. Error	DF	t-value	Pr(> t )
<i>S. scoparium</i>	-0.0186863	0.00940580	663	-1.98667	<b>0.0474</b>
<i>D. purpurea</i>	0.0298957	0.01049889	663	2.84751	<b>0.0045</b>
<i>A. millefolium</i>	0.0216726	0.00909530	663	2.38283	<b>0.0175</b>



Table 4.4. Plant soil feedback strength for germination and biomass for all species growing in 6-species mixtures in pots containing soil inoculum from monoculture field plots. These data can be compared to the final biomass data in Table 2 to see how the strengths of feedbacks change when increasing the diversity of plants in the greenhouse pot.

Species	Estimate of soil x species interaction	Std. Error	DF	t-value	Pr(> t )
<i>S. scoparium</i>	0.0386906	0.02449140	719	1.57976	<b>0.1146</b>
<i>D. purpurea</i>	0.0658845	0.03041845	719	2.16594	<b>0.0306</b>
<i>A. millefolium</i>	-0.0481072	0.02182678	719	-2.20404	<b>0.0278</b>

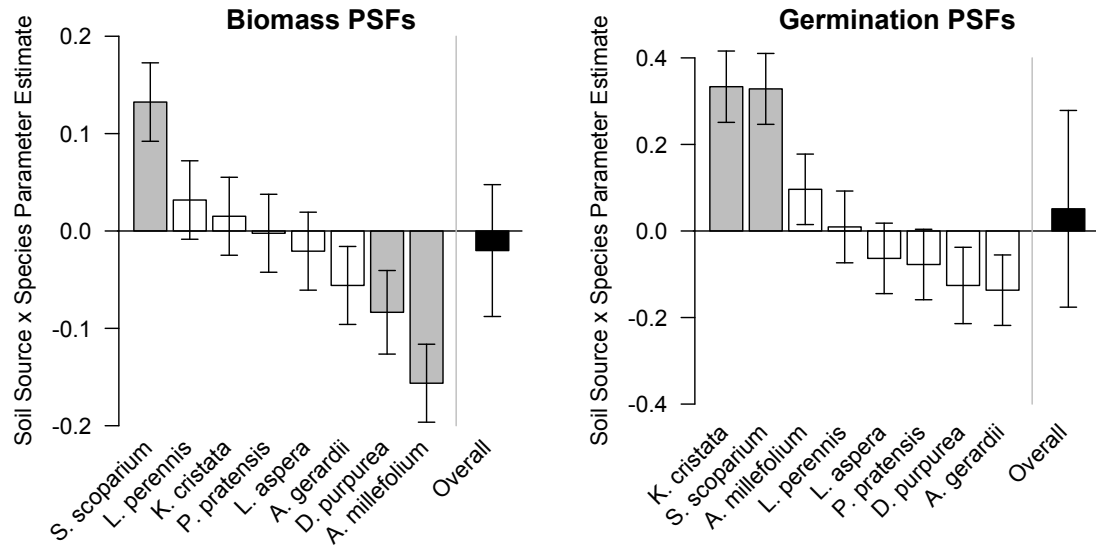


Figure 4.1. Species-specific plant-soil feedbacks for aboveground biomass at 15 weeks (a) and germinant counts at 3 weeks (b). The last bar in each is the overall strength of PSFs across all species. These soil source x species interaction parameter estimates describe how a species responds differentially to its own soil relative to all other monoculture soils. Due to the nature of the multiple regression model, the soil type x species interaction statistic also takes into account how other species respond to the same soils. See Figure 2 for more information. The grey bars indicate significant PSFs. Error bars are standard error of the estimates.

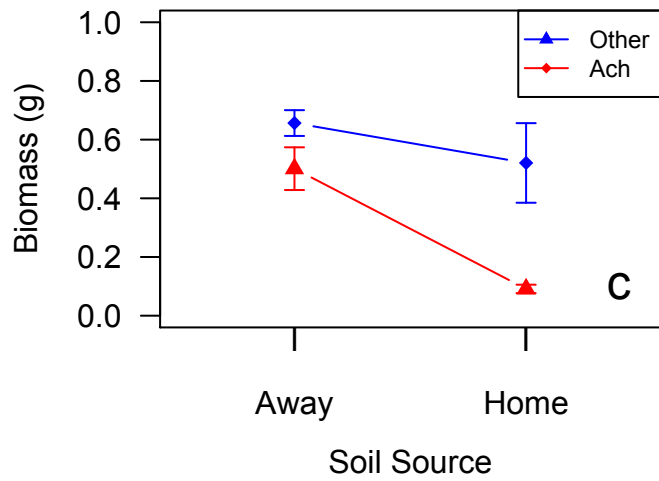
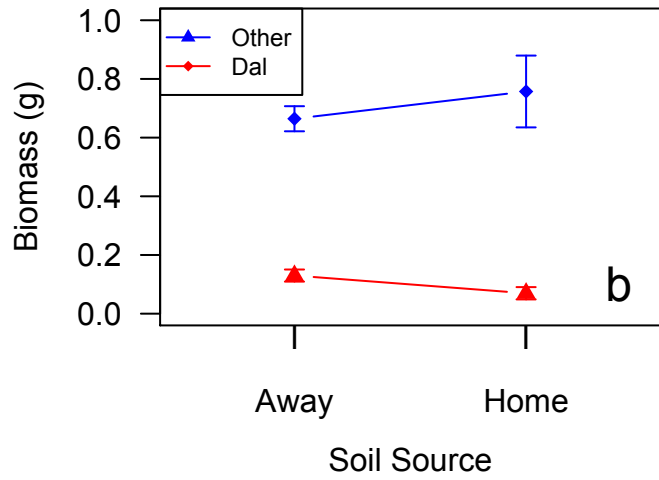
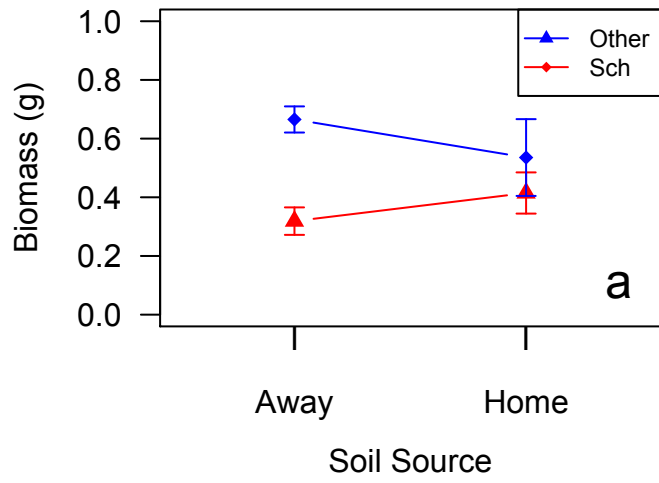


Figure 4.2. Net-pairwise feedbacks for the three species ((a) *S. scoparium* (Sch), (b) *D. purpurea* (Dal), (c) *A. millefolium* (Ach)) with significant biomass PSFs. Blue lines indicate the average of all other species and red lines are the focal species. Away soil includes all monoculture soils except for that of the focal species (home soil). For example, *S. scoparium* has increased growth in it's own soil relative to all other soils, and all other species have the opposite response; this gives the overall positive PSF seen in Figure 1. Error bars are standard error of the mean.

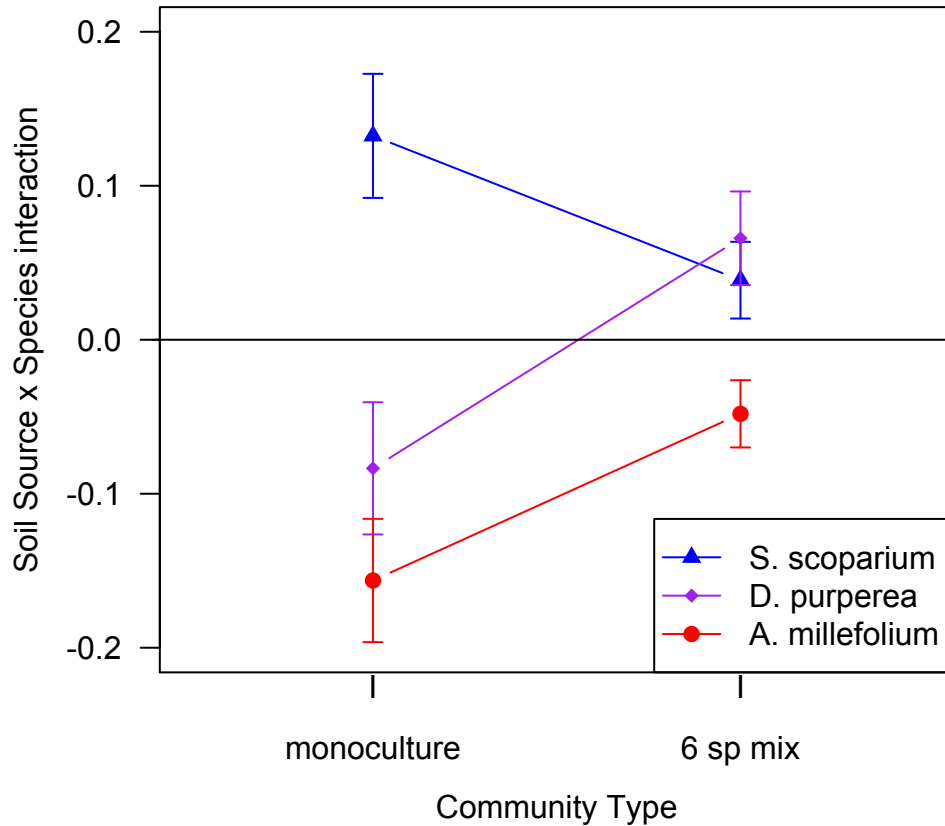


Figure 4.3. The dilution of feedback strength when growing in multispecies communities. Both *A. millefolium* and *S. scoparium* show a less extreme but same signed response in the 6-species communities relative to when growing in monoculture for the same home versus away soils comparison. *D. purpurea* flips signs from a negative PSF when growing in a monoculture pot to a positive PSF when growing with other species. Error bars are standard error of the estimates.

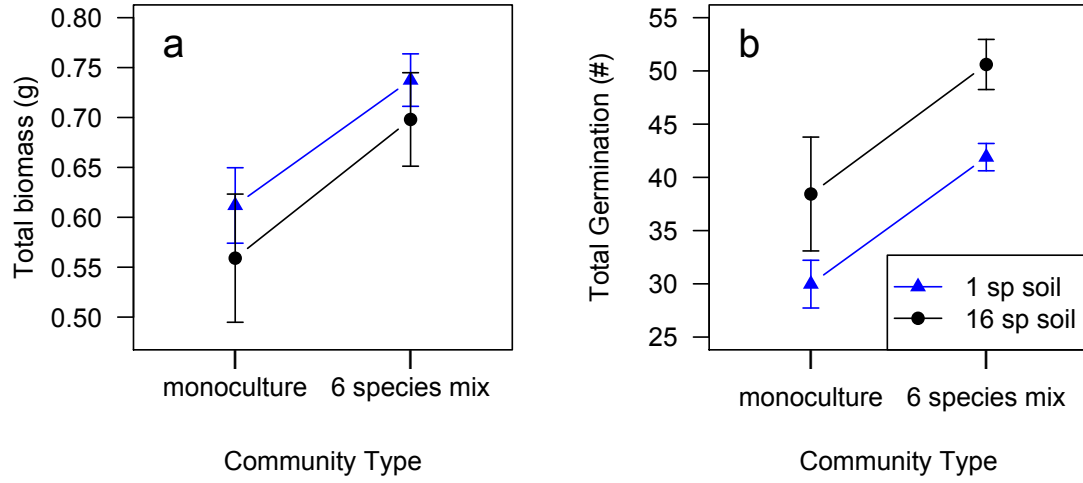


Figure 4.4. The effect of soil history (monoculture or high diversity plots) on growth (a) and germination (b) in all pots. Error bars are standard error of the mean.

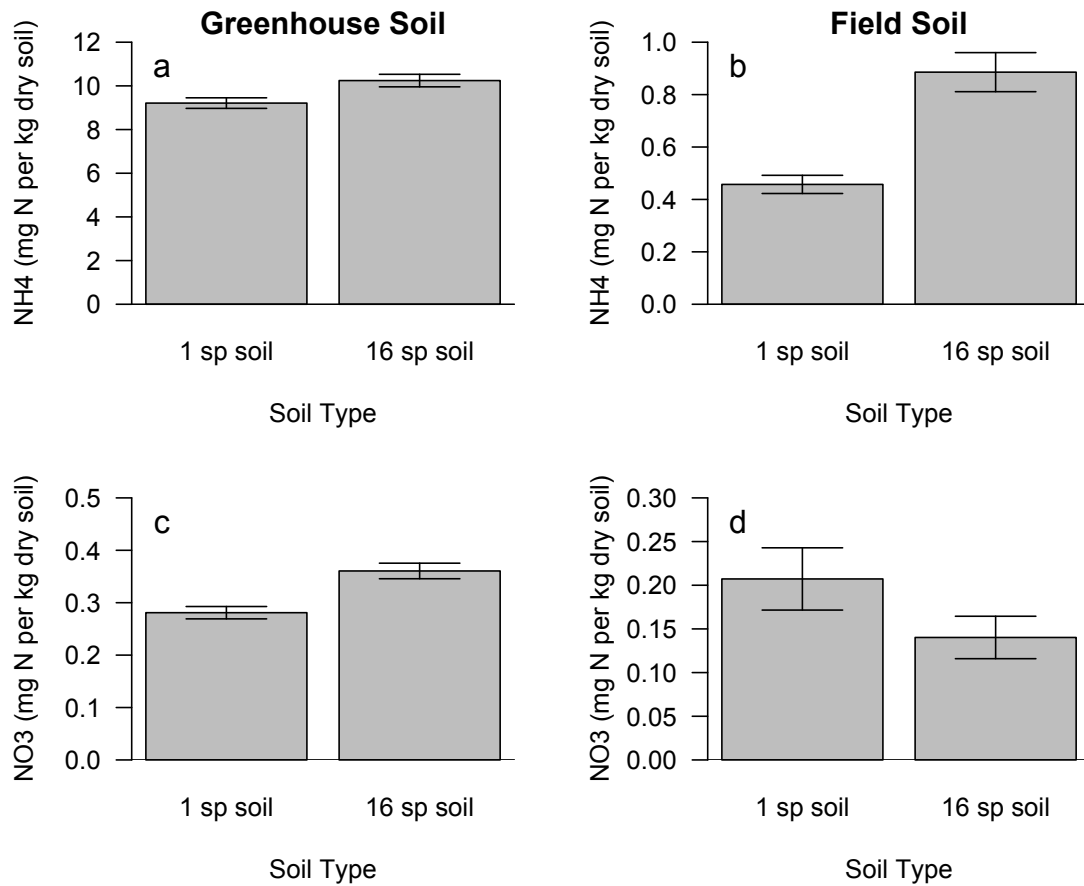


Figure 4.5. Plant diversity effects on nitrogen levels in soil (available NH<sub>4</sub> and NO<sub>3</sub>) in greenhouse pots (a,c) or in inoculum source field plots (b,d). The large difference in scale for greenhouse pots versus field plots is likely due to the greenhouse samples being taken 10 days after potting and before the seeding of plants, and thus uptake was much smaller than in the field samples, taken mid-season.

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