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Mechanisms of Overyielding and Coexistence in Diverse Tallgrass Prairie Communities

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MECHANISMS OF OVERYIELDING AND COEXISTENCE IN DIVERSE TALLGRASS PRAIRIE COMMUNITIES

by

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ABSTRACT

Mechanisms of Overyielding and Coexistence in Diverse Tallgrass Prairie Communities

by

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Despite extensive research, the biodiversity paradox (the tendency of species to coexist despite competition) remains a central ecological enquiry. Coexistence at increasingly high diversity is associated with ecological benefits, including a saturating increase in aboveground biomass produced. Despite decades of research, the mechanisms driving this relationship, known as the biodiversity-productivity relationship, remain unexplained. Spatiotemporal partitioning of resources is a commonly invoked mechanism explaining coexistence and the overyielding associated with the biodiversity-productivity relationship. However, recent research suggests that soil pathogens and soil symbionts may be key players in the biodiversity-productivity relationship, making interactions between plants and soil biota, or plant-soil feedbacks (PSFs), a key area of interest when studying coexistence and productivity.

Our experimentation on the role of PSFs in the biodiversity-productivity relationship in greenhouse conditions found that PSFs improved predictions of biomass production by 9% and accounted for 23% of overyielding due to complementarity.

However, the results from our research on the applicability of greenhouse PSF to field-observed community data call the utility of this research into question. We found no significant correlation between greenhouse- and field-measured PSF at the soil, site, or species level. Thus, field experimentation is needed to understand the biodiversity-productivity relationship as observed in the field. When greenhouse experiments on PSFs in the biodiversity-productivity relationship were replicated in the field, we found PSF-informed models were 5% more accurate in predicting the variation in productivity than Null models, but PSFs could only explain 9% of overyielding due to complementarity. This implies that PSFs play a weak role in the biodiversity-productivity relationship in the field. An investigation of vertical niche partitioning in the same system found that nitrogen and water uptake profiles when adjusted by water-use and nitrogen-use efficiencies were correlated with species productivity on the landscape. This suggest that niche partitioning plays an important role in species growth and productivity in multi-species communities. However, further research on niche partitioning's role in the biodiversity-productivity relationship, and on how mechanisms of overyielding interact, will be needed to fully understand the biodiversity-productivity relationship.

(196 pages)

PUBLIC ABSTRACT

Mechanisms of Overyielding and Coexistence in Diverse Tallgrass Prairie Communities

Leslie E. Forero

Plants compete for the same basic nutrient and water resources. According to the competitive exclusion principle, when a substantial overlap in resource pools exists, the best competitor for resources should drive all other species to extinction. The ability for plants to coexist in violation of the competitive exclusion principle is the "biodiversity paradox". Coexistence is actually beneficial for plants: as species diversity increases, you typically see increases in plant biomass production (known as the biodiversity-productivity relationship). The mechanisms behind coexistence and the biodiversity-productivity relationship remain an ecological mystery. One hypothesis is that plants obtain water and nutrients from different places in the soil, which reduces competition and results in plants coexisting and thriving by exploiting more spaces in the soil. Another hypothesis is that plants alter the soil in which they grow to their own detriment by accumulating species-specific soil pathogens or reducing soil nutrient levels. These plant-altered soils reduce the growth of species that are becoming too dominant in a plant community, creating a plant-soil feedback (PSF) effect that maintains biodiversity and increases productivity. I explored the role of PSFs and niche partitioning in coexistence and the biodiversity-productivity relationship. I investigated 1) how PSFs affect the biodiversity-productivity relationship in controlled greenhouse experiments, 2) whether greenhouse experiments are the best method to measure the role of PSFs in biodiverse communities in the field, 3) how PSFs affect the biodiversity-productivity relationship in diverse plant communities in the field, and 4) how partitioning of soil nitrogen and soil

water affect coexistence and plant productivity.

Greenhouse experimentation suggested PSFs influence productivity and the biodiversity-productivity relationship, but PSFs when measured in the greenhouse were not correlated with PSFs that were measured in the field. This implies PSFs should be measured in the field when trying to predict coexistence or the biodiversity-productivity relationship as observed in the field. Our ability to predict coexistence and productivity in the field was slightly improved by the inclusion of PSFs. However, partitioning of soil water and soil nitrogen was strongly correlated with landscape productivity in the same system, indicating that PSFs are not the dominant mechanism of these phenomena.

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CHAPTER 1

INTRODUCTION

Plants compete for the same resources: soil nutrients, soil water, and light. They acquire these same resources in similar ways (Silvertown 2004). Classical ecological theory suggests that when organisms compete for the same resources using the same strategies, stable coexistence is impossible (Gause 1932; Hardin 1960). Despite competing for the same resources using the same strategies, different plant species coexist, and even thrive (Tilman *et al.* 2001). This is termed the "biodiversity paradox."

There are ecological benefits associated with coexistence. Compared to monocultures, diverse plant communities are more stable over time, more resistant to ecological invasion, and on average produce more biomass in a given year (Tilman & Downing 1994; Tilman *et al.* 2001; Kennedy *et al.* 2002). The tendency for diverse plant communities to produce more biomass in a given year than monocultures, known as the biodiversity-productivity relationship, has a long history in ecological research, and was even mentioned in *The Origin of Species* (Darwin 1859). Observational studies remarking on the greater productivity of biodiverse ecosystems like deciduous forests when compared to low-biodiversity ecosystems like cornfields proliferated in the 50's, 60's, and 70's (Odum 1953; Whittaker 1969; Trenbath 1974). However, the first controlled experiments manipulating biodiversity and observing productivity were not established until the early 90's (Hooper & Vitousek 1997; Hector *et al.* 1999; Tilman *et al.* 2001).

The results from controlled biodiversity-productivity experiments were unequivocal: more diverse plant communities typically produce more biomass than monocultures. However, the mechanisms behind the observed biodiversity-productivity

relationships were hotly debated. Adherents to the concept of biodiversity enhancing ecosystem function hypothesized the biodiversity-productivity relationship was due to complementarity effects, or interactions between species that enhanced productivity (Tilman *et al.* 1997). Skeptics argued that biodiversity-productivity relationships reported by these newly installed biodiversity-productivity experiments could be a selection effect, caused by the greater probability of high-biodiversity treatments to contain a high-yielding species (Aarssen 1997; Garnier *et al.* 1997). A modified Price Equation was developed to parse biodiversity effects into complementarity and selection effects. The model found that the selection effect is typically zero on average, while complementarity is typically positive overall (Loreau & Hector 2001; Cardinale *et al.* 2007). This method for parsing selection effects from complementarity is unable to identify exactly what complementarity mechanisms are responsible for the observed biodiversity effect. Potential interactions between species generating the biodiversity-productivity relationship are myriad and include niche partitioning, and pathogen accumulation (van Ruijven & Berendse 2005; Schnitzer *et al.* 2011; Kulmatiski *et al.* 2012).

The role of niche partitioning as a mechanism of coexistence and productivity is not as clear in plant communities as in animal communities because unlike animals, plants compete for the same resources using the same strategies (Silvertown 2004). One possible niche partitioning strategy available to plants is spatial or temporal diversification in resource acquisition; if plants obtain resources from different soil depths or at different times of year, they can minimize competition with each other (McKane *et al.* 2002). However, studying uptake of soil resources is difficult because rooting distributions are not associated with resource uptake. It is also near impossible to

determine species-specific identities if roots do not have a unique trait such as color variation (Tobar *et al.* 1994; Marulanda *et al.* 2003). Stable isotope tracer approaches suggest that spatiotemporal niche partitioning can play a role coexistence (McKane *et al.* 2002; Kulmatiski & Beard 2013; Mazzacavallo & Kulmatiski 2015). However, the role of spatiotemporal niche partitioning in community productivity is not well resolved, with some studies suggesting it is important for productivity across a landscape, and other studies suggesting that niche overlap and community productivity are not related (von Felten *et al.* 2009; Barry *et al.* 2020; Kulmatiski *et al.* 2020).

Plant-specific soil pathogens have been suggested as a potentially important mechanism explaining coexistence and the biodiversity-productivity relationship (Maron *et al.* 2011; Schnitzer *et al.* 2011). In highly diverse plant communities, an individual plant has a low chance of growing near another plant of the same species, and thus a low chance of contracting a species-specific soil pathogen from conspecifics. Conversely, in monocultures, an individual plant has a high chance of growing near another plant of the same species, and a high chance of contracting species-specific soil pathogens that may be present. This may contribute to the maintenance of coexistence and the biodiversity-productivity relationship by density-dependent limitations on survival and growth (respectively) of conspecifics and enhanced survival and growth of heterospecifics (Schnitzer *et al.* 2011; Eck *et al.* 2019). However, microbial life in soils is incredibly complex with many functional groups and trophic levels (Wall *et al.* 2010). Species-specific symbionts like *Rhizobia* are common within certain functional groups and arbuscular mycorrhizal fungi are near-ubiquitous in the soil (Rillig 2004; Revillini *et al.* 2016). Decomposers can change plant growth and survival by altering litter quality

(Ayres *et al.* 2009). Suggesting that species-specific plant pathogens are the only microbe influencing coexistence and the biodiversity-productivity relationship is an oversimplification; it also fails to explain the full range of variation within the biodiversity-productivity relationship. For example, pathogens as a mechanism of the biodiversity-productivity relationship only explain high-yielding high diversity plots and low-yielding low-diversity plots. However, approximately 20% of biodiversity-productivity observations do not follow this trend and are high-yielding at low diversity or low-yielding at high diversity (Cardinale *et al.* 2007). Effects of species-specific symbionts and mutualists could explain these high-yielding low diversity and low-yielding high diversity observations, but because of the complexity of life in the soil, separating the effects of individual mutualists, decomposers, and pathogens is difficult (Kulmatiski *et al.* 2012).

Despite this complexity, it is possible to describe the net effect of both positive and negative interactions between plants and soil organisms using a plant-soil feedback (PSF) bioassay. PSF experiments are typically implemented in two phases. During the first phase, plant-specific soil types are created by growing target species in a common-garden soil. As the target species grow, they alter the soil microbial community within the soil. At the end of Phase 1, the target species are removed, but the altered soil microbial community remains. During Phase 2, target species are grown on soils cultivated either by the same target species ("home" or "self" soils), or on soils that were cultivated by a different target species ("away" or "other" soils). Plant growth on "self" soils is compared to growth on "other" soils; plants that experience increased growth on "self" soils compared to "other" soils have a positive PSF and plants that experience

decreased growth on "self" soil compared to "other" soils have a negative PSF (Bever 1994). Plant species with a negative PSF would be anticipated to produce low biomasses at low diversity and high biomasses at high diversity; conversely, plant species with a positive PSF would produce high biomasses at low diversity and low biomasses at high diversity. A meta-analysis of PSF literature suggests that roughly 75% of species experience negative PSF and 25% of species experience positive PSF (Kulmatiski *et al.* 2008). This is broadly consistent with the patterns of overyielding (80%) and underyielding (20%) observed in a meta-analysis of biodiversity-productivity literature (Cardinale *et al.* 2007). In addition, mathematical models predicting patterns of overyielding and underyielding from observed PSFs suggest a direct negative relationship between PSF and overyielding, i.e. a community consisting of plants with an average PSF of -0.5 would be predicted to overyield by 50% (Kulmatiski *et al.* 2012).

Although PSFs are a compelling potential mechanism of coexistence and the biodiversity-productivity relationship, they are notoriously variable (De Long *et al.* 2019). PSFs have been observed to vary depending on time of experiment, amount of biotic and/or abiotic stress, and experimental methodology (Hawkes *et al.* 2013; Schittko *et al.* 2016; Beals *et al.* 2020). It has been suggested that experiments investigating PSFs as a putative mechanism of plant community dynamics in the field should be implemented in the field (Heinze *et al.* 2016). Despite this, the majority of PSF experiments take place in short-term, low-stress greenhouse conditions (Kulmatiski & Kardol 2008).

The overarching goal of this research was to advance our understanding of the mechanisms driving coexistence and the biodiversity-productivity relationship. To do

this, we 1) performed a pilot greenhouse study assessing the role of PSF in the biodiversity-productivity relationship, 2) determined the applicability of PSF measured in the greenhouse toward PSF measured in the field, 3) assessed the role of PSFs in the biodiversity-productivity relationship using a field experiment, and 4) measured niche partitioning within the same study system to quantify niche differentiation and its role in coexistence and biomass production.

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CHAPTER 2

PLANT-SOIL FEEDBACKS HELP EXPLAIN BIOMASS PRODUCTION IN COMMUNITIES[1]

ABSTRACT

1. Plant productivity often increases with species richness, but the mechanisms explaining this biodiversity-productivity relationship are not fully understood. We tested whether or not plant-soil feedbacks (PSF) can help explain how biomass production changes with species richness.
2. Using a greenhouse experiment, we measured all 240 possible PSFs for 16 plant species. A suite of plant community growth models, parameterized with (PSF) or without PSF (Null) effects, was used to predict plant growth in 49 separately grown unique plant communities with assigned species richnesses of one to 16 species. Selection effects and complementarity effects in modeled and observed data were separated.
3. Plants created soils that changed subsequent plant growth by 25%, but because PSFs were negative for C_3 and C_4 grasses, neutral for forbs, and positive for legumes, the net effect of all PSFs was a 2% decrease in plant growth. Complementarity caused 16-species communities to produce 10.7 g (i.e. 34%) more biomass than monocultures. Null models incorrectly predicted that 16-species communities would overyield due to selection effects. Adding PSF effects to Null models decreased selection effects, increased complementarity effects,

[1] Forero L. E., Kulmatiski, A., Grenzer, J., & Norton, J .M. Plant–soil feedbacks help explain biomass production in communities. *In preparation.*

and improved correlations between observed and predicted community biomass. PSFs explained 2.5 g of the 10.7 g (23%) of complementarity-caused overyielding observed in experimental communities. Relative to Null models, PSF models improved predictions of the magnitude and mechanism of the biodiversity-productivity relationship. Results provide clear support for PSFs as one of several mechanisms that determine biodiversity-productivity relationships and help close the gap in understanding how biodiversity enhances ecosystem services such as biomass production.

4. *Synthesis*. Plant-soil feedbacks are a minor contributor to the biodiversity-productivity relationship, explaining 23% of complementarity-caused overyielding. Further research quantifying the role of other complementarity mechanisms will be needed to fully understand the biodiversity-productivity relationship.

1. INTRODUCTION

Experimental plant communities with high species richness often produce twice as much aboveground biomass as monocultures (Jochum et al., 2020; Tilman et al., 2001). Research on this diversity-productivity relationship began as a test of niche partitioning as a mechanism of overyielding, but it has become clear that other mechanisms are also important (Mahaut et al., 2020; Weisser et al., 2017). Overyielding occurs when species produce more biomass in communities than would be predicted from monocultures. In addition to niche partitioning, selection effects have been suggested as a mechanism of overyielding. Selection effects occur because productivity species are more likely to be present in more-diverse communities than less-diverse communities

(Aarssen, 1997). While important, niche partitioning and selection effects remain difficult to quantify (Barry et al., 2019; Clark et al., 2019; Mueller et al., 2013) and explain only a modest proportion of the large amount of variation in biomass production in diversity-productivity experiments (Cardinale et al., 2007; Hector et al., 2002). For example, niche partitioning and selection effects are unlikely to explain why roughly 20% of plant communities underyield (Cardinale et al., 2007; Kulmatiski et al., 2012). Despite more than two decades of research, the relative contributions of distinct mechanisms generating the diversity-productivity relationship remains unresolved (Cardinale et al., 2012; Mahaut et al., 2020; Tilman et al., 1996).

In addition to complementarity and selection effects, soil pathogens have been suggested as an additional mechanism of overyielding (Maron et al., 2011; Schnitzer et al., 2011). Species-specific soil pathogens can be expected to cause overyielding if they decrease plant growth more in monocultures than species-rich communities (Maron et al., 2011; Schnitzer et al., 2011; van Ruijven et al., 2020). While promising, a focus on species-specific pathogen effects ignores the complex array of interactions that occur between plants and soils (i.e. plant-soil feedbacks or PSFs, Bever 1994; van der Putten & Peters 1997; Ehrenfeld et al. 2005). Species-specific plant symbionts, generalist pathogens, and even decomposers can have large effects on plant productivity (Eisenhauer et al., 2012; Helander et al., 2018; Revillini et al., 2016). The suite of positive and negative interactions between plants and soil organisms are typically described using a bioassay approach in which plant growth on ‘self-cultivated’ soils is compared to plant growth on soils cultivated by other plant species (Brinkman et al., 2010; Rinella & Reinhart, 2018; van der Putten et al., 2013). This PSF approach provides

a quantitative measure of the net effect of positive and negative plant-soil interactions (Bennett & Klironomos, 2019; Bever, 1994).

While two-phase experiments remain the standard in PSF research (Rinella & Reinhart, 2018), several approaches have been used to describe the role of plant-soil interactions in diversity-productivity relationships (Cowles, 2015; Guerrero-Ramírez et al., 2019; Hendriks et al., 2013; Wang et al., 2019). Schnitzer et al. (2011), Maron et al. (2011), and Wang et al. (2019) found that diversity-productivity relationships develop in high-diversity microbial communities but not in low-diversity microbial communities. Similarly, Guerrero-Ramirez et al. (2019) found that plant growth was suppressed in soils from monoculture communities but not in soils from polyculture communities. These findings support a role of for plant-soil interactions in the diversity-productivity relationship, but complete removal of soil pathogens and shifts from high-diversity to low-diversity plant and microbial communities used in those experiments are likely to exaggerate PSF effects (Forero et al., 2019; Kulmatiski et al., 2008). We are aware of one study that used a two-phase PSF approach to support a role for PSF in diversity-productivity relationships, but that study only tested effects in three-species community (Kulmatiski et al., 2012). There remains a need for a test of PSF effects in diversity-productivity relationships for many plant species under experimental conditions that do not make dramatic changes to soil communities through sterilization and inoculation.

The overarching goal of this research was to determine the role of PSFs in the diversity-productivity relationship. Using a factorial, two-phase greenhouse experiment, we measure all 240 possible PSFs for 16 plant species (Brinkman et al., 2010; Kulmatiski & Kardol, 2008; Rinella & Reinhart, 2018). A suite of plant community growth models

was parameterized either with or without PSF values to describe PSF effects on the diversity-productivity relationship (Kulmatiski et al., 2012). Model predictions were compared to plant growth observed in a new greenhouse diversity-productivity experiment and to an existing field-based diversity-productivity experiment.

2. MATERIALS AND METHODS

Two greenhouse experiments were performed: a PSF experiment and a diversity-productivity experiment. Both experiments were designed to match a diversity-productivity experiment at Cedar Creek Ecosystem Science Reserve Long Term Ecological Research Site, East Bethel, Minnesota, U.S.A. Pairing studies allowed us to test whether greenhouse data provided inference to the field experiment. Four C_3 grasses, four C_4 grasses, four legumes, and four non-leguminous forbs used in the Biodiversity II Experiment were selected for use in this study (Table 2-1, Tilman et al. 2001). Five species that together represented less than 3% of the biomass in the Biodiversity II experiment were excluded from our PSF and diversity-productivity experiments due to lack of seed availability (*Asclepias tuberosa* L., *Dalea villosa* Nutt., *Dalea candida* Michx.) and poor growth in previous experiments (*Quercus macrocarpa* Michx., *Quercus ellipsoidalis* E. J. Hill) (Ownbey & Morley 1991). Seeds were purchased from Prairie Moon Nursery (Minnesota, USA), Granite Seed (Utah, USA), and Prairie Restorations Inc. (Minnesota, USA). Seeds were treated with a 5% bleach solution for two minutes and germinated on paper in growth chambers before being planted in the greenhouse. *L. capitata*, *L. perennis*, *D. purpurea* and *A. canescens* were scarified prior to being placed in the growth chamber. *A. gerardii*, *A. canescens*, *S. rigida*, *P. pratensis*,

A. millefolium, *K. macrantha*, *E. canadensis*, *M. fistulosa*, and *D. purpurea* were germinated under a cool-season treatment (12 hours in light at 20° C and 12 hours in darkness at 15° C). *S. scoparium*, *L. perennis*, *P. smithii*, *P. virgatum*, *S. nutans*, and *L. capitata* were germinated under a warm-season treatment (12 hours in light at 30° C and 12 hours in darkness at 20° C).

2.1 PSF Experiment

Roughly 600 L soil from an area adjacent to the Biodiversity II experiment was dried in a 31° C room for two months, then shipped to the Utah State University Crop Physiology Lab, Utah, U.S.A. A 6:1 mixture of loamy sand and sphagnum peat (Miller Companies, LLC, Hyrum, Utah) was steam sterilized twice for three hours. After cooling, field soil was added to this sand/peat mix to create a growth medium with 10% field soil by volume. This growth medium was used to fill 2,720 pots (950 mL, 7.6 cm x 7.6 cm x 20.3 cm; Steuwe & Sons, Oregon, USA). Four seedlings of each species were planted into 170 randomly-assigned pots. Plants were grown at 25° C under an equal photoperiod under 1000W double-ended high pressure sodium lamps (Gavita Pro 1000 DE, Aalsmeer, The Netherlands). Once a month, 70 mL of a modified Hoagland solution (Peter's 21-5-20, 100 ppm N) was applied to each pot to prevent chlorosis. Plants were watered when the soil surface was dry, at least once per week. After one month, the two smallest seedlings were removed. Trays holding 25 pots were rotated through the greenhouse once per month, although treatments of trays were not blocked in any way and species were randomly distributed within the trays. Plants were grown 6 months, then clipped. Re-sprouting roots were pulled and placed back into the pot of origin. Of the 2,720 planted

pots, 254 were discarded due to a lack of target plant growth and 2,466 were re-planted in Phase 2. In Phase 2, each species was planted in 16 to 35 replicate pots that had grown the same species in Phase 1 (i.e., self-cultivated soils or 'self' treatment) and three to nine replicate pots with soils cultivated by each of the 15 other species in the experiment (i.e., other-cultivated soils or "other" treatment), depending on the success of the Phase 1 treatment. Plants were grown for six months, aboveground biomass was clipped and dried to constant weight at 60 °C and weighed to the nearest 0.1 g.

2.2 Biodiversity-Productivity Experiment

In the same greenhouse and at the same time, 190 12-L pots (22.9 cm in diameter x 39.4 cm high, Stuewe & Sons, Oregon, USA) were planted with a total of 16 seedlings. Each pot contained species from one of five different species richness levels (1, 2, 4, 8, or 16 species). Monocultures were planted in 4 replicate pots and 16-species communities were planted in 30 replicate pots. Thirty-two different communities with two, four or eight species were each planted in three replicate pots. Forty of the 49 communities were identical to communities planted in Biodiversity II, seven lacked the uncommon species noted above, and monocultures of *S. rigida* and *M. fistulosa* were added to this experiment because they were accidentally excluded from the original Biodiversity II experiment due to a seeding error (Fargione, 2004). Plants were grown for eight months, then aboveground biomass was clipped, sorted by species, dried to constant weight at 60 °C, and weighed to the nearest 0.1 g.

2.3 Calculating and Analyzing PSFs

The PSF experiment was primarily used to parameterize growth rates in the community growth models, but we also report PSF values because they are a common metric that allow comparisons among PSF experiments. PSFs were calculated using the method $\bar{S} - \bar{O}/\max(\bar{S}, \bar{O})$ where S represents biomass produced in the response phase on "self" soils and O represents biomass produced in the response phase on "other" soils (Brinkman et al., 2010). Because 'self' and 'other' sample sizes differed and 'self' and 'other' pots were not inherently paired, bootstrapping was used to calculate mean and confidence intervals for PSFs using the command sample_n from the R package 'dplyr' (Schittko et al., 2016; Wickham et al., 2020). Values with confidence intervals that did not overlap zero were considered positive or negative, as appropriate. Bootstrapping was used to describe the 240 soil*species PSF values. To describe species-level PSF (i.e., across 15 soil types), we report the mean and standard error of PSF values across soil types. To test whether or not species-level PSF differed from zero a one-way student's t-test in R was used. The same one-way student's t-test was used to test whether or not soil types resulted in negative or positive PSF and to test if plant functional groups demonstrated positive or negative PSFs.

2.4 Modeling Approach

Mathematical models founded on logistic equations were used to describe species and community biomass over time. Plant community models described in Kulmatiski et al. (2011) and Kulmatiski et al. (2016) were used. To test PSF effects on plant community growth, models were parameterized so that each plant species had either one growth rate for all soil types (Null models) or a different growth rate for each soil type

(PSF models). For both PSF and Null models, a suite of simulations was performed in which five different carrying capacities were used. Both PSF and Null models describe plant growth logistically in a discrete time-step model, where growth is a function of abundances of different soil types. For example, the growth rate of species A on soil α is described as $\Gamma_{A\alpha} = \sqrt[52]{A\alpha/I}$, where Aα = the final biomass of plant A on soil α, I = initial seed mass, and 52 represents the number of timesteps in the discrete model. Plant growth in any timestep is the mean of growth rates associated with each soil type present in the previous timestep, weighted by the relative abundance of each soil type (where 'soil type' is defined by the identity of the plant species that cultivated that soil). For example, for plant A across soil types α through ι, growth would be ΓAt = ΓAα Pα + ΓAβ Pβ + … + ΓAι Pι; and for plant B across all soil types, growth would be ΓBt = ΓBα Pα + ΓBβ Pβ + … + ΓBι Pι. A carrying capacity κ limited plant growth; the carrying capacity could be unique to either a species or to the community as a whole. Changes in each plant's biomass can be described as $A_{t+1} = A_t + \Gamma A_t ((\kappa - A_t)/\kappa)$, $B_{t+1} = B_t + \Gamma B_t ((\kappa - B_t)/\kappa)$.

Soil types grow as a function of plant growth in the previous timestep: for example, soil type α growth is calculated as $\alpha_{t+1} = (1 + \mu\Gamma_{At}A_t)\ \alpha_t$; for soil type β growth is calculated as $\beta_{t+1} = (1 + \mu\Gamma_{Bt}B_t)\ \beta t$. The parameter μ represents a conversion factor between microbial biomass and plant biomass (Appendix A Table A-1). Microbial growth was not limited by a carrying capacity because microbial proportional abundances (i.e., 0 to 1) are used to determine plant growth responses to soil type, so the absolute size of different microbial communities does not affect plant growth.

Null models included only one growth rate on all soil types. This growth rate was derived from species biomass on "other" soil types. $A = f(\Gamma A_{other})$, $B = f(\Gamma B_{other})$

(Kulmatiski et al. 2016, Appendix A Table A-1).

The five different carrying capacities were determined by 1) the maximum observed growth in any plot in the community experiment, 2) the maximum mean observed growth in any community, 3) the maximum species-specific growth in community plots, 4) the maximum observed growth in any PSF plot, and 5) the maximum species-specific growth in any PSF plot. Model results were compared to observed growth in greenhouses using generalized additive mixed models (gam in the mgcv package in R programming; Wood 2017)

2.5 Parsing Selection and Complementarity Effects

A modified Price Equation was used to calculate net biodiversity effects, complementarity effects, and selection effects for predicted and observed diversity-productivity relationships (R package 'partitionBEFsp" (Clark et al., 2019; Loreau & Hector, 2001). This method uses an additive model and monoculture growth to calculate over- or under-yielding of a polyculture: $\Delta RY_i = Y_i - (M_i/N) = (Y_i/M_i) - (1/N)$, where ΔRY_i is the over- or under-yielding of species i in mixture, Y_i is the yield of species i in mixture, M_i is the yield of species i in monoculture, and N is the species richness of the polyculture. The net biodiversity effect is the difference between the total observed yield of a mixture and the expected yield, or average monoculture biomass of all species in a plot ($\Delta Y = Y_O - Y_E$). This net effect can be partitioned into a selection effect and a complementarity effect. Complementarity effects can be calculated as $N\overline{\Delta RY}\overline{M}$, where N is the species richness of the mixture, $\overline{\Delta RY}$ is the average deviation from the expected

relative yield, and $\bar{M}$ average yield in monoculture. Selection effects can be calculated as $Ncov(\Delta RY, M)$.

3. RESULTS

3.1 PSF Experiment

Across the 240 soil*species combinations, the mean PSF was -0.02 and the mean of the absolute value of PSFs was 0.25. Nineteen values were positive, 23 values were negative and 198 values were neutral (Fig. 2-1a). Fifteen of 16 species demonstrated a PSF on at least one soil type.

At the species-level (i.e., across soil types), the mean PSF was -0.12, and the mean of the absolute value of PSFs was 0.22. Five PSFs were negative, four were positive, and seven were neutral (Fig. 2-1b). At the functional-group level, C_3 and C_4 grasses demonstrated negative PSF, forbs demonstrated neutral PSF and legumes demonstrated positive PSF (Fig. 2-1c). PSFs for the three most productive species in monoculture (*L. capitata*, *D. purpurea*, and *A. canescens*) were 0.19, 0.12 and 0.42.

3.2 Biodiversity-Productivity Experiment

Aboveground biomass increased with species richness: 16-species communities produced 34% more aboveground biomass than monocultures (Fig. 2-2). This was caused by a negative selection effect that increased with species richness (i.e., underyielding due to selection effects decreased as species richness increased) and a positive complementarity effect that increased with species richness (i.e. overyielding due to complementarity increased as species richness increased) (Fig 2-3a).

3.3 Model Predictions

The PSF model predicted 24% more biomass in 16-species communities than in monocultures. The Null model predicted 19% more biomass in 16-species communities than in monocultures, (Fig. 2-2). There was no support for separating biomass, overyielding, and selection effects from observed, Null or PSF data (Table 2-2). There was support for separating complementarity effects from Null and PSF model predictions (Table 2-2). The PSF model predicted a constant positive complementarity effect that was large relative to the Null model (Fig. 2-3b and c).

Predictions of community biomass by the PSF ($y = 0.729x + 9.946$, $R^2 = 0.674$, $P < 0.001$, RMSE = 6.334) and Null models ($y = 0.772x + 8.550$, $R^2 = 0.582$, $P < 0.001$, RMSE = 7.170) were correlated with community biomass observed in the 49 experimental plant communities (Fig. 2-4). Predictions of species-level biomass in monocultures by the PSF ($y = 0.883x + 5.709$, $R^2 = 0.907$, $P < 0.001$, RMSE = 4.938) and Null models ($1.058x + 1.840$, $R^2 = 0.895$, $P < 0.001$, RMSE = 5.253) were also correlated with observed monoculture biomass (Appendix A Fig. A-2a). However, predictions of species biomass in multi-species communities from the PSF and Null models were not correlated with observations (Appendix A Fig. A-2b, A-2d, A-2f, A-2h) because both models over-predicted growth in three legume species with positive PSFs. When these three species were removed, PSF model predictions of species biomass in 2-species ($y = 1.131x + 3.428$, $R^2 = 0.207$, $P = 0.02$, RMSE = 4.514), 4-species ($y = 2.833x - 0.712$, $R^2 = 0.380$, $P < 0.001$, RMSE = 5.007), 8-species ($y = 2.543x - 0.488$, $R^2 = 0.297$, $P < 0.001$, RMSE = 2.464), and 16-species ($y = 4.23x - 0.987$, $R^2 = 0.474$, $P = 0.009$, RMSE = 1.499) communities were correlated with observations. Null model

predictions of species biomass were correlated with observations in 4-species ($y = 2.612x - 0.636$, $R^2 = 0.295$, $P = 0.002$, RMSE = 5.339), 8-species ($y = 2.109x - 0.216$, $R^2 = 0.244$, $P < 0.001$, RMSE = 2.515), and 16-species ($y = 4.294x - 1.092$, $R^2 = 0.454$, $P = 0.01$, RMSE = 1.528) communities but not in 2-species communities (Appendix A Fig. A2-c, A-2e, A-2g, A-2i).

4. DISCUSSION

Plant communities produced more biomass than monocultures due to complementarity effects. More specifically, 16-species communities produced 34% more biomass than monocultures. Null models incorrectly predicted 19% overyielding due to selection effects. Adding PSFs to the Null model improved predictions by decreasing selection effects, increasing complementarity effects and predicting 23% overyielding in 16-species communities relative to monocultures. Further, PSF model predictions of community biomass were better correlated with observations than Null model predictions (*i.e.*, $R^2 = 0.67$ and 0.58, respectively). Thus, adding PSFs to a plant community model improved predictions of plant community biomass production, and produced predictions where the mechanism of the diversity-productivity relationship was consistent with observations. Results provide clear support for PSFs as one of several mechanisms that determine diversity-productivity relationships.

The diversity-productivity relationship observed in 16-species communities was caused primarily by 10.7 g of overyielding due to complementarity. Relative to Null models, PSF models increased complementarity effects from -0.5 to +2.1 g suggesting that PSF explained 23% (*i.e.*, 2.6 / 10.7) of the overyielding observed in experimental communities. PSF causes overyielding due to complementarity when negative PSFs

decrease plant growth more in monocultures than in communities (Maron et al., 2011). This is likely to occur because 'self' soils and, therefore, species-specific soil pathogens are less common in diverse plant communities (Kulmatiski et al., 2012; van Ruijven et al., 2020). For example, communities with C_3 grasses had neutral to negative PSF and the greatest overyielding due to complementarity (*i.e.*, the largest deviations from expected monoculture yield ΔRY). Conversely, underyielding due to complementarity occurs when positive PSF effects increase plant growth in monocultures more than in communities (Kulmatiski et al., 2012). This is anticipated to occur because species-specific soil symbionts are less common in diverse plant communities.

After a six-month cultivation phase, plants created soils that changed subsequent plant growth by 25%. However, because PSFs were both positive and negative, the net effect of PSFs was only a 2% decrease in growth. When all the plants in a community demonstrate similar growth rates and PSF values, the plant community growth models used in this study predict that overyielding will be directly proportional to net PSF values (Kulmatiski et al. 2012). In other words, a net PSF of -10% would be expected to produce +10% overyielding. However, because intrinsic growth rates and PSF vary widely among species, the net effect of PSF on overyielding is a more complicated function of the specific plant-soil-plant interactions that occur in the community. In other words, the average PSF produced across the 16 species measured is not as important for the diversity-productivity relationship as the PSFs for the species in a biodiverse community, and the amount of species-specific soil being cultivated within a biodiverse community. For example, low-biomass plants with large PSFs and high-biomass plants with small PSFs will have little effect on overyielding. In this experiment, the net PSF value was -

2%, but because some dominant and common species in the experiment realized relatively large negative PSF, models predicted 23% overyielding due to complementarity. For example, *P. smithii, P. pratensis,* and *D. purpurea* made up the largest proportion of the 16-species communities with 24%, 16%, and 7% abundances, respectively. *P. smithii* had a neutral PSF, *P. pratensis* a negative PSF, and *D. purpurea* a positive PSF, but their weighted average PSF was -0.09, which would be associated with 9% overyielding, rather than the 2% suggested by the -0.02 average PSF value. Further, PSF varied widely among soil types. For example, though *P. smithii* had a neutral PSF at the species level it had PSFs of -0.46 and -0.59 on certain soil types.

This experiment provided rare insight into factorial vs. species-level PSF (Kulmatiski 2016; Rinella & Reinhart 2018). Factorial experiments with more than two to three species have been uncommon, because factorial designs require large sample sizes. For example, this study produced 240 PSF values which are nearly as many as reported in several PSF meta-analyses that included 300 to 1000 PSF values (Crawford et al., 2019; Kulmatiski et al., 2008; Lekberg et al., 2018). This 16-species factorial design allowed us to examine how PSF varies across a relatively wide range of soil types (Fig. 2-1a, b). Five species produced positive PSF on one soil type and negative PSF on another soil type. Further, PSF within a soil type varied widely, with only three soil types producing consistently negative or positive feedbacks (Fig. 2-1a). It should not be surprising that PSF vary widely on soils cultivated by different species (for example a legume vs. a C_4 grass soil), but it has been difficult to demonstrate due to sample size requirements (Crawford et al., 2019; Rinella & Reinhart, 2018). In contrast to results from a previous study with three-species communities (Kulmatiski, 2016), results from

this experiment suggest that 'other' soil types are important to determining PSF and the role of PSF in plant community growth (Rinella & Reinhart, 2018). Additional factorial studies will be needed to gain a better understanding of how variable PSF values are across soil cultivation types and how important this is to plant community development.

PSFs were well associated with plant functional group. Legumes and forbs produced 11 and seven of 19 positive PSFs. C_3 and C_4 grasses produced eight and seven of 20 negative PSFs (Fig. 2-1b). These results are consistent with previous PSF research; despite considerable variation grasses often produce negative PSFs and legumes produce positive PSF (Cortois et al., 2016; Lekberg et al., 2018; Mehrabi & Tuck, 2015). Similarly, among grasses, trees, shrubs, and forbs, grasses have the most negative PSFs (Kulmatiski et al., 2008). Negative PSF in grasses has been attributed to both soil nutrient depletion and species-specific pathogens (Bennett & Klironomos, 2019; Bezemer et al., 2006; De Long et al., 2019). Positive PSF in legumes have been attributed to associations with species-specific rhizobia, although arbuscular mycorrhizal fungi are another likely candidate (Wagg et al., 2015).

Across species richness levels, the PSF and Null models produced similar predictions of species biomass, though PSF predictions were consistently slightly better (Table 2-2, Fig. 2-4). Similar patterns were observed within species richness levels. In monocultures, both PSF and Null model predictions were highly accurate (Appendix A Fig. A-2a). In multi-species communities, both PSF and Null models overpredicted legume growth, with (Appendix A Fig. A-2b, A-2d, A-2f, A-2h). Legumes produced positive PSF which should decrease their growth in communities relative to monocultures if their associated symbionts are diluted in polyculture (Kulmatiski et al., 2012). The fact

that PSF models overpredicted legume growth suggests the way positive PSFs were incorporated into models underestimated their effects. In this experiment, PSF was measured as the growth of a species on a soil previously occupied by the same or a different species; however, in our diversity-productivity experiment plant-soil interactions were occurring between live species within the community. It is possible that measuring the legacy effects caused by the previous occupation of a pot with either the same or a different species underestimates the effects they have in an actively growing community (Eisenhauer et al., 2012). Alternatively, it is possible that emergent plant-soil interactions associated with diverse plant communities may result in larger PSF effects and overyielding in species-rich than species-poor soils (Latz et al. 2012).

This greenhouse experiment was replicated in the field in a separate study (Chapter 4). A recent review found that greenhouse and field measured PSFs are not correlated (Forero et al., 2019), yet we did note some similarities between our greenhouse and field experiments. Both PSF and Null model predictions from this greenhouse experiment were correlated with community biomass in the field experiment (Chapter 4). Though, not surprisingly, predictions from greenhouse data were better correlated with observations of plant community biomass in the greenhouse than in the field (Chapter 4). Individual species-specific and species*soil specific greenhouse and field PSFs were not correlated (Forero et al., 2019). No model predictions were correlated with community biomass from a diversity-productivity experiment performed with the same species in 1997 (data not shown). This is not surprising as herbivory, stress, microbial community composition and competition are also important in plant community productivity (Forero et al., 2019; Hawkes et al. 2013; Mahaut et al., 2020). Results suggest that PSF effects

may vary considerably over time and space.

By measuring individual PSFs, this experiment took a reductive approach to understanding PSF effects in diversity-productivity relationships. We found that, when measured in plant monocultures, PSFs explained roughly 25% of observed complementarity effects. In contrast, several recent studies have highlighted the potential for emergent properties in community-level PSF (Guerrero-Ramírez et al., 2019; Latz et al., 2012; Wang et al., 2019). Latz et al. (2012) found that some beneficial soil organisms are only found in diverse microbial communities. Both Guerrero-Ramírez et al. (2019) and Wang et al. (2019) found that diversity-productivity relationships develop in microbially-rich, but not microbially-poor soils. New experimental approaches will be needed to incorporate the role of soil microbial diversity in PSF and provide a clearer picture of how PSFs function in diverse communities.

There is great interest in the diversity-productivity relationship because continued species diversity losses can be expected to decrease plant productivity and carbon sequestration (Isbell et al., 2015; Tilman et al. 2006). A better understanding of diversity-productivity relationships can help constrain this outcome, and can additionally be harnessed for human well-being, for example by design plant communities that maintain plant diversity and increase plant productivity (Tilman et al., 2006). Our results suggest that PSFs are important in the diversity-productivity relationship, at least under conditions where stress, competition, and herbivory are tightly controlled (Beals et al., 2020; Heinze & Joshi, 2018; Maestre et al., 2009). Results were also broadly consistent with a paired field experiment, thus providing clear support for PSFs as one of several mechanisms that determine the diversity-productivity relationship (Chapter 4).

DATA ACCESSIBILITY

Upon acceptance data will be archived with a DOI.

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TABLES

Table 2-1. Plant species and functional groups used in the plant-soil feedback and biodiversity productivity experiments. Species included *Pascopyrum smithii* Rydb., *Poa pratensis* L., *Koeleria macrantha* (Ledeb.) Schultes, *Elymus canadensis* L., *Andropogon gerardii* Vitman, *Panicum virgatum* L., *Schizachyrium scoparium* (Michx.) Nash, *Sorghastrum nutans* (L.) Nash, *Amorpha canescens* Pursh, *Lupinus perennis* L., *Lespedeza capitata* Michx., *Dalea purpurea* (Vent) Rydb., *Achillea millefolium* L., *Liatris aspera* Michx., *Solidago rigida* L., and *Monarda fistulosa* L. (Ownbey & Morley, 1991).

Species	Functional group	Code
Amorpha canescens	Legume	Ac
Andropogon gerardii	C_4	Ag
Achillea millefolium	Forb	Am
Dalea purpurea	Legume	Dp
Elymus canadensis	C_3	Ec
Koeleria macrantha	C_3	Km
Liatris aspera	Forb	La
Lespedeza capitata	Legume	Lc
Lupinus perennis	Legume	Lp
Monarda fistulosa	Forb	Mf
Poa pratensis	C_3	Pp
Pascopyrum smithii	C_3	Ps
Panicum virgatum	C_4	Pv
Sorghastrum nutans	C_4	Sn
Solidago rigida	Forb	Sr
Schizachyrium scoparium	C_4	Ss

Table 2-2. Akaikie's Information Criterion (AIC) from general additive mixed models for measures of the biodiversity-productivity relationship with different groupings. The lowest AIC for each measure of the biodiversity-productivity relationship are bolded. Generally, the best-performing model was model 3: PSF and Null model grouped and Observations separate, however, for the complementarity effect, model 2: PSF Model, Null Model, and Observations separate, was the best model.

Model	Random-effects groups	Biomass Production AIC	Overyielding AIC	Selection Effect AIC	Complementarity Effect AIC
M1	None (global model)	1145.866	604.7794	570.5035	573.5636
M2	Group 1: PSF Model Group 2: Null Model Group 3: Observations	1144.376	601.6686	556.2153	**513.3917**
M3	Group 1: PSF Model and Null Model Group 2: Observations	**1142.419**	**600.9543**	**555.1877**	520.8377
M4	Group 1: PSF Model and Observations Group 2: Null Model	1146.084	601.3273	563.6283	542.1270
M5	Group 1: Null Model and Observations Group 2: PSF Model	1145.958	603.6236	570.0151	571.8514

FIGURES

Plant	C_3				C_4				Forb				Legume			
Soil	Ec	Km	Pp	Ps	Ag	Pv	Sn	Ss	Am	La	Mf	Sr	Ac	Dp	Lc	Lp
Ec Soil		-0.01	0.06	0.00	-0.09	-0.40	-0.08	-0.28	-0.09	-0.15	-0.12	0.12	0.21	0.09	-0.10	-0.44
Km Soil	-0.20		-0.27	-0.08	0.11	-0.01	-0.16	-0.13	-0.20	0.23	-0.13	-0.09	0.27	0.13	0.15	-0.15
Pp Soil	-0.01	-0.14		0.29	0.15	0.34	-0.11	-0.33	-0.03	**0.80**	0.18	**0.65**	0.44	**0.47**	**0.59**	0.29
Ps Soil	0.14	0.05	-0.05		0.21	0.18	0.09	0.28	**-0.22**	**0.82**	**-0.35**	-0.29	0.37	**0.48**	**0.54**	-0.27
Ag Soil	0.06	-0.08	-0.28	0.09		0.34	-0.05	-0.48	-0.26	**0.72**	-0.12	0.19	**0.54**	-0.10	0.27	-0.37
Pv Soil	0.16	-0.12	-0.37	0.28	-0.22		0.01	0.00	0.07	**0.88**	-0.27	0.01	**0.54**	-0.08	**-0.27**	-0.25
Sn Soil	0.13	0.07	-0.16	**0.43**	-0.19	0.23		0.28	-0.24	0.32	0.05	0.34	**0.56**	0.26	0.35	-0.41
Ss Soil	0.13	-0.03	0.11	0.16	-0.07	0.08	0.08		0.14	0.22	0.15	0.05	0.27	-0.07	0.49	-0.43
Am Soil	-0.04	-0.28	-0.20	-0.01	-0.04	-0.12	-0.02	-0.03		0.60	-0.01	-0.38	0.29	0.38	0.06	-0.03
La Soil	0.04	0.14	-0.63	0.00	-0.01	-0.35	**-0.56**	**-0.33**	-0.22		0.00	-0.13	**0.87**	0.27	0.09	-0.48
Mf Soil	-0.04	-0.02	-0.33	0.12	-0.02	0.17	0.16	-0.09	0.01	-0.10		0.15	0.37	-0.08	0.17	0.23
Sr Soil	-0.48	-0.17	-0.18	0.14	-0.17	0.16	-0.11	-0.07	-0.07	**0.80**	-0.13		**0.63**	-0.16	-0.03	-0.02
Ac Soil	**-0.37**	-0.26	**-0.72**	**-0.46**	-0.36	-0.27	-0.29	-0.05	**-0.49**	0.14	**-0.53**	**0.65**		0.00	0.34	0.07
Dp Soil	**-0.37**	0.00	-0.33	-0.36	-0.24	-0.58	**-0.49**	**-0.64**	-0.48	-0.28	**-0.33**	-0.21	0.10		0.02	0.38
Lc Soil	-0.37	-0.39	-0.61	-0.10	-0.08	-0.43	**-0.38**	0.03	**-0.36**	-0.22	-0.39	-0.37	0.29	0.32		**0.72**
Lp Soil	**-0.66**	**-0.49**	**-0.62**	**-0.59**	-0.44	**-0.59**	**-0.43**	-0.15	-0.24	-0.20	**-0.45**	0.26	**0.51**	-0.01	0.19	

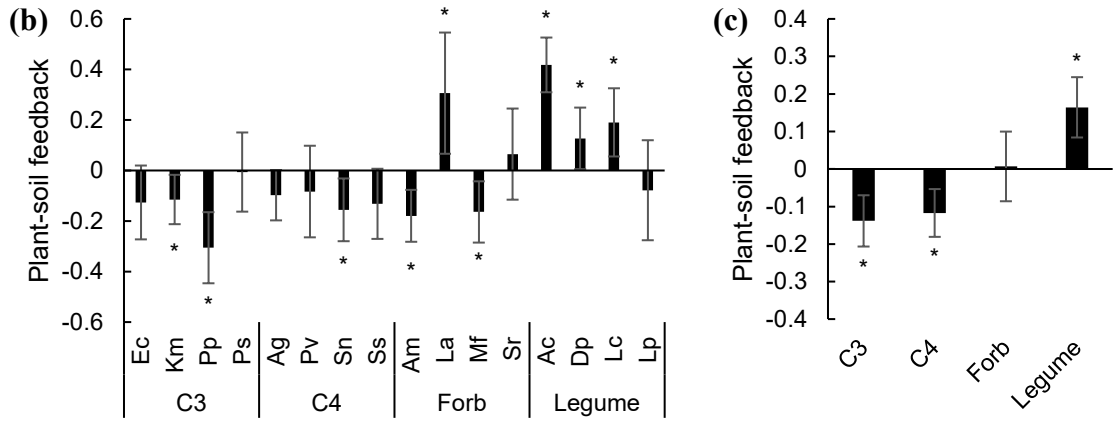

Figure 2-1. Factorial plant-soil feedbacks (PSFs) (a), non-factorial species PSFs (b) and non-factorial functional group PSFs (c) as measured in the greenhouse. 95% confidence intervals were created via bootstrapping (a) or a one-way student's t-test (b, c). Asterisks indicate PSFs with a mean significantly different from zero.

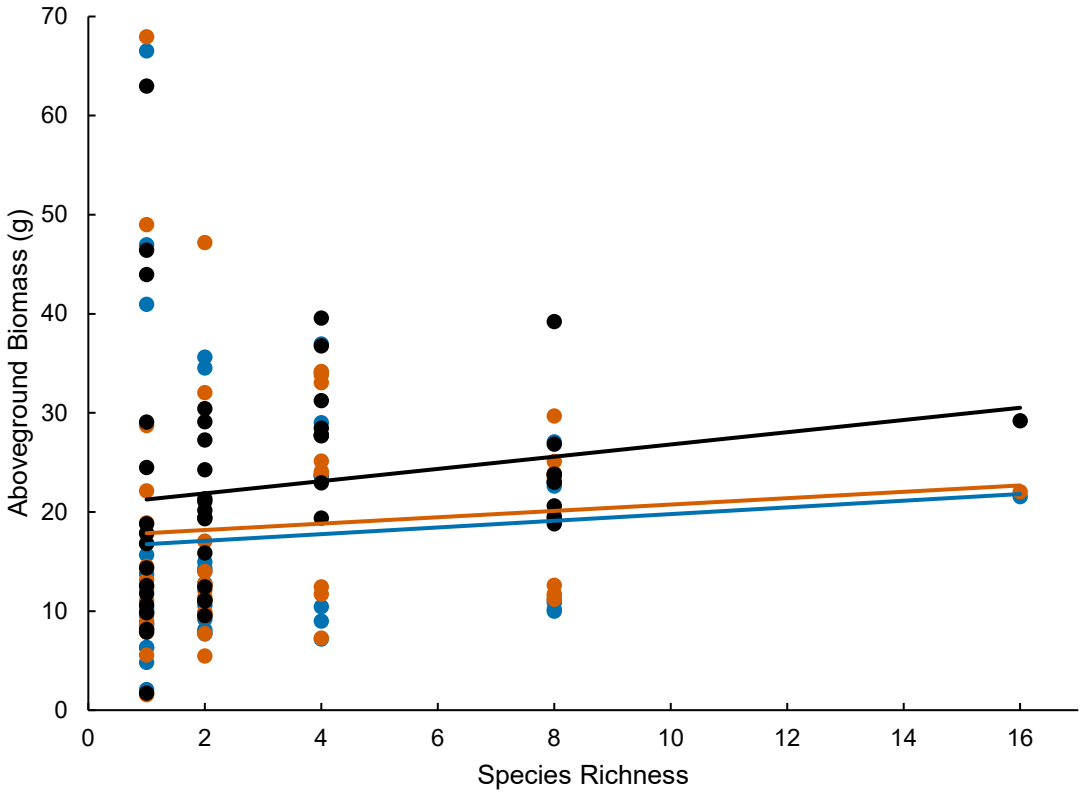

Figure 2-2. Biodiversity-productivity relationships predicted from a PSF-informed model (blue), a model without PSF effects parameterized with growth on other soils (orange); and biodiversity-productivity relationships observed in 12-L pots across community replicates in a greenhouse experiment (black) as analyzed by linear regression.

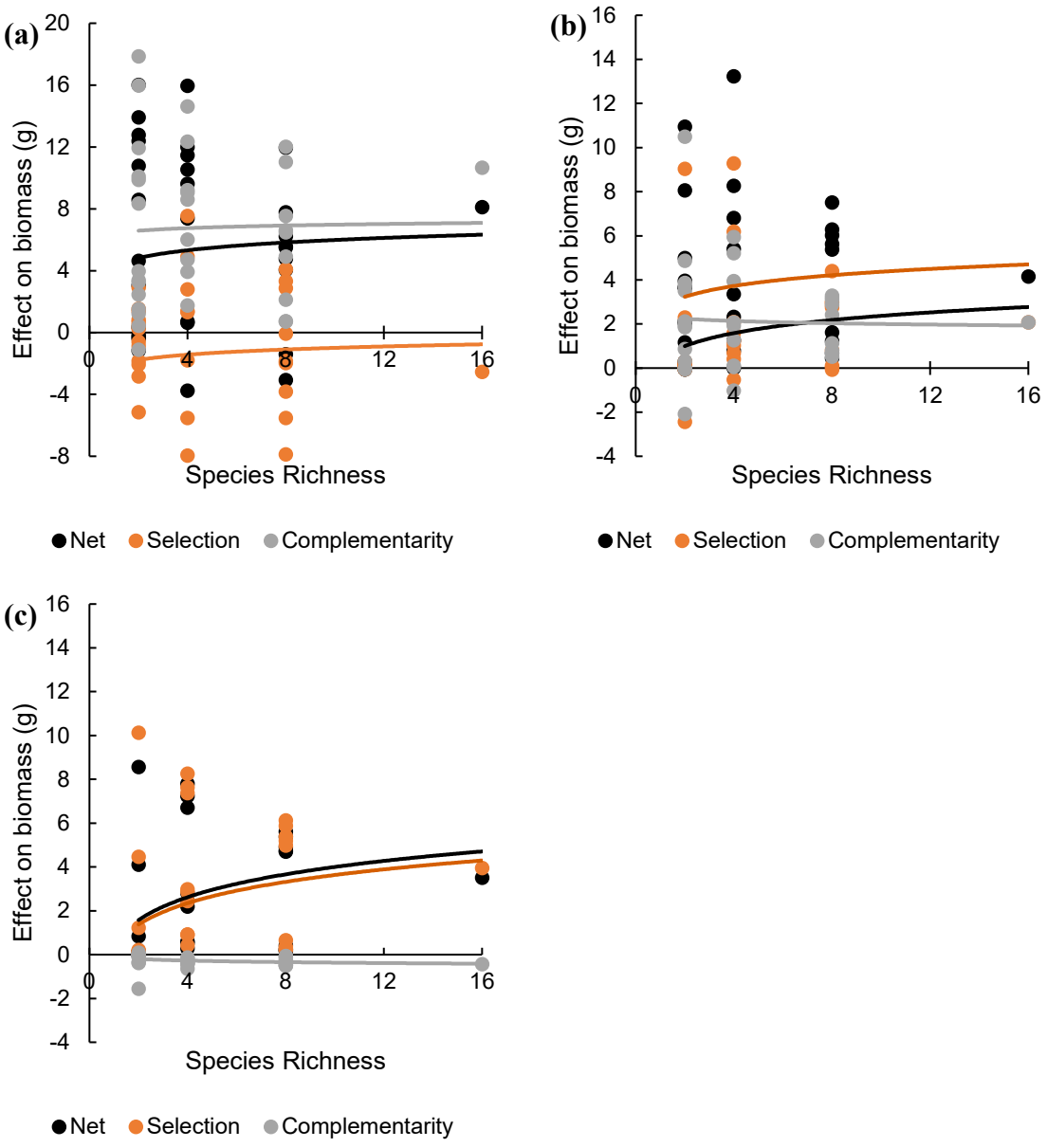

Figure 2-3. Net biodiversity effects (black) partitioned into selection effects (orange) and complementarity effects (gray) from observations in a greenhouse experiment (a), modeled predictions incorporating plant-soil feedbacks (b) and modeled predictions without PSF data (c).

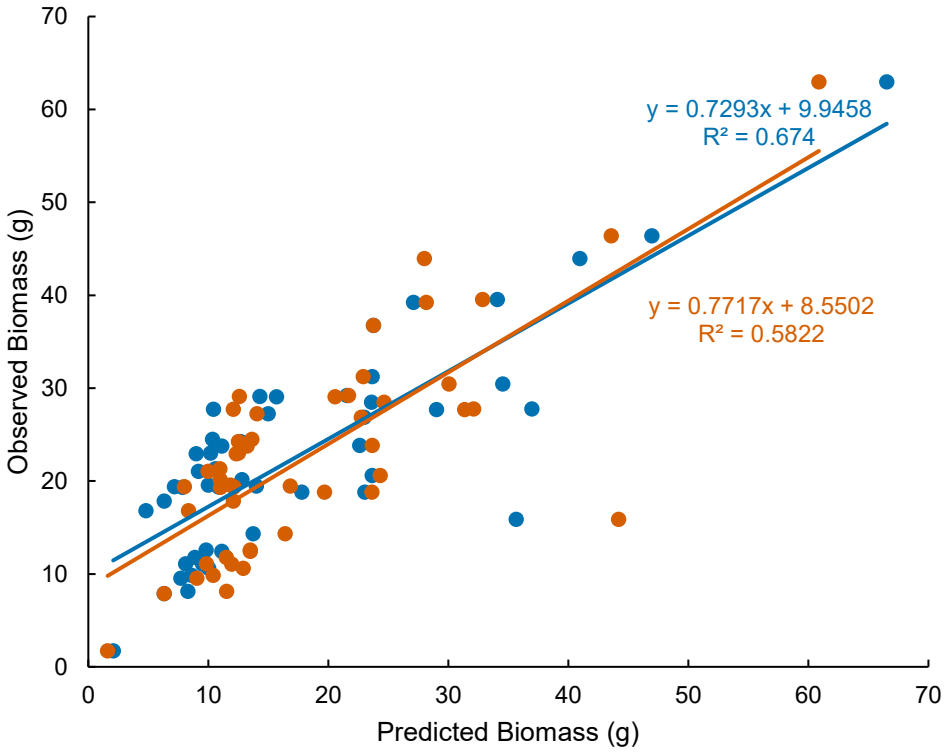

Figure 2-4. Predicted community biomasses (g per 12 L pot) from PSF-informed models (blue) and models without PSF effects parameterized with growth on other soils (orange) biomasses observed in a greenhouse experiment.

CHAPTER 3

GREENHOUSE- AND FIELD-MEASURED PLANT-SOIL FEEDBACK ARE NOT CORRELATED[2]

ABSTRACT

Plant-soil feedbacks (PSFs) have become a commonly invoked mechanism of plant coexistence and abundance. Yet, most PSF experiments have been performed in greenhouse conditions. To test whether or not greenhouse-measured PSF values are of similar magnitude and positively correlated with field-measured PSFs, we compared PSF values from five different studies that measured PSF values in both greenhouse and field conditions. For 36 plant species, greenhouse-measured PSF values were larger than and not positively correlated with field-measured PSF values. Similarly, these 36 species produced 269 soil-specific PSF values, and for each site there was no positive correlation between these greenhouse- and field-measured PSF values. While PSFs were observed in both greenhouse and field conditions, results provided no support at the soil, site or species level that a positive correlation exists between greenhouse- and field-measured PSF. Further, greenhouse-measured PSF appear to overestimate field-measured PSF. Although from five studies, results strongly suggest that field experiments are needed to understand the role of PSFs in plant communities in natural settings.

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[2] Forero, L. E.,Grenzer, J., Heinze, J., Schittko, C., and Kulmatiski, A. (2019). Greenhouse- and field-measured plant-soil feedbacks are not correlated. Frontiers in Environmental Science. Doi: 10.3389/fenvs.2019.00184

1. INTRODUCTION

Plant-soil feedbacks (PSFs) are increasingly used to explain plant community dynamics including succession, invasion, legacy effects, landscape abundance, coexistence, and biodiversity (Kardol et al., 2006; Klironomos, 2002; van der Putten et al., 2013). However, PSF research continues to rely mostly on greenhouse experiments (Figure 3-1). Greenhouse PSF studies are useful for developing conceptual models of plant community dynamics (Aguilera, 2011; Bever et al., 1997; Bonanomi et al., 2005), however, it remains largely untested whether or not PSFs measured in the greenhouse are correlated with PSFs measured in the field (Kulmatiski and Kardol 2008, Schittko et al. 2016).

Plants can alter soil biota, and these changes in soil biota may subsequently affect their own growth and the growth of neighboring plants (Ehrenfeld et al., 2005; Reynolds et al., 2003). PSFs are typically investigated by testing a plant's growth response to soils cultivated by different plant species (Bever, 1994). Many approaches have been used to test PSF effects (Kulmatiski and Kardol 2008) including unsterilized versus sterilized soils, comparisons among different field soil inoculum into sterilized soils, microbial filtrate inoculations, and two-phase experiments in which soil types are cultivated during an experiment. The two-phase approach remains a standard approach (Bever et al., 1997; van der Putten et al., 2013). In a two-phase experiment, during the conditioning phase of the bioassay (Phase 1), plants are used to create a soil with biota specific to that species. In the response phase (Phase 2), phytometers are planted to test the growth response of a species to the altered soil biota. Growth of the Phase 2 species on soil previously conditioned by the same plant ("home") is compared to growth on soil previously

conditioned by a different plant (“away”). By using soil from Phase 1 to inoculate sterilized soils, this approach can isolate microbial from soil chemical (Ehrenfeld et al., 2005; Ke et al., 2015; Morris et al., 2009) and physical (Kulmatiski et al., 2017; Kyle, 2005) effects.

PSF studies are typically executed in the greenhouse for several reasons. Greenhouse studies allow for many isolated replicates and can be performed throughout the year in rapid growth conditions. Because it is relatively easy to sterilize greenhouse soils, greenhouse studies more easily control legacy effects and separate soil nutrient effects from soil microbial effects, relative to field studies. However, completely isolating microbial from nutrient PSF may be unrealistic (Ke et al., 2015; Kulmatiski and Kardol, 2008). Greenhouse studies also lack microsite variability which can increase the likelihood of detecting PSFs in the greenhouse (Burns et al., 2015; Rinella and Reinhart, 2017).

Abiotic and biotic conditions can be very different between the greenhouse and the field (Heinze et al., 2016; Schittko et al., 2016). Greenhouse soils are typically sterilized and inoculated with small amounts of live soil; this likely creates soil conditions favoring fast-growing microbes and fast-growing plant species (De Deyn et al., 2004; Eno and Popenoe, 1964; Howard et al., 2017). Frequent fertilization and watering can cause arbuscular mycorrhizal fungi to become parasitic as conditions change from low to high fertilization regimes, and dry to wet water regimes (Johnson et al., 2003; Schmidt et al., 2011). This could cause PSF to appear neutral or positive in dry field conditions, and negative or neutral in a greenhouse with a consistent water regime (Mohan et al., 2014). Large soil organisms are typically absent in greenhouses which

would affect plant-soil interactions (Kuťáková et al., 2018) such as below-ground herbivory (Bezemer et al., 2013; Hol et al., 2010). More broadly, stressful conditions found in field studies may induce greater facilitation and a more positive PSF in the field (Maestre et al., 2009). These differences have led several authors to recommend greater field experimentation (Heinze et al., 2016; Kulmatiski and Kardol, 2008; Schittko et al., 2016).

Here, our goal was to test whether or not greenhouse-measured PSFs are of a similar magnitude and positively correlated with field-measured PSFs. We predicted that greenhouse- and field-measured PSF would be positively correlated because we expected that plants have a dominant effect on soil microbial community composition and subsequent PSF; these effects should be similar in both settings due to similar plant species and soil microbial communities. A negative correlation or a lack of correlation between greenhouse- and field-measured PSF suggests that greenhouse conditions change plant-soil interactions in ways that reverse or change PSF values. To test this prediction, we compared greenhouse- and field-measured PSF values from published studies and publicly available datasets. To assess whether PSF is overestimated in greenhouse or field conditions, we compared the magnitude of PSF values (regardless of sign) by taking the absolute values of greenhouse- and field-measured PSF.

2. METHODS

A Scopus search for PSF studies with the term “plant-soil feedback” or “plant-soil feedbacks” in the title, abstract, or keywords was performed on March 19, 2019. Of the resulting 515 studies, meta-analyses, modeling papers, reviews, and non-English studies were removed. The remaining studies were reviewed to identify studies containing 1) a

home/away PSF method (Brinkman et al., 2010), 2) aboveground biomass or cover as the response variable, and 3) grasslands as the study ecosystem (Burns et al., 2017; Teste et al., 2017), which left 297 studies. Species from grassland ecosystems were selected as the focal organisms because most PSF research has been conducted in grassland ecosystems, so sufficient sample sizes from non-grassland ecosystems were unlikely (Kulmatiski et al., 2008; van der Putten et al., 2013). Of these 297 studies, 237 occurred in the greenhouse, 50 in the field, seven in mesocosms, and three included both greenhouse and field approaches. Of these three studies, data was collected from two, but one possible study did not respond to requests for data. An additional three datasets produced by the authors, which are publicly available at the USU Digital Commons, were also included.

The resulting dataset contained paired greenhouse-measured and field-measured PSF values for 36 species derived from 2975 field observations and 2907 greenhouse observations at five different study sites. We used the paired dataset to 1) calculate PSF values using a single method for all data, 2) test for correlations between greenhouse- and field-measured PSF, and 3) compare PSF values and PSF magnitudes (absolute values) between greenhouse- and field-measured PSF.

2.1 Study Sites

Of the five study sites included, three were from Europe (Berlin, Potsdam, and Jena in Germany) and two were from North America (Winthrop, Washington and Cedar Creek, Minnesota in the United States). At all sites, the focal species selected were abundant in local plant communities. Four species were common among at least two study sites (Appendix A).

All five studies compared phytometer growth responses to “home” and “away”

conditioned soil (Bever, 1994). When more than two species are used in a PSF experiment, this comparison can be undertaken by mixing all conditioned "away" soils together to create a single "away" treatment. This approach was used in the Berlin study; it eliminates site-by-site variation in soil microbes (Reinhart and Rinella, 2016; Rinella and Reinhart, 2017), and can be useful when the research question is not focused on spatial variability (Cahill et al., 2017; Gundale et al., 2017). Alternately, phytometer responses can be measured on each "away" soil creating a species*soil-level design. This approach was used in the studies at Cedar Creek, Jena, Potsdam, and Winthrop. Data from species*soil-level PSF experiments were converted to species-level PSF values by averaging a species' growth across "away" soil types.

2.2 Greenhouse Experiments

The experiments at Cedar Creek, Jena, and Winthrop implemented a cultivated two-phase approach (Rinella and Reinhart, 2018). The experiments at Berlin and Potsdam collected conditioning soils from underneath monotypic stands in the field (Table 3-1; Kulmatiski and Kardol, 2008).

For Phase 1, the Cedar Creek greenhouse experiment steam-sterilized a six-to-one mixture of sand and sphagnum peat inoculated with ten percent field soil. The prepared 1-L pots were planted and grown for a six-month Phase 1. The Jena greenhouse experiment inoculated a three-to-one mixture of compost and sand with ten percent field soil. The prepared 1-L pots were planted and grown for an eight-month Phase 1. The Winthrop greenhouse experiment steam-sterilized a six-to-one mixture of coarse sand and sphagnum peat and inoculated with five percent field soil. The prepared 1-L pots were planted and grown for a three-month Phase 1. At the end of Phase 1, plants were removed

by hand-clipping; 2282 pots at Cedar Creek, 239 pots at Jena, and 216 pots at Winthrop had growth.

For Phase 1, the greenhouse experiment at Potsdam collected field soil from underneath three different species' monotypic stands and filled 90 0.41-L pots with 100% field soil (Heinze et al., 2016). In Berlin Schittko et al. (2016) collected field soil from underneath eight different species' monotypic stands. The soil for the "away" treatment was mixed, where the soil for the "home" treatment was not mixed. A steam-sterilized sandy loam soil was inoculated with 23% "home" or "away" soils collected in the field and used to fill 240 pots, 80 of which were retained for the greenhouse experiment.

For the greenhouse experiment at Cedar Creek, the Phase 2 length was six months; at Jena three months; at Winthrop, three months; at Potsdam two and one-third months; and at Berlin four months. Pots were clipped and aboveground biomass weighed for all species at the end of Phase 2 (Table 3-1).

2.3 Field Experiments

At Cedar Creek and Jena, the field site area was sprayed with glyphosate and disked. Experimental plots (0.35 m by 0.75 m) were established with 0.75 mm thick HDPE root barrier inserted to 35 cm deep between each plot. For Phase 1 at Cedar Creek, ten grams of pure live seed per m^2 was applied to each of the plots. At Jena, 2000 total pure live seeds per m^2 were applied to each of the plots. After a two-year Phase 1, the area was sprayed with glyphosate and hand-tilled using a garden claw. Non-target species were removed by hand-weeding. At Cedar Creek, plots containing C_3 grasses and forbs were hand-tilled using a garden claw, but vigorous root growth in the C_4 grasses necessitated tilling using a miniature tiller on plots containing that functional group. Seed

was re-applied at the same respective rates. After a 2-year Phase 2, aboveground biomass was clipped, dried and weighed; 2066 Cedar Creek plots and 345 Jena plots had growth.

At Winthrop, the top 10 cm of vegetation and soil was removed (Kulmatiski, 2019). A one-to-one mix of native soil inoculum and sand was applied to the prepared site, and disked to 15 cm to homogenize. A grid of 1.2 m wide geotextile cloth was laid down to create 315 1.5 by 1.5 m PSF plots in the area. Ten grams of pure live seed per m^2 was applied to each plot, and allowed to grow for a four-year Phase 1. After four years, Phase 1 plants were sprayed with glyphosate. Seed was re-applied for Phase 2 and plots were allowed to grow for three years. Growth was estimated using percent cover in June 2013.

At Potsdam, 30 (0.4 m by 0.4 m) plots were prepared by cutting the first 25 cm of roots under three different monotypic stands to create three Phase 1 treatments (Heinze et al., 2016). Three individuals of each species were planted in each plot. Individuals were spaced 10 cm apart and allowed to grow for 10 weeks. After the 10 weeks, aboveground biomass was harvested, and 89 individuals had growth.

At Berlin, at week 14 of the greenhouse experiment, 160 pots were transferred to the field and left to sit on top of the soil for a period of two weeks (Schittko et al. 2016). After two weeks, the aboveground biomass was harvested. Extended methods for Cedar Creek and Jena are in Appendix A; for Potsdam, Winthrop, and Berlin extended methods are in Heinze et al., 2016; Kulmatiski et al., 2011, 2017; and Schittko et al., 2016.

2.4 Statistical Analyses

To avoid bias from different calculation methods, original plant growth data on “home” and “away” soils was used to calculate PSF values using a single method

for all data (Brinkman et al. 2010). PSFs were calculated as (H-A)/maximum(H,A), where H is the aboveground growth (ground cover or biomass) produced by a species in Phase 2 on "home" soils, and A is the aboveground growth produced by a species in Phase 2 on "away" soils. The denominator refers to the maximum aboveground growth produced by a species regardless of soil type. This calculation has similar mathematical properties to the commonly used ln(H/A) metric (i.e., values that are symmetric around zero and bounded between +1 and -1). In addition, it has the advantage of being easily interpretable as the proportion increase or decrease in growth due to soil type (Brinkman et al. 2010). Plots or pots where the Phase 1 or the Phase 2 realized no growth were removed from the dataset. To prepare the data from species*soil-level PSF studies for a species-level analysis, one PSF value was calculated for each "away" species by taking the mean PSF value for each species across soil types.

To determine if the mean PSF value for each experiment was different from zero, we took the standard error of the mean. For data from species-level PSF studies, one home versus away PSF was calculated for each species. For species-level PSF values, we used linear models to test for a correlation between greenhouse- and field-measured PSF within each study site and overall. For species*soil-level PSF values, we used linear models to test for a correlation between greenhouse- and field-measured PSF within each study site only, to control for the outsized effect of Cedar Creek's data on the overall dataset. Linear models were performed using the polyfit and fitlm scripts in MATLAB (MathWorks, Inc, 2015b). Residuals for the species-level data were checked for normality using the Shapiro-Wilk test.

2.4.1 Comparisons

To compare PSF values and PSF magnitudes (absolute values) among study sites and regions, we performed a one-way analysis of variance (ANOVA) using the script anova1 in MATLAB. Significance was evaluated at $\alpha = 0.05$. When significant, differences were explored with a Tukey's Honest Significant Difference test in MATLAB using the script multcompare.

3. RESULTS

From species-level data, 36 paired PSF values were compared. Of these 36 values, eight came from the mixed-soil PSF experiment at Berlin, and the remainder from species*soil-level studies where the mean PSF value across all soil types was calculated to create a single PSF value per plant species: 16 PSF values came from Cedar Creek, five from Jena, three from Potsdam, and four from Winthrop. Greenhouse PSF values were positive in Berlin and Potsdam, and neutral in Jena, Winthrop, and Cedar Creek (Figure 3-2a). Field PSFs were positive in Berlin and Winthrop, neutral in Jena and Potsdam, and negative in Cedar Creek (Figure 3-2a). For the species-level greenhouse-measured data the average PSF was 0.046 and the coefficient of variance was 5.14; for the field-measured data the average PSF was -0.008 and the coefficient of variance was 24.01.

A total of 269 PSF values from species*soil-level field/greenhouse paired experiments were compared. Of these values, 239 came from the Cedar Creek study, 20 from the Jena study, six from the Potsdam study, and four from the Winthrop study. PSF values for Berlin were excluded from the species*soil-level dataset because the study was not species*soil-level in design. Greenhouse PSF values were positive in Potsdam and

Jena, and neutral in Winthrop and Cedar Creek (Figure 3-2b). Field PSF values were positive in Winthrop, neutral in Jena and Potsdam, and negative in Cedar Creek (Figure 3-2b). For the species*soil-level greenhouse-measured data the average PSF was -0.007 and the coefficient of variance was 50.59; for the field-measured data the average PSF was -0.064 and the coefficient of variance was 4.81.

We tested for correlations in species-level data both within and among sites. For species*soil-level data we tested within but not among sites because 86% of species-level data was from one site. For species-level data, there was no correlation between greenhouse- and field-measured PSF values across all study sites ($F_{1,34} = 0.179$, $P = 0.675$, Figure 3-3a). Similarly, there was no correlation between greenhouse- and field-measured PSF values within study sites ($P > 0.05$, Figure 3-3a). For the species*soil-level PSFs, there was no correlation between greenhouse- and field-measured PSF values at the Cedar Creek, Jena, and Winthrop sites ($F_{1,237} = 0.001$, $P = 0.972$; $F_{1,18} = 0.003$, $P = 0.959$; and $F_{1,2} = 0.039$, $P = 0.801$; respectively; Figure 3-3b). There was a negative correlation between greenhouse- and field-measured data from the Potsdam site ($F_{1,4} = 10.129$, $P = 0.034$, $R^2 = 0.717$; Figure 3-3b).

We tested for differences in magnitude (absolute value) for species-level data only because of the strong effects Cedar Creek had on species*soil-level data. While there were few correlations between greenhouse- and field-measured PSF values, there were differences between the magnitude of greenhouse- and field-measured PSF values, indicating that PSF (either positive or negative) were larger in greenhouse than field conditions ($F_{1,70} = 5.056$, $P = 0.028$).

4. DISCUSSION

Although PSFs are commonly invoked as a mechanism to explain complex plant community dynamics in the field, the majority of PSF experiments take place in controlled greenhouse conditions. We had predicted that greenhouse- and field-measured PSF would be positively correlated due to the dominant effects of plants on their soil microbial communities, but found no evidence to suggest that greenhouse-measured PSF data are positively correlated with field-measured PSF. We also found greenhouse-measured PSF values were exaggerated relative to field-measured PSF values. Together, results suggest that the greenhouse-measured PSFs that predominate in the literature both overestimate and provide little direct inference into PSF effects in the field. Although our dataset is derived from only five sites, our results strongly suggest that PSFs are sensitive to growth conditions (Casper et al., 2008). Consequently, field experiments are likely to be needed to fully understand the role of PSFs in natural systems.

There are several potential reasons that could explain why PSF values were smaller in the field than in the greenhouse. More stressful growing conditions (for example, competition, drought, or herbivory) may minimize PSF effects (Crawford and Knight, 2017; Fry et al., 2018; van der Putten et al., 2016). Although researchers in all five field experiments attempted to decrease competitive effects by hand-weeding, it is likely that competitive pressure was still greater in the field than greenhouse experiments due to the larger seed bank in unsterilized field soils (Lekberg et al., 2018). Similarly, greater aboveground herbivory in the field was likely to decrease PSF values directly by removing aboveground biomass and potentially indirectly by inducing increased belowground growth (Heinze and Joshi, 2018). Drought in the field may also decrease

PSF values by decreasing plant growth, microbial growth, and nutrient cycling rates (van der Putten et al., 2016). With only five studies and many potential factors affecting differences between greenhouse and field results, it was not possible to test these hypotheses, but they are consistent with our observation of larger PSF values in the greenhouse.

Methodological differences were likely to explain why there was no positive correlation between field and greenhouse PSF values, though we were unable to isolate any specific methodological difference that would explain our results. Compared to the field, growing space is restricted, experiment length is shorter, and dominant soil microbes differ in the greenhouse. Excepting Berlin, greenhouse pots were smaller than field plots. Yet, we did not observe a qualitatively different relationship between greenhouse and field PSF values at Berlin. The Winthrop site had the largest difference between field plot and greenhouse pot size, yet PSF values were not notably different from other sites.

Differences in temporal scales among sites similarly did not appear to drive our results. PSFs have been suggested to accumulate over time (Diez et al., 2010; Kardol et al., 2006; Kulmatiski et al., 2008; Lepinay et al., 2018), but Potsdam, which had similar greenhouse and field experiment lengths, did not have a positive correlation between greenhouse- and field-measured PSF. Sterilized soils, which were used at three of the five reviewed experiments, often have higher nutrient availability and promote faster plant growth, changing PSF values and soil microbial communities that drive PSF (De Deyn et al., 2004). However, sites using sterilized soils and sites using unsterilized soils both had uncorrelated PSF values. Little can be inferred from the five studies reviewed, but results

did not provide strong evidence to suggest that pot size, experiment length, or sterilization technique provided a strong explanation for the difference between greenhouse and field results.

The only correlation observed between greenhouse- and field-measured PSF, was a negative correlation at the Potsdam site. This site was the only site to use 100% field soil in the greenhouse experiment. It is possible that a negative correlation occurred because under decreasing light conditions PSF can be reversed (Smith and Reynolds, 2015), but it is not clear why this effect would only appear when 100% field soils were used. To the contrary, we would have expected that the use of 100% field soil would produce more similar results to the field.

Although our results and results from previous studies suggest that PSF values are very context-dependent (Casper and Castelli, 2007), the PSF concept remains relevant to plant community ecology. Greenhouse-measured PSFs have been found to improve predictions of plant growth in communities in the greenhouse (Kulmatiski et al., 2011, 2017) and field-measured PSFs have been found to improve predictions of plant growth in communities in the field (Klironomos 2002, Kardol et al. 2006, Mangan et al. 2010, Kulmatiski 2019; Mariotte et al. 2018). Thus, our results suggest that while greenhouse studies are useful for conceptual model development and predicting plant growth in greenhouse conditions, ecologists who wish to understand the role of PSFs for specific plant species in the field should rely on field studies.

While from five studies, our results suggest that the PSF literature, which is predominantly derived from greenhouse experiments, overestimates PSF effects and while it may provide insight into general patterns of interactions that occur in plant

communities in the field, it provides little insight into the specific PSFs that determine the growth and abundance of specific plants in natural communities. Our findings are consistent with results from previous studies (Heinze et al., 2016; Schittko et al., 2016), and suggest that although greenhouse-measured PSFs are important for conceptual models, field experiments will likely be needed to understand the role of PSFs in complex plant community dynamics in the field.

DATA ACCESSIBILITY

The Potsdam dataset analyzed for this study is available on request from Johannes Heinze. The Cedar Creek, Jena, and Winthrop datasets analyzed for this study can be found at USU Digital Commons (https://doi.org/10.26078/52k0-jr94, https://doi.org/10.15142/T3XM19). The Berlin dataset analyzed for this study can be found in the Dryad Digital Repository (https://doi.org/10.5061/dryad.r7c23).

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TABLES

Table 3-1. Methods for the paired greenhouse and field experiments.

	Berlin	Cedar Creek	Jena	Potsdam	Winthrop
Field plot size	1.4-L pots except for *Cichorium intybus* and *Medicago x varia*, which were in 3.1-L pots	0.75 by 0.35 m	0.75 by 0.35 m	0.4 by 0.4 m	1.5 by 1.5 m
Greenhouse pot size	1.4-L and 3.1-L (see above)	1-L	1-L	0.41-L	1-L
Phase 1 Type	Inoculum	Cultivated	Cultivated	Inoculum	Cultivated
Greenhouse live soil rate	23%	10%	10%	100%	5%
Greenhouse experiment length	four-month Phase 2	six-month Phase 1 and six-month Phase 2	eight-month Phase 1 and three-month Phase 2	two and one-third month Phase 2	three-month Phase 1 and three-month Phase 2
Field experiment Length	0.5 months spent in the field out of a four-month experiment	24-month Phase 1 and 24-month Phase 2	24-month Phase 1 and 24-month Phase 2	two and one-half month Phase 2	48-month Phase 1, 32-month Phase 2
Greenhouse N	80	2282	239	90	216
Field N	160	2066	345	89	315

FIGURES

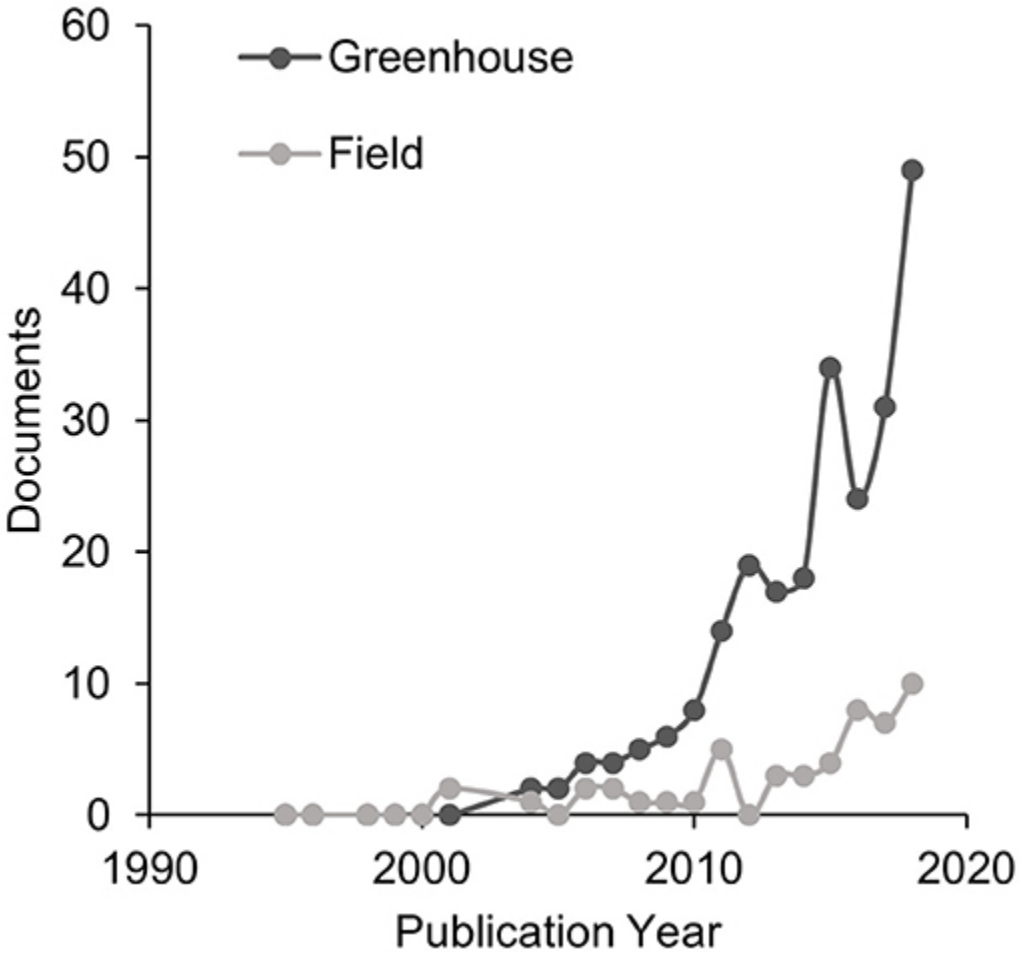

Figure 3-1. A Scopus search performed on March 18, 2019 for "plant-soil feedback" OR "plant-soil feedbacks" in abstract, title, or keyword of published articles from 1995 to 2018 demonstrated an exponential increase in PSF research over the past 20 years. Dark gray indicates greenhouse studies and gray indicates field studies.

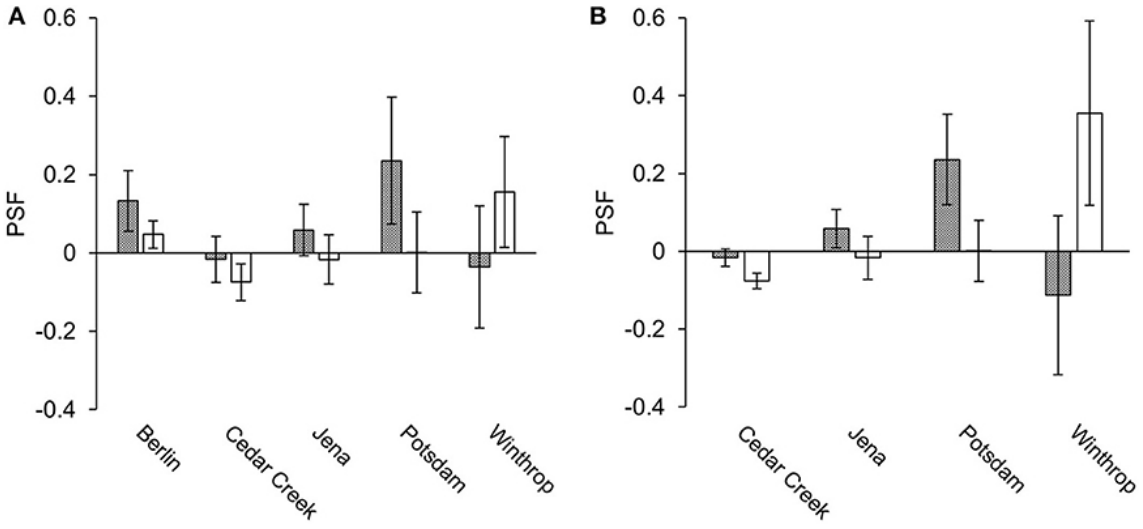

Figure 3-2. Average greenhouse- and field-measured species-level **(A)** and species*soil-level **(B)** PSF values (mean ± SE). Gray indicates greenhouse studies and white indicates field studies.

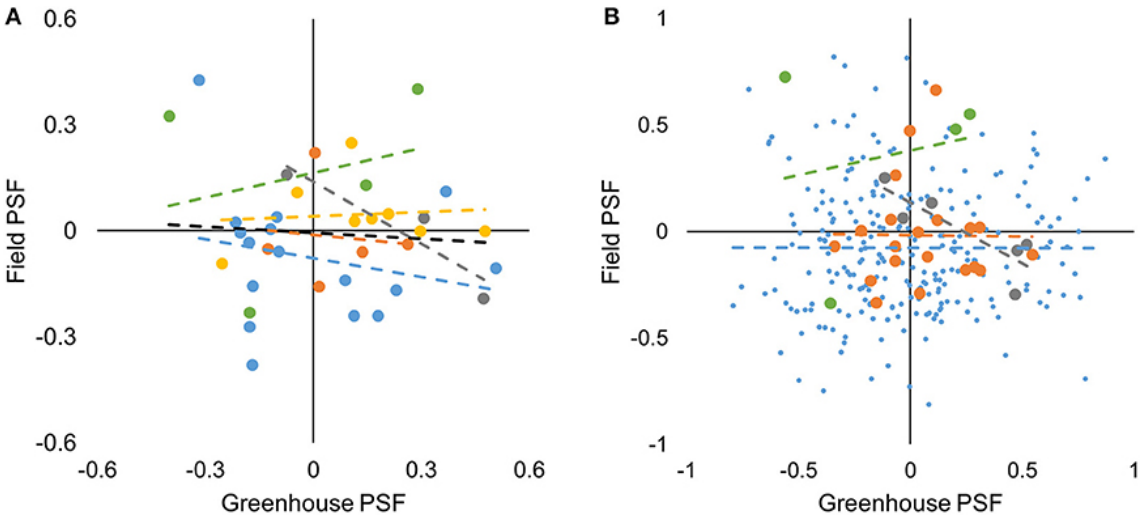

Figure 3-3. Greenhouse- vs. field-measured species-level **(A)** and species*soil-level **(B)** PSF values from five study sites. Yellow, Berlin; blue, Cedar Creek; orange, Jena; gray, Potsdam; green, Winthrop; black is a best-fit line for all study sites. Though only significant for the Potsdam species*soil-level data, best-fit regression lines are shown for each site to demonstrate that slopes were close to zero.

CHAPTER 4

PLANT-SOIL FEEDBACKS HELP EXPLAIN BIODIVERSITY-PRODUCTIVITY RELATIONSHIPS[3]

ABSTRACT

- Species-rich plant communities often produce twice as much aboveground biomass as monocultures, but the mechanisms causing these biodiversity-productivity relationships remains unresolved.
- We tested whether or not plant-soil feedbacks (PSFs) can help explain the biodiversity-productivity relationship. Using a field experiment, we measured all possible PSFs for 16 species in a tallgrass prairie system, Minnesota, USA. A suite of plant community growth models was parameterized with or without this PSF data and model predictions were compared to biomass in plant communities with one to 16 species, that were grown separately.
- Across 240 PSF values, plants created soils that changed subsequent plant growth by 27%. Plant community growth models parameterized with these PSFs predicted 27% overyielding due to complementarity. Without these PSFs, Null models incorrectly predicted 17% overyielding due to selection effects. In experimental communities, complementarity resulted in 185% overyielding.
- PSFs improved predictions of the magnitude and mechanism of overyielding relative to Null models. Results were consistent with theoretical models that

[3] **Forero LE, Kulmatiski A, Grenzer J, Norton JM**. **2020**. Plant-soil feedbacks help explain biodiversity-productivity relationships. In review.

predict a negative relationship between PSF and overyielding. Results, therefore, clearly demonstrated that PSFs can be one of several mechanisms that determine biodiversity-productivity relationships.

INTRODUCTION

Plant productivity typically increases with species richness (Cardinale *et al.*, 2007). Efforts to understand this fundamental aspect of ecosystem function (i.e., overyielding; Jochum *et al.*, 2020) have understandably focused on mechanisms of overyielding such as complementarity and selection effects (Jing *et al.*, 2015). Complementarity effects are often attributed to niche partitioning which allows species-rich communities to capture more resources than species-poor communities (Tilman *et al.*, 2006). Selection effects occur when more productive species are over-represented in species-rich relative to species-poor communities. However, niche complementarity and selection effects do not fully explain biodiversity-productivity relationships (Hector *et al.*, 2002; Mueller *et al.*, 2013; Barry *et al.*, 2019). For example, while most plant communities overyield, some communities underyield and niche-partitioning and sampling effects generally do not help explain this wide range of responses (Cardinale *et al.*, 2007).

Mechanisms that explain both over- and underyielding are likely to improve understanding of biodiversity-productivity relationships (Huston *et al.*, 2000; Loreau & Hector, 2001). Plant-soil interactions offer the potential to explain both overielding and underyielding (Kulmatiski *et al.*, 2012). Species-specific soil pathogens, for example, can be expected to be more abundant in monocultures than species-rich communities resulting in overyielding (Maron *et al.*, 2011; Wright *et al.*, 2017; Wang *et al.*, 2019).

Conversely, species-specific soil symbionts can be expected to be more abundant in monocultures than species-rich communities resulting in underyielding (Bever *et al.*, 2012; Bauer *et al.*, 2020). Although it is near-impossible and likely inappropriate to individually characterize the effect of each species-specific soil pathogen and symbiont on plant productivity, it is possible to summarize the net effect of negative and positive plant-soil interactions using PSF experiments (Bever, 1994; van der Putten & Peters, 1997). Thus, PSFs offer the potential to help explain both overyielding and underyielding in biodiversity-productivity relationships (Kulmatiski *et al.*, 2012; Wang *et al.*, 2019).

Several experimental approaches have been used to explore the role of plant-soil interactions in biodiversity-productivity relationships (Maron *et al.*, 2011; Schnitzer *et al.*, 2011; Hendriks *et al.*, 2013; Jing *et al.*, 2015; Guerrero-Ramírez *et al.*, 2019; Wang *et al.*, 2019). Perhaps the best support comes from field (Maron *et al.*, 2011) and potted (Schnitzer *et al.*, 2011) studies that used fungicide and microbial inoculations to demonstrate soil organism effects on the biodiversity-productivity relationships (Maron *et al.*, 2011; Schnitzer *et al.*, 2011), but these types of sterilization and inoculation experiments have been found to exaggerate PSF effects (Kulmatiski *et al.*, 2008; Lekberg *et al.*, 2018). Several studies have used greenhouse experiments (Kulmatiski *et al.*, 2012; Cowles, 2015; Guerrero-Ramírez *et al.*, 2019; Wang *et al.*, 2019), but greenhouse experiments have been found to produce PSFs that are not correlated with field-measured PSF (Forero *et al.*, 2019).

Two-phase, factorial field experiments remain the preferred approach for describing PSF (Kulmatiski & Kardol, 2008; Brinkman *et al.*, 2010; van der Putten *et al.*, 2013; Reinhart & Rinella, 2016). In these experiments, each plant in a community is

grown on soils cultivated by each other plant in the community. Due to the sample sizes required by factorial designs, these experiments have rarely been performed with more than a few species in the field (Hendriks *et al.*, 2013; Crawford *et al.*, 2019). We are not aware of any two-phase, field experiments that have tested the effects of PSF in biodiversity-productivity relationships.

The overarching goal of this research was to quantify the role of PSFs in a biodiversity-productivity relationship. To do this, we measured PSFs for 16 species using a factorial, two-phase field experiment. We then parameterized a suite of plant community growth models with or without PSF plant growth data. Model predictions were compared to biomass in new and existing experimental communities with 1 to 16 plant species. To better explain the mechanisms causing the biodiversity-productivity relationship, net biodiversity effects in model predictions and observed data were separated into complementarity and selection effect components (Loreau & Hector, 2001; Clark *et al.*, 2019).

MATERIALS AND METHODS

Research was conducted in the Cedar Creek Ecosystem Science Reserve Long Term Ecological Research site, East Bethel, Minnesota, USA (45.403290 N, 93.187411 W). Previous research at the study site demonstrated large increases in community biomass with species richness (*i.e.*, biodiversity-productivity relationships) that increase over time and are caused by complementarity (Fargione *et al.*, 2007). Soils are sandy and of the Nymore series: mixed, frigid, Typic Udipsamment. During the four years of the study, mean annual precipitation and temperature were 723.0 mm and 6.5° C, which is consistent with the 1963 to 2019 records at the site (769.3 mm and 6.6° C, respectively).

We performed two experiments: a PSF experiment and a biodiversity-productivity experiment. Each experiment included 16 species used in an existing biodiversity-productivity experiment at the site (the Biodiversity II experiment; Table 1(Tilman *et al.*, 1997). Five species that together represented less than 3% of the biomass in the biodiversity-productivity experiment from 1997 (henceforth, BP_{1997}) were excluded from our PSF and biodiversity-productivity experiments due to seed availability (*Asclepias tuberosa* L., *Dalea villosa* Nutt., *Dalea candida* Michx) and poor growth in previous experiments (*Quercus macrocarpa* Michx., *Quercus ellipsoidalis* E. J. Hill) (Ownbey & Morley, 1991). Seeds were purchased from Prairie Moon Nursery (Minnesota, USA), Granite Seed (Utah, USA), Prairie Restorations Inc. (Minnesota, USA) and Minnesota Native Landscapes (Minnesota, USA).

In October 2014, a 1750 m^2 fallow area adjacent to the BP_{1997} experiment was sprayed with a 5% glyphosate solution (Monsanto, Missouri, USA) and disc-harrowed to 15 cm to incorporate vegetation and homogenize soils. For the PSF experiment, 2,720 plots (0.75 m x 0.35 m) were established. For the biodiversity-productivity experiment, 232 plots (1.5 m by 1.5 m) were established. For all plots, a 35-cm deep by 4-cm wide trench was dug and lined with a root barrier (1-mm thick high-density polyethylene; Global Plastic Sheeting, California, USA). Throughout the PSF and biodiversity-productivity experiments, non-target plants were removed by hand several times each year.

PSF Experiment

A two-phase, factorial PSF experiment was used (Brinkman *et al.*, 2010). Phase I began in April 2015. For each of the 16 target species, 10 g live seed m^{-2} was planted by

hand in 170 replicate plots. During 2015, plots were watered weekly to promote establishment, and during the first two years plots were weeded once every two weeks to ensure the conditioned soils were monospecific. Seeded plant species grew in 2608 of the 2720 plots in Phase I. After two growing seasons, in late summer 2016, vegetation was killed with a 5% glyphosate treatment and aboveground biomass removed. To prevent resprouting in Phase II, plots were hand-tilled with a garden claw (~75% of plots) or rototiller as necessary (~25% of plots; Stihl Inc., Delaware, USA), November 2016. To further limit resprouting, a 5% glyphosate solution was applied again in April 2016 prior to seeding for Phase II.

For Phase II, each target species was to be planted in 35 replicate plots with 'self' soils and nine replicated plots with each of the 15 'other' soils. Because some target species failed to establish in Phase I, actual replication ranged from 27 to 35 replicates on 'self' soils and five to nine replicates on each 'other' soil (Appendix C Table C-2). Further, each target species was randomly assigned to five to nine replicate plots that had no Phase I growth. These 'control' plots were used to parameterize one of the Null models. During Phase II, plots were weeded once per month.

Plant cover in every plot was assessed by visual estimation in August 2017 and September 2018 and plant aboveground biomass was clipped, dried and weighed in October 2018. The 2017 percent cover data was converted to biomass values using the 2018 percent cover to biomass relationship.

Calculating and Analyzing PSFs

PSF values were calculated from aboveground biomass data as follows: PSF = (S-O)/maximum(S,O) where S is the aboveground biomass produced in Phase II on 'self'

soils, where O is the aboveground biomass produced in Phase II on 'other' soils (Brinkman *et al.*, 2010). This value is symmetrical around zero, bound by -1 and 1 and easily-interpretable as the proportion change in growth among soil types. The mean and error associated with these values was estimated using bootstrapped confidence intervals calculated using the sample_n command from the R package 'dplyr' (Wickham *et al.*, 2020). Because PSFs were measured for 16 species on 15 soil types, analyses yielded 240 species*soil level PSF values.

While the PSF experiment was performed primarily to produce plant growth rates on different soil types for use in plant community growth models, we also report PSF values. The 240 species*soil-level PSF values were considered positive or negative when their 95% confidence interval did not overlap zero. Variation in species*soil PSF values is derived from the 27 to 35 replicate "self" and 5 to 9 replicate "other" field plots. Species-level PSF values were then calculated as the mean PSF value across 15 soil types. Variation in species-level PSF is derived from the 15 soil types. To determine if species-level PSF values differed from zero, one-way t-tests were used. Species-level PSF were considered different from zero when $P < 0.05$. To test whether or not PSF values changed between the first and second year of Phase II, a one-way ANOVA with year as a factor was used ('aov' and 'TukeyHSD' in R programming). Differences among years were considered significant when $P < 0.05$.

Biodiversity-Productivity Experiment

In April 2015, 63 plant communities containing 1 to 16 plant species were planted in 232 plots. Plant communities with 1, 2, 4, 8, 14, and 16 species were established with 16, 14, 9, 9, 14, and 1 unique community compositions for each richness level,

respectively. Each unique community composition was planted in three replicate plots, except monocultures which were each planted in four replicates plots, and 16-species communities which were planted in 30 replicate plots. Community compositions were designed to replicate those in the BP_{1997} experiment (Tilman *et al.*, 2001; Fargione *et al.*, 2007; Appendix C Table C-3). For 40 of 63 communities, species composition in the new and existing experiments were identical. The remaining 23 communities differed in that they did not include the five species described above, but again, these species represent less than 3% total biomass in BP_{1997}.

Each plot received 10 g live seed m^{-2}, with each seeded species in the community representing equal proportions of the seed mix. Plots were watered in the first year of the study (2015), and were weeded every two weeks for the first two years of the study. Thereafter, plots were weeded once per month. In August 2017, percent plant cover by species was assessed by visual estimation to the nearest percent. Rather than removing thatch by burning (as in BP_{1997}), total biomass was harvested and removed to prevent melting the plastic root barrier. In August 2018, plant cover in each plot was assessed by visual estimation, then randomly-selected 15 cm by 150 cm strips were clipped, sorted to species, dried to constant weight at 60 °C and weighed to the nearest 0.1 g. The remaining biomass was then clipped, dried and weighed. Percent cover to dry biomass correlations were used to transform percent cover values to biomass values.

To provide an additional test of the role of PSF in the BP relationship, we also used published data from the fourth year of the BP_{1997} experiment (49; https://www.cedarcreek.umn.edu/research/data). Cover to biomass relationships reported for 2007 were used to convert species-level cover data to species-level biomass that were

then scaled to match observed community biomass (Tilman *et al.*, 1997).

Modeling Approach

Plant species biomass in communities were predicted using the best-performing discrete plant community growth models in a similar previous study (i.e., the 'logistic species-level-K model' and the 'logistic constant-K model'; Kulmatiski *et al.*, 2011, 2016). In this logistic growth model, species-conditioned soils 'grow' as a function of plant biomass, plant species growth rates, and a plant-to-microbe conversion factor (Appendix C Table C-1). Plant growth rates are a function of the proportion of different conditioned soil types present. To prevent run-away growth, biomass is limited by a carrying capacity, which can be either unique to a species or to the community. Null model simulations are the same except that they include only one soil type and one plant growth rate (Appendix C).

Growth rates were derived from a) growth on control soils (control Null model), b) growth on 'self' soils (self Null model), or c) growth on each soil type (PSF model). Competition coefficients were assigned a value of '1', but each species could affect the growth of other species due to community-level carrying capacities (Kulmatiski *et al.* 2016). Each of these three model parameterizations (i.e., growth on control, growth on self, or growth on each soil type) was run with five different carrying capacities: 1) the maximum observed growth in any plot in the community experiment, 2) the maximum mean observed growth in any community, 3) the maximum species-specific growth in community plots, 4) the maximum observed growth in any PSF plot, and 5) the maximum species-specific growth in any PSF plot. Mean Null model predictions of community biomass were calculated from the 10 model simulations (Control Null, Self

Null each with five carrying capacities). Mean PSF model predictions were calculated from the five simulations with different carrying capacities.

Because growth rates were derived from the second year of growth, we assumed that growth rates represented two years of growth. To simulate the four years of growth in the biodiversity-productivity experiment, model simulations were executed for 52 timesteps, after which plant biomass was reduced to 1% of the previous timestep and allowed to run for another 52 timesteps. Model simulations for 52 or 208 time steps produced qualitatively similar results but only results from the 104 timestep approach described immediately above are reported since they best represented conditions in the field. Mean model output for the sum of species growth from the suite of Null or PSF model simulations are reported.

Parsing Selection and Complementarity Effects

For observed and predicted data, the relationship between species richness and community biomass was described using a best-fit log-linear regression (Proc Reg; SAS V9.4). To parse complementarity from selection effects from these biodiversity-productivity relationships, we used the modified Price equation (R package 'partitionBEFsp"; Loreau & Hector, 2001; Clark *et al.*, 2019). Complementarity effects can be either positive or negative, depending on whether species on average have higher or lower yields than the expected relative yield. Selection effects can be either positive or negative, depending on whether species have a positive or negative covariance between relative yield and biomass. This method is easily interpretable, comparable to other results, and remains the standard practice (Clark *et al*. 2019). Data from outlier communities with total biodiversity effects greater than five times the interquartile range

were removed. Because *S. rigida*, *D. purpurea*, *D. villosa*, and *D. candida* did not grow in monoculture communities in BP_{1997}, when partitioning biodiversity effects for BP_{1997} their monoculture growth was assumed to be twice biculture growth.

Testing PSF and Biodiversity-Productivity Data

Patterns in the observed and predicted biomass with species richness were described with simple, best-fit log linear regressions (Proc Reg; SAS V9.4). The relationship between predicted and observed biomass in different plant communities was assessed by ordinary least squares regression. Plant community biomass was the response variable that was predicted by either Null-or PSF-model-predicted biomass. Similarly, relationships between community-level PSF and overyielding were assessed by ordinary least squares regression (Kulmatiski *et al.*, 2011). Community-level PSF for each species in the community was calculated as $PSF_i = (S_i - O_i)/\text{maximum}(S_i, O_i)$, where O_i represents the average growth of species i on any "other" conditioned soil type present in the community and S_i represents the growth of species i on "self" conditioned soil (20). Community-level PSF across the entire community was calculated as the average of community-level PSF across each species in the community.

RESULTS

PSF Experiment

PSFs were predominantly negative (Fig. 4-1). Across the 240 species*soil-level PSFs, 23 were negative, and 13 were positive (i.e., 95% confidence interval did not overlap zero; Fig. 4-1a). These 39 PSFs occurred across species so that 14 of 16 species demonstrated a PSF on at least one soil type (Fig. 4-1a). Because PSF were both positive

and negative, the mean absolute value of PSFs at the end of the experiment (0.27 in 2018) was larger than the mean of all values at the same time (-0.10). In other words, after a two-year training phase, plants created soils that changed subsequent plant growth by 27%, but because some plants created soils that increased plant growth and other plants created soils that decreased plant growth, the net effect was that plants created soils that decreased plant growth by 10%. PSFs became more negative in the second year of Phase 2 and were 0.00 and -0.10 in 2017 and 2018, respectively ($T_{239} = 5.4$, $P < 0.001$). The absolute value of PSF also increased from 0.23 in 2017 to 0.27 in 2018 (0.23; $T_{239} = -3.1$, $P = 0.002$). For conciseness, only 2018 species*soil level PSF values are shown in Fig. 4-1a.

When species*soil-level PSFs were averaged across soil types to produce one PSF for each species, there were five negative and three positive species-level PSFs in 2017 and five negative and one positive species-level PSFs in 2018 (Fig. 4-1b).

Biodiversity-Productivity Experiments

After four years, community biomass in the concurrent biodiversity-productivity experiment increased with species richness (Fig. 4-2; $F_{1,59} = 36.4$, $P < 0.001$) from 55.6 g m^{-2} in monocultures to 187.3 g m^{-2} in 16-species communities (Fig. 4-2). This 131.8 g m^{-2} difference represented a 237% increase in biomass production. Complementarity effects explained 172.5 g m^{-2} overyielding and selection effects explained 40.8 g m^{-2} underyielding in 16-species communities (Fig. 4-3a).

These results were consistent with those from a similar experiment performed at the site in 1997. In that experiment, after 4 years growth, biomass increased with species richness ($F_{1,59} = 12.66$, $P < 0.001$) from 78.5 g m^{-2} in monocultures to 183.4 g m^{-2} in 16-

species communities (Fig. 4-2). This 104.8 g m^{-2} difference represented a 133% increase in biomass production. On average, 16-species communities experienced a 95.0 g m^{-2} biodiversity effect. Complementarity effects explained 84.5 g m^{-2} overyielding. Selection effects explained 10.5 g m^{-2} overyielding (Fig. 4-3b).

Model Predictions

Plant-community growth models informed with PSF data (i.e., PSF models) predicted that biomass would increase with species richness ($F_{1,59}$ = 7.81, P = 0.007), from 60.1 g m^{-2} in monocultures to 76.1 g m^{-2} in 16-species communities (Fig. 4-2). This 16.0 g m^{-2} difference represented a 27% increase in biomass production. Complementarity effects explained 15.0 g m^{-2} overyielding. Selection effects explained 1.0 g m^{-2} overyielding (Fig. 4-3c). Overyielding was negatively correlated with community-level PSF for 16-species communities (PSF = -5.2*overyielding + 0.15; $F_{1,14}$ = 9.57, P = 0.009, R^2 = 0.42), but not for 2-, 4-, or 8-species communities (P > 0.05).

Null models predicted that biomass would increase with species richness ($F_{1,59}$ = 7.33, P = 0.009) from 60.7 g m^{-2} in monocultures to 70.7 g m^{-2} in 16-species communities (Fig. 2). This 10.3 g m^{-2} difference represented a 17% increase in biomass production. Complementarity explained 1.5 g m^{-2} underyielding. Selection effects explained 11.8 g m^{-2} overyielding (Fig. 3d).

Both PSF and Null model predictions were correlated with community biomass in the concurrent biodiversity-productivity experiment, though PSF model predictions were closer to 1:1 (Observed biomass = 0.97*PSF predicted biomass + 47.3) and had a stronger predictive ability (R^2 = 0.20, P < 0.001, RMSE = 77.6; Appendix C Fig. C-1a) than Null model predictions (Observed biomass = 0.81*Null predicted biomass + 61.5,

$R^2 = 0.14$, $P = 0.003$, RMSE = 81.6; Appendix C Fig. C-1a). Neither PSF nor Null model predictions were correlated with biomasses from the biodiversity-productivity experiment performed in 1997 ($P > 0.05$; Appendix C Fig. C-1b).

DISCUSSION

PSFs improved understanding of the magnitude and mechanism of the biodiversity-productivity relationship. PSF models predicted greater overyielding than Null models, and the mechanism of this overyielding was correct in PSF models and incorrect in Null models. Explaining 12 to 15% of overyielding in new and existing biodiversity-productivity experiments, PSF effects were not large, rather, results provided clear support for PSFs as one of several mechanisms that determine the biodiversity-productivity relationship (van Ruijven & Berendse, 2005). Results were consistent with previous modeling (Kulmatiski *et al.*, 2012), greenhouse (Schnitzer *et al.*, 2011; Guerrero-Ramírez *et al.*, 2019; Wang *et al.*, 2019) and soil pathogen (Maron *et al.*, 2011) studies, but the factorial, two-phase PSF field experiment and biodiversity-productivity experiments used here were expected to provide better insight into how plant-soil interactions affect biodiversity-productivity relationships in the field (Rinella & Reinhart, 2017; Forero *et al.*, 2019).

Null and PSF models predicted that 16-species communities would produce 10.3 g m^{-2} and 16.0 g m^{-2} more biomass than monocultures, respectively. However, Null models incorrectly predicted this overyielding was due to selection effects, while PSF models correctly predicted this was due to complementarity (Fig. 4-3). In new and existing biodiversity-productivity experiments, 16-species communities produced 104.8 g m^{-2} to 131.8 g m^{-2} more biomass than monocultures due to complementarity (Fig. 4-3).

Thus, PSFs predicted 12-15% of the observed biodiversity-productivity effect and the mechanism of this effect was consistent with the mechanism in the observed data.

Null models predicted overyielding due to selection effects because faster growing species realized a competitive advantage and were over-represented in communities relative to mean plant growth in monocultures. In contrast, PSF models predicted overyielding due to complementarity. PSF caused overyielding because the effects of negative PSF (i.e., soil pathogens) were diluted in diverse communities (Maron *et al.*, 2011; Schnitzer *et al.*, 2011). While the net effect of PSF was to increase overyielding, positive PSF also helped improve correlations between predicted and observed community biomass by correctly decreasing the biomass of species with positive PSFs in communities (Appendix C Fig. C-1a; Kulmatiski *et al.*, 2011). For example, a positive PSF for *L. perennis* on *S. nutans* soil, correctly resulted in less *L. perennis* biomass in *L. perennis/S. nutans* bicultures than predicted by the Null model.

The magnitude and direction of PSFs in this study were broadly consistent with those from across the literature, suggesting that PSFs likely also play a small role in the biodiversity-productivity relationship in other systems (Crawford *et al.*, 2019; Beals *et al.*, 2020). The absolute value of PSFs (0.27) indicated that two years of plant growth created soils that changed subsequent plant growth by 27%. However, because PSFs were both positive and negative, the net PSF effect was smaller (*i.e.*, a PSF value of -0.10 in 2018). Absolute PSF values reported across the literature tend to be larger (0.53; Kulmatiski *et al.*, 2008), but the greenhouse experiments commonly reported in the literature are known to produce larger PSF values than field experiments (Forero *et al.*, 2019; Beals *et al.*, 2020). Regardless of whether PSFs change plant growth by 10% or

50%, they are unlikely to explain the 200% overyielding often observed in biodiversity-productivity experiments (Cardinale *et al.*, 2007; Kulmatiski *et al.*, 2012).

The plant community models used in this study produce a relationship between PSF and overyielding that approaches -1:1 as species richness approaches ~16 species (Kulmatiski *et al.*, 2012). In this study, mean PSFs of -10% were associated with 27% observed overyielding. This relationship was closer to -3:1, in part, because it happened to be the case that large negative PSF were important for some dominant plant species (Kulmatiski *et al.*, 2012). For example, *P. virgatum* was a dominant species with a -0.4 PSF on soils cultivated by another dominant species, *S. scoparium*. However, the -5:1 relationship observed between weighted, community-level PSF and overyielding in 16-species communities was notably greater than -1:1, suggesting that the two-phase experimental approach underestimated PSF values or that PSFs may exaggerate or interact with other factors that also encourage overyielding (*e.g.*, niche partitioning or competition; Barry *et al.*, 2019). Regardless of the exact value, results provide clear support for a negative relationship between PSF and overyielding.

PSF experiments are often performed by comparing plant growth on 'self-cultivated' soils to plant growth on 'other-cultivated' soils (Brinkman *et al.*, 2010; Rinella & Reinhart, 2017). At this species level, six species in our study in 2018 realized significant PSFs. However, using a factorial PSF experimental design allowed us to describe how each plant species grows on each of 15 soil types (Kos *et al.*, 2015; Rinella & Reinhart, 2017). At this species*soil level, 14 of 16 plant species realized a significant PSF on at least one soil type. While most species realized either positive or negative PSFs, three plant species demonstrated significantly positive PSFs on one soil type and

significantly negative PSFs on a different soil type. For example, *A. canescens* PSF values ranged from -0.4 to +0.5. For this species, 'self vs. other' PSF experiments could be expected to report strongly positive, strongly negative or neutral PSF depending on the soil types used (Rinella & Reinhart, 2017). It should not be surprising that PSF varies at the level of soil type, but use of the factorial designs needed to demonstrate this pattern with more than two plant species remains less common than the use of 'self vs. other' approaches (Crawford *et al.*, 2019). Thus, results provide a clear example of how broad species-level assessments of PSF can hide important soil-type specific PSFs (Kos *et al.*, 2015; Rinella & Reinhart, 2017; Bauer *et al.*, 2020).

In this study, PSF-informed models improved predictions of plant community biomass for the concurrent biodiversity-productivity experiment but not for the 1997 experiment. Because factors from climate to anthropogenic nitrogen deposition to soil microbial community composition have likely changed in the 20 years between these two experiments, it is impossible to pinpoint why community biomass differs between the two experiments (van der Putten *et al.*, 2016). An implication of the poor correlation between the new and old data is that inference about the effects of PSF on plant community development are likely to be time- or site-dependent (Eisenhauer *et al.*, 2012). However, despite a lack of correlation between predicted and observed biomass for specific communities, the general pattern of increasing aboveground biomass with species richness was consistent across both experiments and PSFs helped explain this pattern. Further, the mechanism of this effect was complementarity in the 1997 experiment, the concurrent experiment and in PSF-informed predictions of this relationship.

PSFs explained 12% to 15% of the net biodiversity effect. Because PSF alone did not fully explain the biodiversity effect, it is likely that several mechanisms interact to produce the observed biodiversity-productivity relationship (Hooper *et al.*, 2016; Lekberg *et al.*, 2018; Pillai & Gouhier, 2019). Niche partitioning has long been thought to be a primary mechanism, but because niche-partitioning remains difficult to measure, the extent to which it determines the biodiversity-productivity relationship remains unresolved (Mueller *et al.*, 2013; Barry *et al.*, 2020; Mahaut *et al.*, 2020). Because PSFs explained a small portion of the total biodiversity effect, results support a large role for niche partitioning. Future research that integrates the effects of niche partitioning, sampling effects and PSF can be expected to improve predictions of the effects of species loss on plant community productivity and resilience with implications for biofuel production and conservation.

DATA ACCESSIBILITY

Upon acceptance data will be archived with a DOI at Utah State University Digital Commons online repository.

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TABLES

Table 4-1. Plant species and functional groups used in the plant-soil feedback and biodiversity-productivity experiments.

Species	Codes	Functional group
Amorpha canescens	Ac	Legume
Andropogon gerardii	Ag	C_4
Achillea millefolium	Am	Forb
Dalea purpurea	Dp	Legume
Elymus canadensis	Ec	C_3
Koeleria macrantha	Km	C_3
Liatris aspera	La	Forb
Lespedeza capitata	Lc	Legume
Lupinus perennis	Lp	Legume
Monarda fistulosa	Mf	Forb
Poa pratensis	Pp	C_3
Pascopyrum smithii	Ps	C_3
Panicum virgatum	Pv	C_4
Sorghastrum nutans	Sn	C_4
Solidago rigida	Sr	Forb
Schizachyrium scoparium	Ss	C_4

FIGURES

(a)

Plant/ Soil	C3					C4			Forb				Legume			
	Ec*	**Km***	Pp	Ps	Ag	Pv	Sn	Ss	Am	**La***	Mf	Sr	**Ac***	**Dp***	Lc	**Lp***
Ec Soil		-0.74	0.01	-0.05	-0.09	-0.06	-0.4	**0.29**	-0.13	-0.3	-0.31	0.35	**0.41**	**-0.41**	0.17	0.17
Km Soil	-0.32		-0.02	0.15	0.23	0.08	-0.01	-0.13	-0.04	-0.4	-0.07	0.27	0.01	-0.17	-0.22	0.19
Pp Soil	-0.23	-0.54		-0.19	0.44	-0.02	-0.6	-0.2	-0.13	-0.64	-0.12	0.13	-0.18	-0.16	-0.25	0.23
Ps Soil	-0.14	-0.49	0.31		-0.33	0.09	-0.25	0.16	0.14	-0.14	0.12	-0.43	0.25	**-0.36**	0.17	0.26
Ag Soil	**-0.39**	-0.6	-0.38	0.04		-0.14	0.18	0.11	-0.02	-0.51	-0.01	0.35	**0.52**	-0.25	**-0.31**	0.43
Pv Soil	**-0.32**	-0.35	-0.71	0.13	-0.08		0.26	-0.21	0.02	-0.07	**-0.54**	-0.07	**0.44**	**-0.52**	-0.33	0.03
Sn Soil	0	-0.57	0.12	0.24	0.11	0.06		0.14	-0.19	-0.52	-0.26	-0.47	0.13	**-0.43**	0.12	**0.68**
Ss Soil	**-0.34**	-0.29	**1**	0.28	0.18	-0.4	-0.37		**-0.27**	0.13	-0.26	0.47	-0.04	-0.3	**-0.32**	-0.12
Am Soil	**-0.39**	-0.28	-0.54	-0.3	-0.06	-0.04	**0.83**	-0.2		-0.43	-0.01	**-0.62**	0.07	**-0.27**	-0.19	**0.71**
La Soil	-0.27	-0.47	-0.06	-0.36	0.06	0.18	-0.42	-0.08	-0.14		-0.33	-0.21	0.32	0.26	-0.2	0.44
Mf Soil	-0.13	**-0.89**	0.66	-0.17	0.01	0.27	-0.23	**-0.25**	**0.46**	0.25		-0.28	**-0.37**	-0.28	-0.33	-0.18
Sr Soil	-0.37	-0.79	-0.31	-0.17	0.16	**0.42**	0.21	-0.01	**0.39**	0.11	-0.12		0	0.1	-0.08	-0.4
Ac Soil	-0.04	-0.08	-0.01	-0.32	0.22	-0.21	**0.74**	0.24	0	-0.74	0.05	0.08		-0.26	-0.27	-0.38
Dp Soil	**-0.45**	**-0.91**	-0.32	**-0.59**	-0.23	-0.3	-0.57	-0.13	0.04	-0.5	0.04	**-0.53**	0.25		-0.22	**0.67**
Lc Soil	-0.26	-0.11	-0.2	-0.11	0.17	-0.38	-0.02	-0.16	-0.17	-0.18	-0.3	-0.37	-0.15	**-0.37**		-0.04

(b)

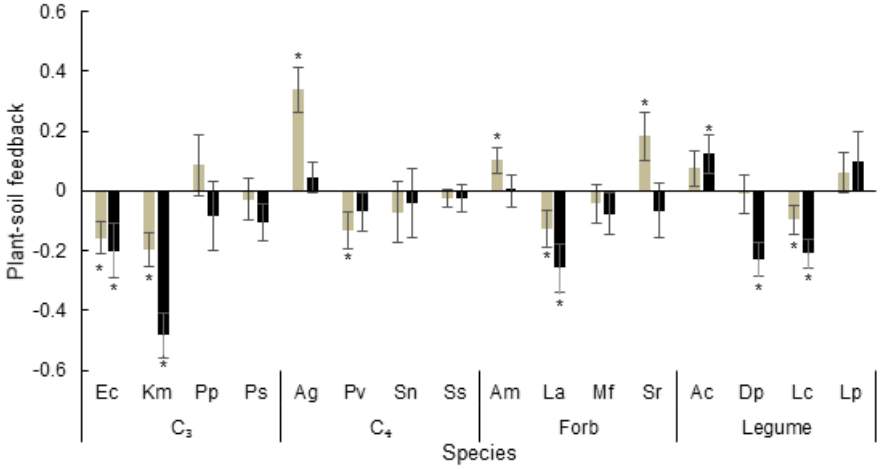

Figure 4-1. Species*soil-level (a) and species-level (b) plant-soil feedback (PSF) values for 16 plant species. Negative species*soil-level PSFs shown in red. Positive species*soil-level PSFs shown in green. For species*soil-level PSFs, bold and underlined values indicate that the 95% confidence intervals (CI) did not overlap zero. CIs reflect variation among replicate field plots. Species-level PSFs represent the mean (+/- SE) of species*soil-level PSFs in 2017 (grey) and 2018 (black). Species-level PSF values that differed from zero in a one-sample t-test at the $\alpha = 0.05$ level indicated with an asterisk.

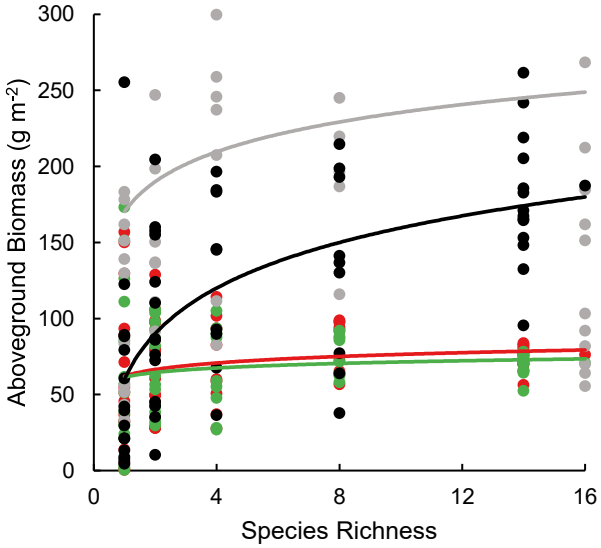

Figure 4-2. Aboveground biomass increased with species richness in a new (black symbols) and existing (grey symbols) field experiments and in both Null (green symbols) and plant-soil feedback (red symbols) model simulations. Each point represents total aboveground biomass in one community type (n = 63) after four years growth. Large values from six outlier plots are not shown.

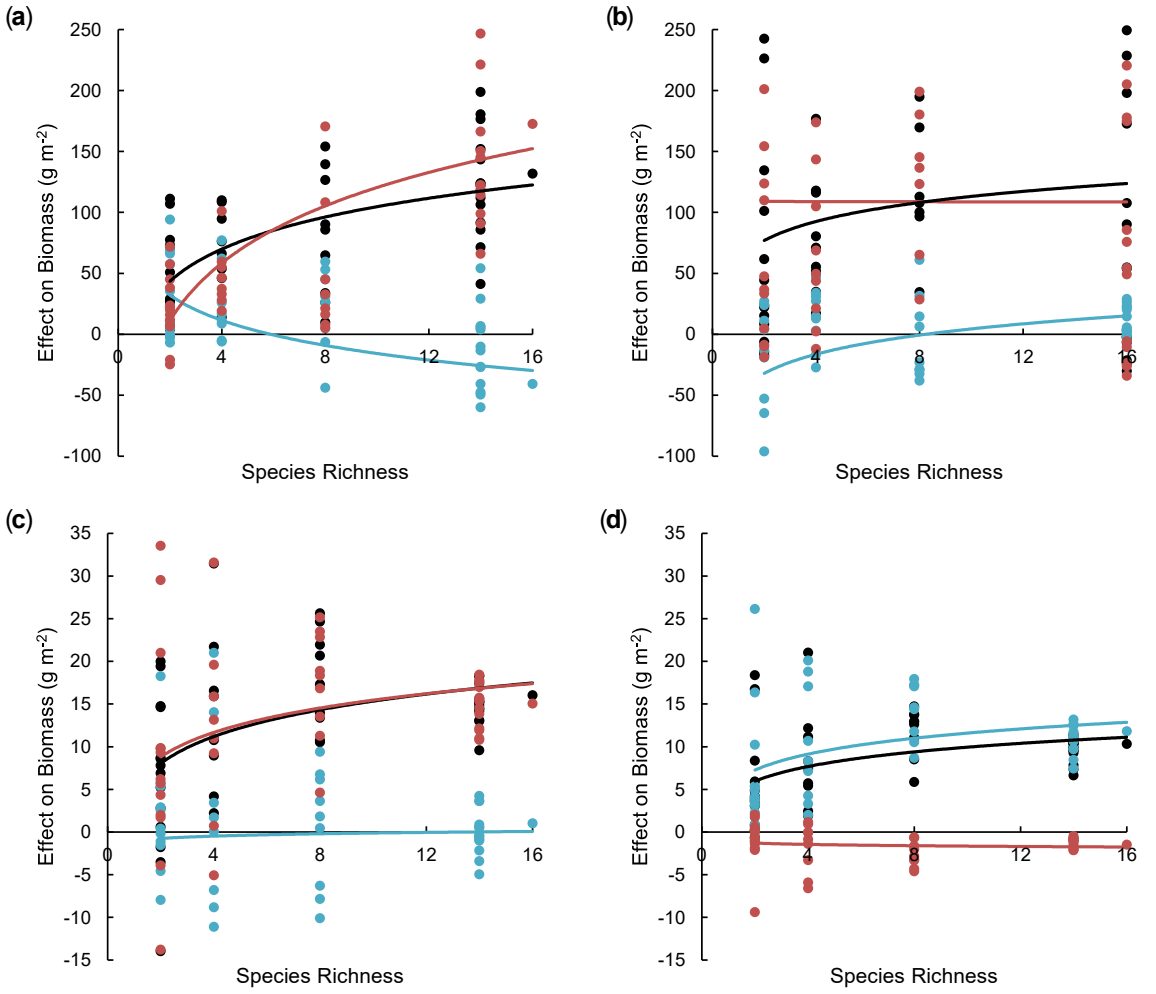

Figure 4-3. Net overyielding (black symbols) increased with species richness due to complementarity (orange symbols) in the new field experiment (**a**) and in plant-soil feedback model predictions (**c**), but in the BP_{1997} experiment (**b**) and Null model predictions (**d**) total biomass increased due to selection effects (blue symbols). Each point represents one of 63 observed (**a, b**) or modeled (**c, d**) plant communities. Outlier values from one to five plots omitted from each panel for clarity.

CHAPTER 5

VERTICAL RESOURCE UPTAKE PATTERNS HELP EXPLAIN OLD-FIELD PLANT ABUNDANCE[4]

Abstract

Vertical root distributions have long been thought to be important to plant growth and coexistence. However, it remains difficult to demonstrate how different root distributions affect resource uptake and plant growth on the landscape. We injected water and N tracers to five depths (5-150 cm) and measured uptake by 11 dominant grassland species, Minnesota, USA. Tracer uptake profiles were adjusted by depth-specific resource availability and resource-use efficiencies to estimate biomass production associated with each rooting distribution. These biomass estimates were compared to plant landscape abundance. Among species, water uptake ranged from 52 cm to 67 cm of soil water resulting in 1 to 94 g m^{-2} biomass. Rooting distributions that could absorb more water and produce more biomass were more abundant on the landscape (R^2 = 0.31 and 0.48, respectively). While on average 50% of water uptake occurred between depths 1 and 19 cm, mean N uptake was deeper (occurring between depths 1 to 38 cm) and differed more among species than water uptake. However, N uptake and biomass resulting from N uptake were not correlated with plant landscape abundance. Within-season temporal variation, not measured in this study, appeared to be important to N uptake, but less important to water uptake. It has long been difficult to demonstrate how

[4] Forero LE, Kulmatiski A. Vertical resource uptake patterns help explain prairie plant abundance. In preparation.

root distributions affect plant abundance, but here we provided clear links between root distributions, water uptake, plant growth and landscape abundance.

Introduction

Different rooting distributions are likely to provide plants with different amounts of soil resources (Holdo and Nippert 2015; Guderle et al. 2018; Newman et al. 2020). Rooting distributions that provide more resources are likely to allow more plant growth and rooting distributions that provide access to resources little used by other species may minimize resource competition, allowing coexistence (Silvertown et al. 2015; Letten et al. 2017; Case et al. 2020). For example, some species produce mostly shallow roots and others produce deeper roots (Schenk and Jackson 2002; Zhou et al. 2020). Shallow roots may have preferential access to precipitation and nitrogen-rich soils; deep roots may have preferential access to larger and more stable soil resource pools (Ryel et al. 2008; Ward et al. 2013; Kulmatiski et al. 2017).

While there is wide agreement that root distributions are likely to affect plant growth and coexistence, data demonstrating the extent of this effect remain difficult to collect (Schenk 2008; Silvertown et al. 2015; Barry et al. 2020; Kühnhammer et al. 2020) leading some to suggest that vertical niche partitioning may not be important (Higgins et al. 2000; Barry et al. 2020). Because it remains difficult to measure, vertical niche partitioning among plant species is often inferred from differences in root biomass distributions (Yu et al. 2007; Nippert and Holdo 2015; Barry et al. 2020). Yet, there is reason to believe that root biomass distributions may not be well-correlated with resource uptake (Chen et al. 2004; Sternberg et al. 2005; Gambetta et al. 2017). For example, large suberized roots can represent a large portion of root biomass but have little effect on

vertical resource uptake (Peek et al. 2005; Kulmatiski et al. 2010). Even fine roots can vary several-fold in their ability to absorb soil resources (Tobar et al. 1994; Marulanda et al. 2003; Kiba and Krapp 2016). Without measurements of soil resource uptake (instead of root biomass distributions), the extent of resource partitioning is unclear (Dubbert and Werner 2019).

Stable isotope techniques offer the potential to describe vertical resource uptake distributions by different species in mixed communities in the field (McKane et al. 2002, Koeniger et al. 2010, Zheng et al. 2018). Natural abundance stable isotope approaches have the advantage that they can quickly sample resource uptake of many plants across the landscape, but inference for rooting distributions is limited by the types of naturally occurring isotope enrichment profiles that are present in the soil (Nippert and Knapp 2007a; Rothfuss and Javaux 2017; Beyer et al. 2018). Isotope tracer techniques do not require naturally-occurring enrichment profiles and they provide more detailed patterns of vertical resource uptake. However, tracer experiments require more effort, and therefore provide more limited sampling (Beyer et al. 2016).

Both natural abundance and tracer techniques can be used to quantify patterns of vertical resource uptake, but not necessarily the amount of water absorbed by different plants (Kühnhammer et al. 2020; Sprenger and Allen 2020). For example, large isotope concentrations can be found in plants with slow or fast sapflow rates (Vargas et al. 2020). Further, in tracer studies, there is potential for plants to absorb injected tracer in otherwise dry soils (Kulmatiski et al. 2017). These problems can be addressed by using tracer-derived root distribution data in soil water flow models (Holdo 2013; Warren et al. 2015; Mazzacavallo and Kulmatiski 2015). This combined tracer and water flow model

approach provides estimates of the depth, timing, and extent of water use by different plant species (Nippert and Holdo 2015; Mazzacavallo and Kulmatiski 2015; Zheng et al. 2018; Beyer et al. 2018). Tracer experiments also offer the potential to simultaneously follow multiple resources through the soil-plant continuum (Hoekstra et al. 2014). This is advantageous because limitations in more than one resource are likely to determine species coexistence in many systems (Swanson et al. 2015; Harpole et al. 2016; Yan et al. 2020).

Our goal was to link vertical root distributions with resource uptake and landscape abundance. To describe resource uptake distributions in a tallgrass prairie, we measured water and N tracer uptake by dominant species in plots that received tracer injections to one of five soil depths (5, 15, 30, 60 and 150 cm). Because water moves quickly through the soil-plant-atmosphere continuum, water tracer uptake profiles were used in soil water flow models to estimate the amount of water each rooting distributions could be expected to absorb across a growing season (Holdo and Nippert 2015; Kulmatiski et al. 2020a). Similarly, N tracer uptake was adjusted by estimates of N availability by depth across the growing season to estimate rooting distribution effects on season-long N uptake (McKane et al. 2002; Kulmatiski et al. 2017). Water and N uptake distributions were used to describe both total uptake (i.e., uptake across the root profile) and to identify depths at which a species could extract more soil resource than any other species (henceforth, ‘unique niches’). Finally, water and N uptake were adjusted by water- and N-use efficiencies to provide an index of how resource uptake may affect biomass production. These indices were then compared to plant landscape rank abundance. We predicted that species with rooting distributions that could absorb more soil resources would be more

abundant on the landscape.

Materials and Methods

Research was conducted during peak growing season, July 20-25 2016 at Cedar Creek Ecosystem Science Reserve, East Bethel, MN, USA (45.396989, -93.191277). The study site is tallgrass prairie located at the boundary of hardwood forest and conifer forest ecosystems. Soils are sandy skeletal and in the Zimmerman soil series (Grigal et al. 1974). Mean annual precipitation is 796 mm and mean annual temperature is 6.7 °C (PRISM Climate Group). Dominant species at the site include the native forbs *Achillea millefolium* (0.93% cover, Nutt.), *Artemesia ludoviciana* (6.13%, Nutt.), *Erigeron canadensis* (0.07%, Cronq.), *Equisetum laevigatum* (0.14%, A. Braun), and *Ambrosia psilostachya* (0.06%, DC.); the native C_3 grass *Panicum oligosanthes* (0.59%, Schultes); the native C_4 grasses *Andropogon gerardii* (0.65%, Vitman), *Schizachyrium scoparium* (3.50%, Nash), and *Sorghastrum nutans* (4.14%, Nash); and the non-native C_3 grasses *Poa pratensis* (36.21%, L.) and *Elymus repens* (31.72%, Beauv.) (Ownbey and Morley 1991). These 11 species represent 84% of the total aboveground biomass in the study system and are the focus of this study. Aboveground biomass data were obtained from Cedar Creek's 2016 Experiment 002 survey, which was performed one week after tracer injections and plant sampling (https://www.cedarcreek.umn.edu/research/data/dataset?ple002).

Tracer injections

The tracer experiment broadly followed the approaches described by Kulmatiski et al. (2017). Briefly, 25 circular plots (3 m diameter), were established 5 m apart in a 20

m by 20 m study area. Each plot was randomly assigned to represent one of five replicates of each of five soil depths (5, 15, 30, 60, and 150 cm). In each plot, 314 pilot holes were drilled to the target depth in a 15 cm by 15 cm grid using a 1-cm drill bit and a hammer-drill (Hilti TE-60, Tulsa, Oklahoma). Custom-made syringes (16-gauge thin-walled hypodermic tubing; Vita Needle Company, Needham, Massachusetts, USA) were used to inject 1 mL of 70% $^{2}H_20$ that contained 1 mg $^{15}NH_4{}^{15}NO_3$ (Cambridge Isotopes, Tewksbury, MA). This tracer injection was followed by 2 mL tap water injection to clear tracer from the syringe. Therefore, each of 25, 7 m^2 plots received 952 mL of tracer plus rinse water across 314 holes. Injections occurred over three days. Due to time constraints, injections to 150 cm were performed in three replicate plots instead of five replicate plots. The two unused plots were used to collect 'control' samples from plots that received no injections.

$^{2}H_2O$ tracer uptake

Two days after injections in each plot (Hoekstra et al. 2014; Warren et al. 2015; Mazzacavallo and Kulmatiski 2015), non-transpiring stem tissues from one to three individuals of each of the 11 target species were clipped with rinsed clippers, placed in pre-made 19-mm wide medium-walled borosilicate tubes, sealed with parafilm and placed on ice (Pyrex, Corning, NY, USA). Samples were moved to a freezer within 6 hours. Water from plant tissues was extracted using cryogenic distillation (Vendramini and Sternberg 2007) within two weeks of sampling. Extracted water samples were analyzed for hydrogen and oxygen isotopes on a wavelength scanned cavity ring-down spectrometer (Picarro L-2120i; Picarro Instruments, CA, USA). Raw hydrogen and oxygen concentrations were normalized to the VSMOW-SLAP scale using three

calibrated waters. These calibrated waters (i.e., standards) were included for roughly every ten samples. The isotopic data for plant extracts were checked and adjusted for spectral contamination using ChemCorrect® software (Picarro Inc.). Isotope concentrations are typically low and reported in delta notation in units of parts per thousand (i.e., [{Rsa – Rstd}/Rstd] * 1,000, where R is the ratio of, for example, ^{2}H to H, sa = sample, and std = standard – typically Vienna mean standard ocean water). Deuterium isotope values [in delta notation (δ)] were converted to deuterium excess values (δ_e) to control for natural isotope enrichment caused by evaporation as follows: $\delta_e = \delta^2H - [(8 * \delta^{18}O) + 10]$ (Craig 1961; Kulmatiski et al. 2010; Mazzacavallo and Kulmatiski 2015). To account for variation in natural enrichment among species, δ_e values for each species in control plots were subtracted from δ_e values for each species in experimental plots. Vials containing control samples for *A. psilostachya* and S. *nutans* broke during stem water extraction. Species control δ_e values ranged from 6.30 to 544.97, while mean treatment δ_e values were 1289.97, so the lack of control values for *A. psilostachya* and S. *nutans* was likely to have little effect on results. To allow a direct comparison among species that was not biased by plant mass, abundance, or lateral rooting distributions, tracer uptake was converted to the proportion of tracer uptake by depth (Kulmatiski et al. 2010; Hoekstra et al. 2014).

Water uptake

$^{2}H_2O$ uptake was used to simulate the vertical distributions of active roots for the 11 focal species by depth. The soil water flow model Hydrus 1D was used to integrate the effect of these vertical rooting distributions on water uptake across a growing season (Hartmann et al. 2018; Zheng et al. 2018; Kulmatiski et al. 2020a). A shortcoming of this

approach is that vertical root distributions for each of the eleven species must be simulated separately as a monoculture. A commonly used set of model parameters was used for all simulations, so parameter selection was not likely to affect interpretation of the effect of different rooting distributions (Kulmatiski et al. 2020a). Detailed parameter definitions and settings are provided in Appendix D Tables D-1 through D-3, but we a brief description is as follows: Evapotranspiration was estimated using the Penman-Monteith equation (Šimunek et al. 2012). The hydraulic sub-model was a van Genuchten-Mualem model with no hysteresis. Neural network predictions of soil hydraulic parameters were made using soil texture and bulk density data for the soil series (Grigal et al. 1974; Appendix D Table D-3). The water flow boundary conditions allowed for surface runoff and free drainage. In the water uptake model, plants were not allowed to shift their root distributions to compensate for a lack of uptake from dry soils by absorbing more water from wet soils because we measured root distributions directly (i.e., the critical stress for water uptake was set to 1). Radiation was considered as solar radiation. Plant height was assigned to the mean value for the site (Appendix D Table D-4) and leaf area index was assigned a value of 1. Assigning a standard leaf area value across species isolated the effect of root distributions on water uptake. Interception was set to 2 mm. Hourly weather data used in Hydrus for summer 2016 was obtained from a weather station on-site at Cedar Creek Ecosystem Science Reserve. Windspeed and solar radiation data were obtained from a weather station at the nearby Carlos Avery Wildlife Management Area. This parameterization was repeated to estimate vertical root distributions in monoculture for each of the 11 species. Because model parameters were the same for all species, so parameter selection was not likely to affect the interpretation

of results. For example, use of the Hargreaves equation would likely result in greater estimates of evapotranspiration, but this greater evapotranspiration estimate would be the same for all rooting distributions and unlikely to significantly change differences in estimated water uptake among different rooting distributions.

Nitrogen tracer uptake

For N analyses, green plant tissue was collected two days after injection and dried at 49° C for 25 hours. Samples were analyzed in the Utah State University Stable Isotope Laboratory for total N and $^{15}N/^{14}N$ ratios by continuous-flow, direct combustion and mass spectrometry using a Europa Scientific 2020 system (PDZ, Crewe UK). Because background soil N pools were larger in shallow than deep soils, ^{15}N enrichments caused by tracer injections were assumed to be smaller in shallow than deep soils (Stark 2000). To account for this background pool dilution effect, ^{15}N enrichments were adjusted using a time-weighted mean ^{15}N excess calculated using a two compartment (NH_4^+ and NO_3^-) isotope dilution model (as in Stark 2000 and Kulmatiski 2017). Initial ^{15}N enrichments were estimated from the mass of soil wet by the tracer addition (based on the measured soil water content, the amount of water added, and texture-based estimates of field capacity), soil inorganic N concentrations measured prior to injection, and the amount of ^{15}N injected. Dilution of the ^{15}N tracer was modeled assuming that turnover times of inorganic N pools were 1 d throughout the soil (Booth et al. 2005). We assumed that plants took up NH_4^+ and NO_3^- at rates proportional to their concentrations in KCl extracts. These calculations produced a dilution-adjusted concentration of $^{15}N/^{14}N$ in leaf tissues.

To standardize ^{15}N uptake across species with different total N concentrations in

their leaf tissues, dilution-adjusted ^{15}N concentrations were converted to μg ^{15}N per gram leaf biomass using sample weight and leaf N concentrations. The μg ^{15}N per g leaf biomass in control samples was subtracted from treatment samples. As with $^{2}H_2O$ tracer values, to μg ^{15}N per gram leaf biomass was converted to the proportion of uptake by soil depth for each species. Because shallow soils have greater soil N concentrations, dilution-adjusted data indicated that tracer concentrations were smaller in shallow than deep soils. As a result, dilution-adjusted ^{15}N uptake data indicated shallower uptake profiles than unadjusted values, though differences among species were similar in unadjusted and dilution-adjusted data (Appendix D Figure D-1).

Soil water, N and root biomass

Gravimetric soil water content was measured in 200 g grab samples from 15, 30, 45, 60, 90, 105, 120, 135, 150, and 160 cm in depth. Soil samples were kept on ice while in the field and refrigerated. Weighed samples were dried at 65° C to constant weight and passed through a 2-mm sieve. Dry roots and rocks were collected by hand and weighed. Ten grams of dried soils were extracted in 100 mL 1 M KCl solution for five minutes and extractable soil N was determined on a Lachat autoanalyzer (Lachat Instruments, Loveland, Colorado, USA).

Biomass production indices

Water- and N-use efficiencies were used to provide an index of water and N-uptake relationships to biomass production. Published water-use efficiencies were collected from Pastore et al. (2019). These values were calculated as the rate of net leaf photosynthesis divided by stomatal conductance as measured using a LICOR 6400

portable gas exchange system (Pastore et al. 2019). Percent leaf N data collected during isotope analyses were used as an N-use efficiency. These estimates were used to produce indices of resource uptake effects on biomass production and not precise estimates of biomass production, so we use them to produce a rank order estimate of resource uptake effects on growth. We compare these rank order estimates to the rank order of plant abundance on the landscape to test the hypothesis that both resource uptake and resource use efficiencies are important for biomass production.

Statistical analyses

To approximate the continuous soil profiles of root biomass and tracer uptake with depth we fit generalized additive mixed effects models (GAMMs) for water and ^{15}N uptake (Wood 2012; Kulmatiski et al. 2017). We let the GAMMs have four "knots" to allow for a smooth interpolation between the five sample depths. We fit nested subsets of the mixed models with different groupings of species-level tracer uptake, which define each model's random effects structure. Groupings included 1) an ungrouped "global" model, 2) a model grouped by C3 grasses, C4 grasses, forbs, and ferns, 3) a model grouped by grasses, forbs, and ferns, 4) a model grouped by grasses and forbs/ferns, and 5) a model grouped by species. We fit models with group level intercepts and slopes (the "effect" of soil depth). All models were fit in R (R Core Research Team 2004) using the gam function from the mgcv package (Metadata S1; Data S1; Wood 2012). We used Akaikie's Information Criterion (AIC) to rank models in terms of their support by the data. The model with the lowest AIC is the best model in terms of predictive ability and in terms of support from the data (Appendix D). Likewise, for any given hypothesis, we can compare two of the models and assess their relative support. Simple regressions were

used to compare observed vs. predicted plant absolute and rank abundance.

Results

By species, the sum of δ_e across depths (i.e., tracer uptake) ranged from 3704‰ in *A. millefolium* to 9037‰ in *A. ludoviciana* (Figure 5-2a). By depth, δ_e (mean ± SE) ranged from 25 ± 12‰ in 150 cm plots to 4147 ± 435‰ in 5 cm plots (Figure 5-2a, 5-3b). GAMMs indicated that the proportion of water tracer uptake by depth differed among species, but not among functional groups (Table 5-2). When water tracer uptake was used in Hydrus 1D to estimate season-long water uptake, the mean depth of water uptake ranged from 26 to 33 cm across the 11 species. The total amount of water uptake ranged from 52 to 67 cm of soil water for the 11 species (Figure 5-2c). When adjusted by water use efficiencies, the variation in water uptake caused by differences in rooting distributions were estimated to result in range of biomass production from 1 to 94 g m^{-2}.

^{15}N uptake ranged from 0.57 µg g^{-1} leaf biomass in *S. nutans* to 4.00 µg g^{-1} leaf biomass in *P. pratensis* (Figure 5-1a). By depth, ^{15}N uptake ranged from 0.12 µg g^{-1} leaf mass in the 150 cm depth to 0.58 µg uptake g^{-1} leaf matter in the 60 cm depth (Figure 5-1a, Figure 5-3a). The depths of 50% N uptake ranged (depending on species) from 18 to 50 cm, with a mean of 38 cm. For ^{15}N, GAMMs suggested that the proportion of tracer uptake by depth differed among functional groups (Table 5-3).

Seven of the 11 species demonstrated depths at which they were estimated to extract more soil water than any other species (i.e., unique niches; Figure 5-2c). Five of the 11 species demonstrated unique niches in their proportion of ^{15}N uptake g^{-1} leaf matter cm^{-1}soil depth (Figure 5-1b). Nine of 11 species demonstrated a unique N niche, a unique water niche, or both. Neither total unique niche space nor rank unique niche space

was correlated with rank plant landscape abundance for water, N, or for a combination of the two.

Rank landscape abundances were not positively correlated with total water uptake (Figure 5-4a). Rank landscape abundances were positively correlated with rank total water uptake (Figure 5a, $y = 0.69x + 1.85$, $R^2 = 0.48$, $P = 0.02$). The rank order of plant production estimated from water uptake and water use efficiencies was also correlated with rank plant landscape abundance (Figure 5-5d, $y = 0.61x + 2.35$, $R^2 = 0.37$, $P = 0.05$).

Rank landscape abundances were not correlated with ^{15}N per gram of leaf biomass or with the rank order of ^{15}N per gram of leaf biomass (Figure 5-4b, 5-5b). When adjusted by leaf percent N (an estimate of N use efficiency), plant mass produced by N uptake was marginally correlated with plant rank landscape abundance (Figure 5-5c, $y = 0.58x + 2.51$, $R^2 = 0.34$, $P = 0.06$). The sum or plant production expected from water and N uptake was correlated with rank plant landscape abundance (Figure 5-5e, $y = 0.74x + 1.53$, $R^2 = 0.44$, $P = 0.03$).

Discussion

Different root distributions are believed to affect plant growth and coexistence by providing plants with different soil resource pools, but it remains difficult to link root distributions, resource uptake and plant growth (Hoekstra et al. 2014; Guderle et al. 2018; Jesch et al. 2018; Barry et al. 2020). By using tracer uptake data in a soil water flow model, we demonstrated how differences in vertical root distributions can result in differences in water uptake. Importantly, these differences were correlated with rank plant landscape abundance ($R^2 = 0.48$). Similarly, when adjusted by water-use efficiencies, these water uptake values produced estimates of plant growth that were also

correlated with rank plant landscape abundance. Results, therefore, provided a mechanistic link between vertical root distributions, resource uptake, plant growth and plant landscape abundance.

Vertical niche partitioning is not likely to be the only factor determining species abundance on the landscape. Horizontal and temporal niche partitioning, as well as herbivory, plant-soil feedbacks, fire competition, and other factors, are likely to affect species abundance (Adler et al. 2010; Levine et al. 2017). Yet, our estimates of resource uptake derived from a mid-season tracer experiment provided surprisingly good predictions of species abundance. Because previous studies have demonstrated that rooting distributions change during the growing season (McKane et al. 1990), we expect that repeated sampling through the growing season would have produced more accurate estimates of resource uptake by different rooting distributions that were better correlated with plant landscape abundance (Kulmatiski et al. 2020a).

Consistent with previous studies, we found that all species relied on shallow roots for water uptake: the depth of 50% $^{2}H_2O$ uptake occurred between 26 cm and 33 cm and the depth of 50% of water uptake was between x and y cm (Nippert and Knapp 2007a; Nippert et al. 2012). Water uptake was slightly deeper than tracer uptake because plants maintained active roots in shallow soils even though those soils dried periodically during the growing season. Shallow rooting distributions resulted in large niche overlap, yet small differences in $^{2}H_2O$ tracer uptake profiles resulted in the amount of water uptake over the year ranging from between 52 and 67 cm across species. These differences appeared to be biologically important because plants with rooting distributions that could take up more water were more abundant on the landscape ($R^2 = 0.48$). When adjusted by

water-use efficiencies, these water uptake values produced estimates of plant biomass that were also correlated with the rank order of plant abundance on the landscape (R^2 = 0.37). Several studies have suggested that vertical root niche overlap is too large to allow species coexistence (Higgins et al. 2000; Barry et al. 2020), but our results demonstrate that even small differences in vertical root distributions can result in large differences in soil water uptake (Kulmatiski et al. 2020b).

Assuming rain-use efficiencies of 0.4 g mm^{-1} rain, our simulations suggest that differences in root distributions would allow species to produce between 208 g and 268 g biomass $year^{-1}$ (Šimunek et al. 2012; Ruppert et al. 2012). The fact that root distributions alone were estimated to provide 29% more biomass to some species than others was somewhat surprising given the large niche overlap in water uptake profiles. This highlights the importance of converting root distributions to resource uptake and supports the idea that even small differences in root distributions can produce biologically important effects on water uptake and plant growth (Kulmatiski et al. 2020b). This is in contrast to previous studies suggesting that vertical root niche overlap is too large to allow coexistence (Jesch et al. 2018; Barry et al. 2020).

N uptake was expected, but not found to be correlated with plant landscape abundance in this N-limited ecosystem. It is important to note, however, that our estimates of N uptake represented uptake during a two-day sampling period. We did not have depth and time specific estimates of N availability to produce season-long estimates of N uptake, but previous research at the site has demonstrated important changes in N uptake across the growing season indicating that these temporal patterns are important (McKane et al. 2002). In contrast, water uptake was estimated from season-long water

uptake. It is likely that repeated measures of N uptake are needed to understand rooting pattern effects on N uptake. Alternatively, our results are consistent with a recent review suggesting that, due to root proliferation, the total N uptake in a plant community is largely determined by N availability and not root activity (Kulmatiski et al. 2017; Dybzinski et al. 2018).

Though not correlated with plant landscape abundance, N uptake data were reasonable and informative. N-uptake patterns differed from water uptake patterns. The mean depth of N-tracer uptake (38 cm) and N availability (58 cm) were both deeper than the mean depth of $^{2}H_2O$ tracer uptake (8 cm) and water availability (13 cm). These results add to a growing body of research showing that plant foraging for water resources occurs somewhat independently of foraging for N resources (McKane et al. 2002; da Silva et al. 2011; Bakhshandeh et al. 2016). An important implication of this research is that root biomass distributions across the soil depth profile are unlikely to provide a good indicator of resource uptake (Kulmatiski et al. 2017).

Together, differences in vertical uptake of water and N provided nine of 11 species with depths at which they could extract either more water or N than any other species. These resource niches could be expected to encourage species coexistence by providing species with preferential access to resources at a certain depth (Kulmatiski et al. 2020a)

Even with measurements of tracer uptake, it is difficult to estimate depth and species-specific resource uptake (Silvertown et al. 2015). For example, it may not be appropriate to compare raw isotope concentrations in stem tissues among plants because low concentrations could reflect small uptake, greater tracer dilution in larger plants, or

greater dilution in plants with more roots outside of the injection area (Hoekstra et al. 2014). For these reasons, we reported tracer uptake as a proportion of uptake by depth – a measure that controls for species-differences. Proportion uptake, however, does not account for water uptake from otherwise dry soils or indicate the amount of water uptake, so the proportion uptake data was used in soil water flow model that accounted for rapid changes in precipitation, evaporation, percolation and transpiration (Holdo 2013; Mazzacavallo and Kulmatiski 2015; Zheng et al. 2018). The use of the soil water model was critical in this study. Tracer uptake alone indicated large niche overlap and tracer uptake was not correlated with plant landscape abundance. However, when tracer uptake patterns were used in a soil water flow model, water uptake was correlated with species landscape abundance. Results, therefore, highlight the importance of understanding root presence, root activity, and resource availability (Holdo 2013).

In our system, previous research has suggested that both hydrologic niches and N niches are important for species coexistence (McKane et al. 1990; Nippert and Knapp 2007b). Specifically, C_3 plants were found to rely more on deep soil water than C_4 plants (Nippert and Knapp 2007b, a), and that the two dominant species had larger niche spaces in shallow (0-12 cm) soils than the four subdominant species (McKane et al. 1990). Overall, our research provides strong support for spatial partitioning of multiple resources as a driver of coexistence and species productivity in diverse tallgrass prairie communities. However, incorporating additional data on temporal variation in spatial niche partitioning, and incorporating additional potential limiting resources in this approach is likely needed to fully understand coexistence and species productivity in diverse plant communities (Beyer et al. 2017; O'Keefe et al. 2019). A better

understanding of vertical resource partitioning can be expected to provide critical insight into both species coexistence and biosphere-atmosphere interactions under both current and changing climate conditions (Bradford et al. 2020; Tague et al. 2020).

Data accessibility

Upon acceptance data will be archived in a public repository with a DOI.

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TABLES

Table 5-1. AIC from general additive mixed models for water uptake for plants with different groupings, model 5 (grouped by individual species) had the lowest AIC.

Model	Random-effects groups	AIC (WaterUptake)
M1	None (global model)	1578.8588
M2	Group 1: C3 grasses (Elyre, Panol, Poapr) Group 2: C4 grasses (Andge, Schsc, Sornu) Group 3: Ferns (Equla) Group 4: Forbs (Achmi, Ambps, Artlu, Erica)	1416.0182
M3	Group 1: Grasses (Andge, Elyre, Panol, Poapr, Schsc, Sornu) Group 2: Forbs (Achmi, Ambps, Artlu, Erica) Group 3: Ferns (Equla)	1416.7418
M4	Group 1: Grasses (Andge, Elyre, Panol, Poapr, Schsc, Sornu) Group 2: Forbs and Ferns (Achmi, Ambps, Artlu, Equla, Erica)	1452.9951
M5	Grouped by each species	**972.8744**

Table 5-2. AIC from general additive mixed models for ^{15}N uptake for plants with different groupings, model 1 (no groupings, general model) had the lowest AIC for ^{15}N not adjusted by depth, followed by model 5 (grouped by species). For ^{15}N adjusted by depth, model 4 (grasses grouped, forbs and ferns grouped) and the general model were the two top performing models.

Model	Random-effects groups	AIC (µg ^{15}N)	AIC (P ^{15}N)	AIC (µg ^{15}N cm^{-1})	AIC (P ^{15}N cm^{-1})
M1	None (global model)	**53.81180**	**-55.45677**	**-242.0021**	**-55.68317**
M2	Group 1: C3 grasses (Elyre, Panol, Poapr) Group 2: C4 grasses (Andge, Schsc, Sornu) Group 3: Ferns (Equla) Group 4: Forbs (Achmi, Ambps, Artlu, Erica)	57.25307	-41.52370	-235.6329	-41.28492
M3	Group 1: Grasses (Andge, Elyre, Panol, Poapr, Schsc, Sornu) Group 2: Forbs (Achmi, Ambps, Artlu, Erica) Group 3: Ferns (Equla)	55.97260	-43.79194	-235.5210	-43.28867
M4	Group 1: Grasses (Andge, Elyre, Panol, Poapr, Schsc, Sornu) Group 2: Forbs and Ferns (Achmi, Ambps, Artlu, Equla, Erica)	55.46894	**-46.17570**	-238.2838	**-48.39272**
M5	Grouped by each species	**54.06140**	-38.42413	**-260.2586**	-29.51704

FIGURES

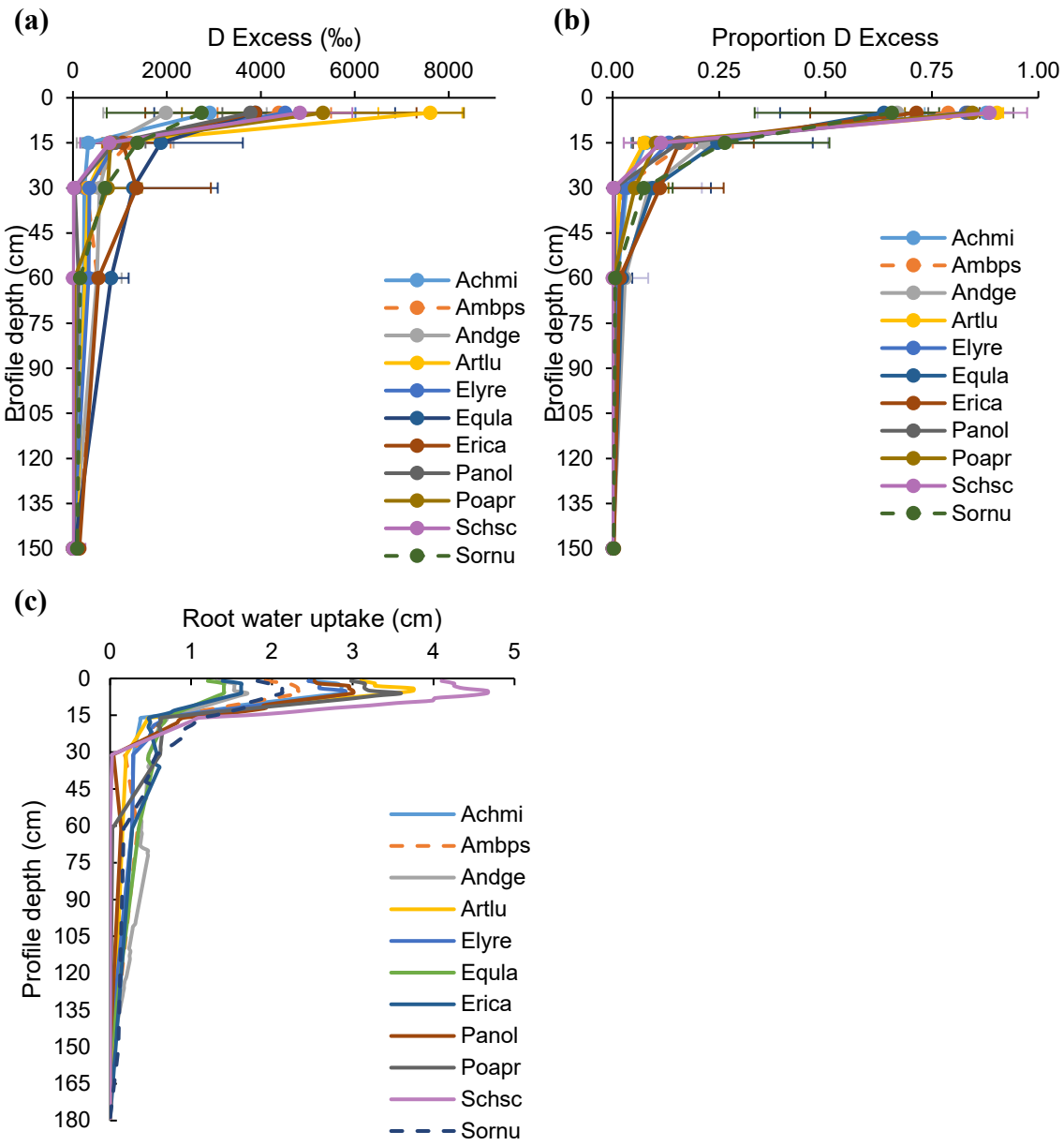

Figure 5-1. Species-level hydrologic profiles by depth, (a) excess deuterium uptake, b) proportion of deuterium uptake by depth adjusted by centimeters in the profile depth range, and (c) root water uptake in centimeters. *Ambrosia psilostachya* and *Sorghastrum nutans* (shown with dashed lines) did not have control samples.

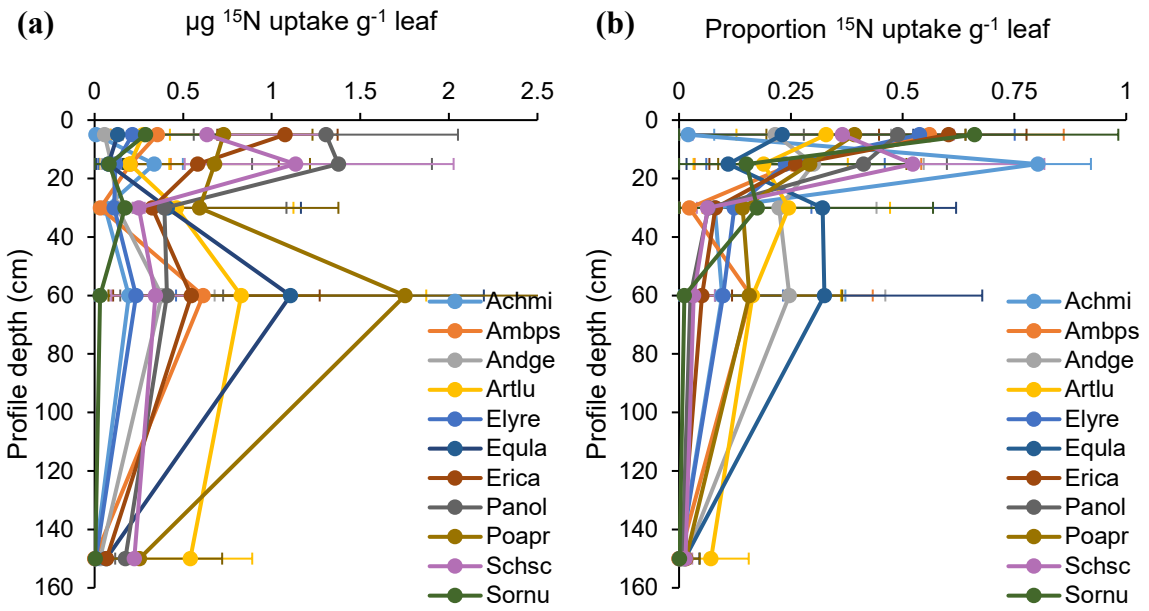

Figure 5-2. µg ^{15}N uptake per gram of leaf (a), and proportion ^{15}N uptake per gram of leaf matter (b).

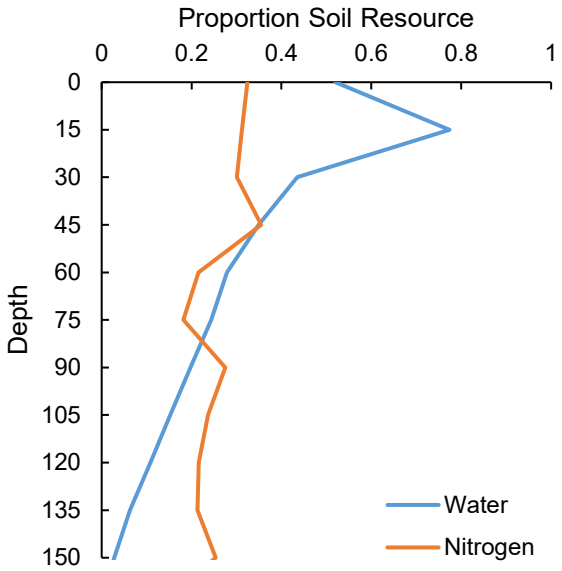

Figure 5-3. Proportion soil water and nitrogen by depth.

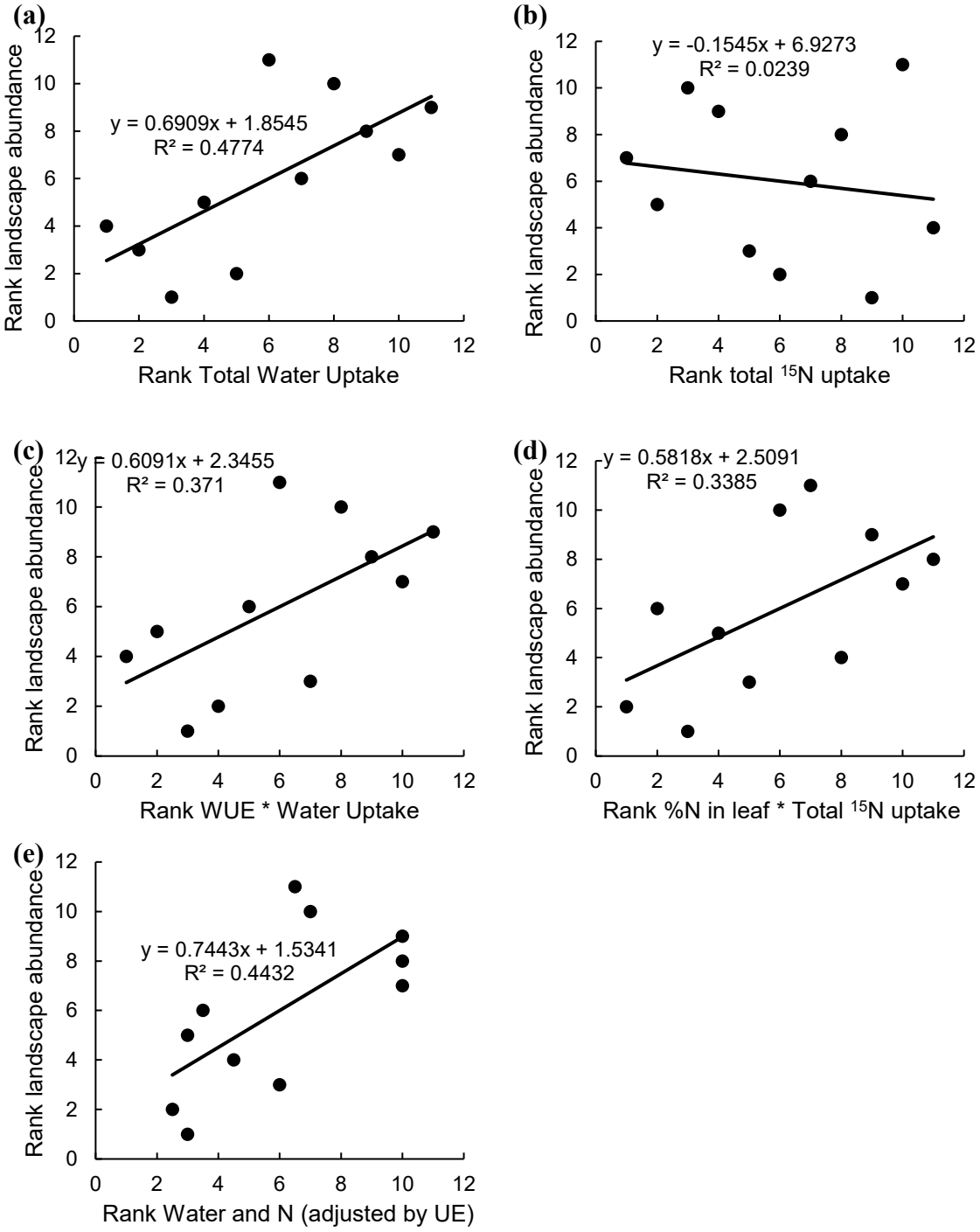

Figure 5-4. Rank total water uptake (a), rank ^{15}N uptake per centimeter of profile depth (b), rank total water uptake adjusted by water use efficiency rank (c), ^{15}N uptake per centimeter of profile depth adjusted by percent nitrogen in leaf (d), and rank percent nitrogen per gram of leaf matter adjusted by nitrogen use efficiency plus rank total water uptake adjusted by water use efficiency (e) versus rank landscape abundance.

CHAPTER 6

SUMMARY AND CONCLUSIONS

Despite decades of research, coexistence and the biodiversity-productivity relationship remain important unresolved phenomena in ecological research (Hooper & Vitousek 1997; Hooper *et al.* 2016). Recent research suggests that interactions between plants and their co-associated microbes may be a key potential mechanism of mediating plant coexistence and biodiversity-productivity relationships, however, niche partitioning of resources is another possible mechanism (Schnitzer *et al.* 2011; Eck *et al.* 2019, Barry *et al.* 2020). Because the extent to which plant-soil feedbacks and niche partitioning play roles in the biodiversity-productivity relationship remains a central unresolved ecological question, we performed a greenhouse study assessing the role of PSF in the biodiversity-productivity relationship in Chapter 2, compared greenhouse and field-measured PSF in Chapter 3, performed a field study assessing the role of PSF in the biodiversity-productivity relationship in Chapter 4, and measured niche partitioning within the same study system to quantify the role of niche differentiation in coexistence and biomass production in Chapter 5.

In Chapter 2, I demonstrated that PSFs improve predictions of biodiversity-productivity relationships using a controlled greenhouse experiment. In Chapter 3, I demonstrated that PSF measured in the greenhouse is not correlated with PSF measured in the field, and therefore it is likely that the biodiversity-productivity relationship as measured in the field will be best predicted by PSF measurements from the field. In Chapter 4, I demonstrated a role for PSFs, albeit weak, as a mechanism of the biodiversity-productivity relationship in the field. In Chapter 5, I investigated niche

partitioning as a mechanism affecting species productivity in the same system and demonstrated that strong vertical partitioning of nitrogen and water uptake is correlated with species productivity.

In Chapter 2, we measured 240 factorial PSFs for 16 species under greenhouse conditions. The average factorial PSF was -0.02 and the average absolute value of factorial PSF was 0.25; i.e. plant-associated soil microbes increased or decreased growth by 25% on average, but the net effect of all PSFs was a 2% decrease in plant growth. Using the soil-specific plant growths measured, we predicted diverse plant community growth for forty-nine unique plant communities with total species richness between one and 16 species. PSF-informed models predicted 24% more biomass in 16-species communities than in monocultures while Null models predicted 19% more biomass; we observed 33% more biomass in 16-species communities than in monocultures. Further, PSF models were able to predict 67% of the variation in total biomass production while Null models were only able to predict 58%, representing a 9% improvement. PSFs improved predictions of the biodiversity-productivity relationship's magnitude. In addition the mechanisms of the biodiversity-productivity relationship predicted by PSF-informed models were consistent with observed mechanisms of the biodiversity-productivity relationship.

Although Chapter 2 suggests that PSFs have a role to play in the biodiversity-productivity relationship, Chapter 3 illustrates that extending greenhouse PSF observations to plant community dynamics in the field is flawed. We compared PSF values measured in the greenhouse to PSF values measured in the field for 36 plant species across five different studies. These PSF values included 269 factorial PSF values

and 36 non-factorial PSF values. For both factorial and non-factorial PSF values, there was no significant correlation between greenhouse- and field-measured PSF at the soil, site, or species level. A major implication of this work is that greenhouse experiments are the most appropriate method for understanding plant dynamics in greenhouse conditions, and field experiments are the most appropriate method for understanding plant dynamics in the field. Thus, field experimentation is needed to understand the biodiversity-productivity relationship as observed in the field.

In Chapter 4, I explored the role of PSFs in the biodiversity-productivity relationship in a field experiment. We measured 240 factorial PSFs and determined that the average factorial PSF value was -0.10 and the average absolute value of PSF was 0.27, suggesting that plant-associated microbes increase or decrease growth on soils by 27%, but the average effect is a 10% decrease in growth. These values (-.10 and 0.27) were broadly consistent with the average factorial PSF value (-0.02) and average absolute value of PSF (0.27) from the greenhouse experiment, suggesting a similar role for PSF in the biodiversity-productivity relationship between the two experimental contexts. The biodiversity-productivity relationship observed in the field was large, with 185% overyielding due to complementarity. PSF-informed models predicted 27% overyielding due to complementarity while Null models predicted 17% overyielding due to selection effects. PSF-informed models were 5% more accurate in predicting the variation in productivity than Null models and were 10% more accurate in predicting the magnitude of overyielding than Null models. However, no model came near predicting the 185% overyielding ovserved. The mechanism of overyielding in PSF-informed models were also consistent with the mechanism of overyielding in the observed biodiversity-

productivity relationship. However, the majority of overyielding observed in the field could not be attributed to PSFs, suggesting a role for other mechanisms of complementarity like niche partitioning in the biodiversity-productivity relationship.

Although niche partitioning through different rooting patterns is frequently invoked as a mechanism of plant growth, productivity, and coexistence, measuring differences is rooting patterns remains difficult and is uncommonly done. In Chapter 5, I used a depth-specific dual-isotope tracer technique to investigate partitioning of belowground water and nitrogen resources for eleven dominant species in an ex-arable field converted to tallgrass prairie. Nine of the 11 species demonstrated unique depths at which they could extract either more soil water, more nitrogen, or both than other species, indicating that most species had unique niches. Further, nitrogen and water uptake profiles when adjusted by water-use and nitrogen-use efficiencies were correlated with species productivity on the landscape. Results suggest that niche partitioning plays an important role in species growth and productivity in multi-species communities.

The overall goal of this research was to quantify potential mechanisms of coexistence and the biodiversity-productivity relationship. We were able to demonstrate a role for PSFs in both the greenhouse (9% improvement in biomass predictions and 23% of the total complementarity observed) and the field (5% improvement in biomss predictions and 9% of the total complementarity observed), but ecologists should take caution when applying PSF findings in the greenhouse to plant community dynamics in the field. With PSFs explaining 23% of complementarity in the greenhouse and 9% in the field, 77-91% of overyielding due to complementarity remains unexplained. It is possible that our measurement of PSF as interaction in monoculture-conditioned soils may be

inappropriate, when we consider that PSFs in biodiversity experiments occur in soils conditioned by highly diverse plant communities. Alternately, it is possible that our measurements were appropriate, and PSFs are simply small in the field, and thus a small component of the biodiversity-productivity relationship. Although we were able to demonstrate an important role for spatial niche partitioning in species productivity in the same system, further experimentation on spatial niche partitioning as a driver of the biodiversity-productivity relationship is likely needed. It is possible and entirely likely that many mechanisms interact to produce a biodiversity-productivity relationship that is more than the sum of its constituent parts.

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APPENDICES

APPENDIX A

CHAPTER 2 SUPPLEMENTAL INFORMATION

Model details

We used discrete logistic growth models to predict plant community growth and the biodiversity-productivity relationship (Table A-1; Kulmatiski et al. 2011). These models use soil-specific plant biomass production observations to calculate growth rates; growth rates were derived from growth on other soils (Null model), or growth on all soil types (PSF model). Competition coefficients were assigned a value of '1', but each species could affect the growth of other species due to community-level carrying capacities. Each of these three models was run with five different carrying capacities: 1) the maximum observed growth in any plot in the community experiment, 2) the maximum mean observed growth in any community, 3) the maximum species-specific growth in community plots, 4) the maximum observed growth in any PSF plot, and 5) the maximum species-specific growth in any PSF plot. Model predictions for the PSF and Null models were calculated as the mean of the five simulations with different carrying capacities.

Plant growth rates were calculated from the initial seed mass (0.002 g) and final observed biomass of each species on each soil. For example, for a model with 52 time steps, the growth rate of species A on soil α, $\Gamma_{A\alpha} = \sqrt[52]{A\alpha/I}$, where Aα = the final biomass of plant A on soil α, and I = initial seed mass (Table 1). A different growth rate is calculated for each plant species on each species-conditioned soil (i.e., soil type):

$$\Gamma_{A_t} = \Gamma_{A\alpha}P\alpha + \Gamma_{A\beta}P\beta + \cdots + \Gamma_{A\iota}P$$

$$\Gamma_{B_t} = \Gamma_{B\alpha} P\alpha + \Gamma_{B\beta} P\beta + \cdots + \Gamma_{B\iota} P$$

$$\vdots$$

$$\Gamma_{I_t} = \Gamma_{I\alpha} P\alpha + \Gamma_{I\beta} P\beta + \cdots + \Gamma_{I\iota} P$$

Species-conditioned soils 'grow' as a function of plant biomass, plant species growth rates, and a conversion factor μ (Table A-1). Conversion factor μ was set to 5 to reflect the assumption that microbial communities grow faster than plants (Kulmatiski et al. 2011). Species-conditioned soil growth was modeled as $\alpha_{t+1} = \left(1 + \mu\Gamma_{A_t} A_t\right)\alpha_t$, $\beta_{t+1} = \left(1 + \mu\Gamma_{B_t} B_t\right)\beta_t$, …, $\iota_{t+1} = \left(1 + \mu\Gamma_{I_t} I_t\right)\iota_t$.

The proportion each conditioned soil type comprises of the total soil community can be described by $P_{\alpha_t} = \alpha_t/(\alpha_t + \beta_t + \cdots + \iota_t)$ (Table A-1). Plant growth rates are a function of the proportion of different conditioned soil types present. To prevent run-away growth, biomass is limited by a carrying capacity κ, which can be either unique to a species or to the community (Table A-1). Changes in each plant's biomass can be described as $A_{t+1} = A_t + \Gamma_{A_t}\left((\kappa - A_t)/\kappa\right)$, $B_{t+1} = B_t + \Gamma_{B_t}\left((\kappa - B_t)/\kappa\right)$, …, $I_{t+1} = I_t + \Gamma_{I_t}\left((\kappa - I_t)/\kappa\right)$.

Although the Null models are similar in their implementation, they do not incorporate growth on all conditioned soil types. For the self Null model, plant species biomass is a function of the average observed plant biomass across "other" soils, i.e. $A = f\left(\Gamma_{A_{other}}\right)$, $B = f\left(\Gamma_{B_{other}}\right)$, ..., $I = f\left(\Gamma_{I_{other}}\right)$ (Table A-1).

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Supplementary Tables

Table A-1. Parameter definitions for the PSF and Null models.

Parameter	Definition
A, B, …, I	Plant A through I
A_t, B_t, …, I_t	Biomass of plant A through I at time t
α, β, …, ι	Conditioned soil types α through ι, cultivated by plants A through I
Γ_{At}, Γ_{Bt}, … , Γ_{It}	Growth rate of plant A through I at time t
P_α, P_β,…,P_ι	Proportion of conditioned soil type α through ι
μ	Conversion factor
κ	Carrying capacity
A_{other}, B_{other}, …, I_{other}	Plant A through I's biomass on "other" soil
Aα, Aβ, …, Aι	Plant A through I's biomass on conditioned soil types α through ι
Bα, Bβ, …, Bι	
⋮	
Iα, Iβ, …, Iι	
$\Gamma_{A\alpha}$, $\Gamma_{A\beta}$, … , $\Gamma_{A\iota}$	Growth rates of species A through I on conditioned soil types α through ι
$\Gamma_{B\alpha}$, $\Gamma_{B\beta}$, … , $\Gamma_{B\iota}$	
⋮	
$\Gamma_{I\alpha}$, $\Gamma_{I\beta}$, … , $\Gamma_{I\iota}$	

Table A-2. Planting treatments for the 1997 biodiversity-productivity (BP) and the contemporary greenhouse (GH) experiment. The first digit indicates whether the species is planted (1) or not planted (0) in the BP_{1997} community. The second digit indicate whether a species is planted (1) or not planted (0) in the BP community. Thus, a 1 1 indicates that the species occurs in the mixture in both experiments, a 0 0 indicates that the species occurs in the mixture in neither experiment, a 1 0 indicates it was planted in the mixture in BP_{1997} but not in GH, and a 0 1 indicates it was planted in GH but not in BP_{1997}. Codes indicate the reason the community was changed from the original community in BP_{1997}. Code A indicates no difference between the BP_{1997} and GH communities. Code B indicates that *Dalea candida* and *Dalea villosa* were removed from the community when seeding GH plots. Code C indicates that woody species were removed from the community when seeding GH plots. Code D indicates *A. tuberosa* was removed from the community when seeding GH plots. For one community, after woody species and *A. tuberosa* were removed, the diversity would be 7 species, so *S. scoparium* was randomly selected to create a diversity of 8 species; this community is indicated by code CDE. Two novel communities, community A and community B were created specifically for the GH study because *M. fistulosa* and *S. rigida* co-occur in the intended monoculture plots in BP_{1997} due to a seeding error.

Com	Div. BP_{1997}	Div. GH	Am	Ac	Ag	At	Dc	Dv	Dp	Ec	Km	Lc	La	Lp	Mf	Pp	Ps	Pv	Qe	Qm	Ss	Sn	Sr	Code
2	1	1	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	1 1	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	A
5	1	1	0 0	0 0	1 1	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	A
6	2	2	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	1 1	0 0	0 0	1 1	0 0	0 0	A
11	1	1	1 1	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	A
12	10	8	1 1	0 0	0 0	0 0	**1 0**	**1 0**	0 0	0 0	1 1	0 0	0 0	1 1	1 1	1 1	0 0	0 0	0 0	0 0	1 1	1 1	1 1	B
20	1	1	0 0	1 1	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	A
26	4	4	0 0	0 0	0 0	0 0	0 0	0 0	1 1	1 1	0 0	0 0	0 0	0 0	0 0	0 0	1 1	0 0	0 0	0 0	0 0	1 1	0 0	A
31	1	1	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	1 1	0 0	0 0	A
48	2	2	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	1 1	1 1	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	A
56	2	2	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	1 1	0 0	0 0	0 0	0 0	0 0	0 0	1 1	0 0	0 0	A
57	10	8	1 1	0 0	0 0	0 0	**1 0**	**1 0**	0 0	0 0	1 1	1 1	0 0	0 0	1 1	1 1	1 1	0 0	0 0	0 0	1 1	0 0	1 1	B
81	8	8	1 1	0 0	0 0	0 0	0 0	0 0	1 1	1 1	0 0	1 1	1 1	0 0	0 0	1 1	1 1	0 0	0 0	0 0	1 1	0 0	0 0	A
83	1	1	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	1 1	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	A
87	1	**1**	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	1 1	0 0	A
93	4	4	0 0	0 0	0 0	0 0	0 0	0 0	1 1	0 0	1 1	0 0	0 0	1 1	0 0	0 0	1 1	0 0	0 0	0 0	0 0	0 0	0 0	A

110	4	4	0 0	0 0	0 0	0 0	0 0	0 0	0 0	1 1	0 0	1 1	0 0	1 1	0 0	0 0	0 0	1 1	0 0	0 0	0 0	0 0	0 0	A
115	9	8	0 0	0 0	1 1	**1 0**	0 0	0 0	0 0	1 1	1 1	0 0	0 0	0 0	1 1	1 1	1 1	0 0	**1 0**	0 0	**0 1**	0 0	1 1	CDE
125	2	2	0 0	0 0	0 0	0 0	0 0	0 0	1 1	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	1 1	0 0	0 0	A
129	1	1	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	1 1	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	A
138	4	4	1 1	0 0	0 0	0 0	0 0	0 0	0 0	1 1	1 1	0 0	1 1	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	A
139	4	4	0 0	0 0	0 0	0 0	0 0	0 0	0 0	1 1	1 1	1 1	0 0	0 0	0 0	0 0	1 1	0 0	0 0	0 0	0 0	0 0	0 0	A
142	1	1	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	1 1	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	A
168	2	2	0 0	0 0	1 1	0 0	0 0	0 0	0 0	0 0	1 1	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	A
171	2	2	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	1 1	0 0	0 0	1 1	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	A
175	2	2	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	1 1	0 0	0 0	0 0	0 0	0 0	0 0	0 0	1 1	0 0	A
176	4	4	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	1 1	0 0	0 0	1 1	1 1	1 1	0 0	0 0	0 0	0 0	0 0	A
178	9	8	1 1	0 0	0 0	0 0	0 0	0 0	0 0	1 1	1 1	0 0	10	0 0	1 1	0 0	1 1	1 1	0 0	0 0	1 1	0 0	1 1	A
185	1	1	0 0	0 0	0 0	0 0	0 0	0 0	1 1	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	A
193	2	2	0 0	0 0	1 1	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	1 1	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	A
202	17	16	1 1	1 1	1 1	**1 0**	0 0	0 0	1 1	1 1	1 1	1 1	1 1	1 1	1 1	1 1	1 1	1 1	0 0	0 0	1 1	1 1	1 1	D
206	9	8	0 0	0 0	1 1	**1 0**	0 0	0 0	0 0	0 0	0 0	1 1	0 0	1 1	1 1	1 1	1 1	0 0	0 0	0 0	0 0	1 1	1 1	D
208	8	8	1 1	0 0	0 0	0 0	0 0	0 0	0 0	0 0	1 1	1 1	0 0	1 1	0 0	1 1	1 1	1 1	0 0	0 0	1 1	0 0	0 0	A
210	8	8	1 1	0 0	0 0	0 0	0 0	0 0	0 0	1 1	1 1	1 1	1 1	1 1	0 0	1 1	0 0	0 0	0 0	0 0	1 1	0 0	0 0	A
224	2	2	1 1	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	1 1	0 0	A
229	4	4	0 0	0 0	1 1	0 0	0 0	0 0	1 1	0 0	0 0	0 0	0 0	0 0	0 0	1 1	0 0	0 0	0 0	0 0	1 1	0 0	0 0	A
230	1	1	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	1 1	0 0	0 0	0 0	0 0	0 0	A
234	2	2	0 0	0 0	0 0	0 0	0 0	0 0	0 0	1 1	0 0	0 0	0 0	1 1	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	A
237	1	1	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	1 1	0 0	0 0	0 0	0 0	0 0	0 0	0 0	A
256	1	1	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	1 1	0 0	0 0	0 0	0 0	0 0	0 0	A
286	4	4	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	1 1	0 0	0 0	0 0	1 1	0 0	0 0	0 0	0 0	1 1	1 1	0 0	A
290	2	2	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	1 1	0 0	0 0	0 0	0 0	0 0	0 0	0 0	1 1	A
300	2	2	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	1 1	0 0	0 0	0 0	1 1	0 0	0 0	0 0	0 0	0 0	A

302	5	4	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	1 1	1 1	1 1	0 0	0 0	0 0	0 0	**1 0**	0 0	0 0	1 1	C
303	10	8	1 1	0 0	0 0	0 0	**1 0**	**1 0**	0 0	0 0	1 1	0 0	1 1	1 1	1 1	1 1	1 1	0 0	0 0	0 0	0 0	0 0	1 1	A
311	2	2	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	1 1	0 0	0 0	0 0	0 0	0 0	0 0	1 1	0 0	0 0	0 0	0 0	0 0	D
330	2	2	0 0	0 0	1 1	0 0	0 0	0 0	0 0	0 0	0 0	0 0	1 1	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	A
333	1	1	0 0	0 0	0 0	0 0	0 0	0 0	0 0	1 1	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	A
A	None	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	
B	None	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	

Supplementary Figures

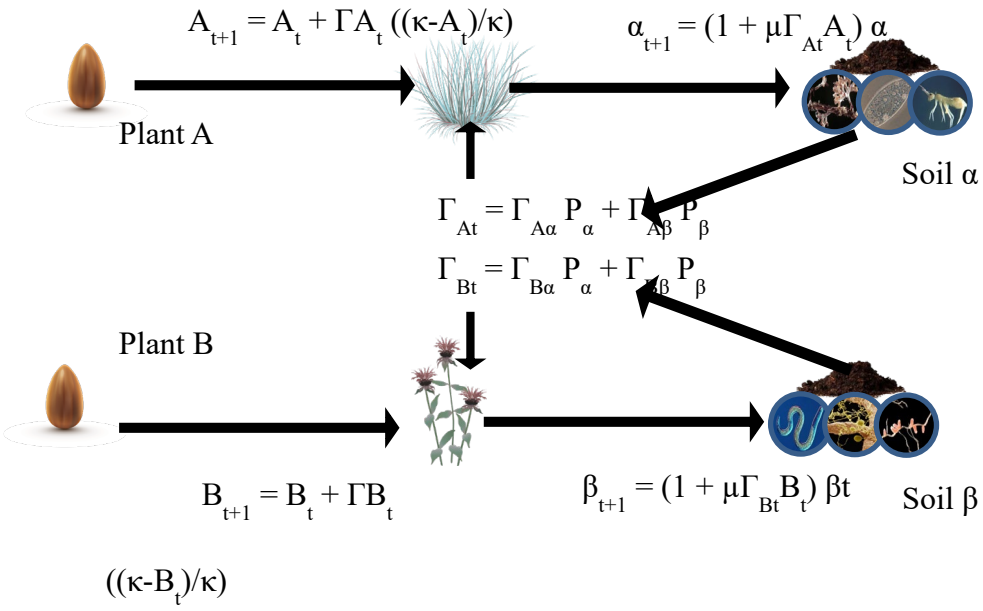
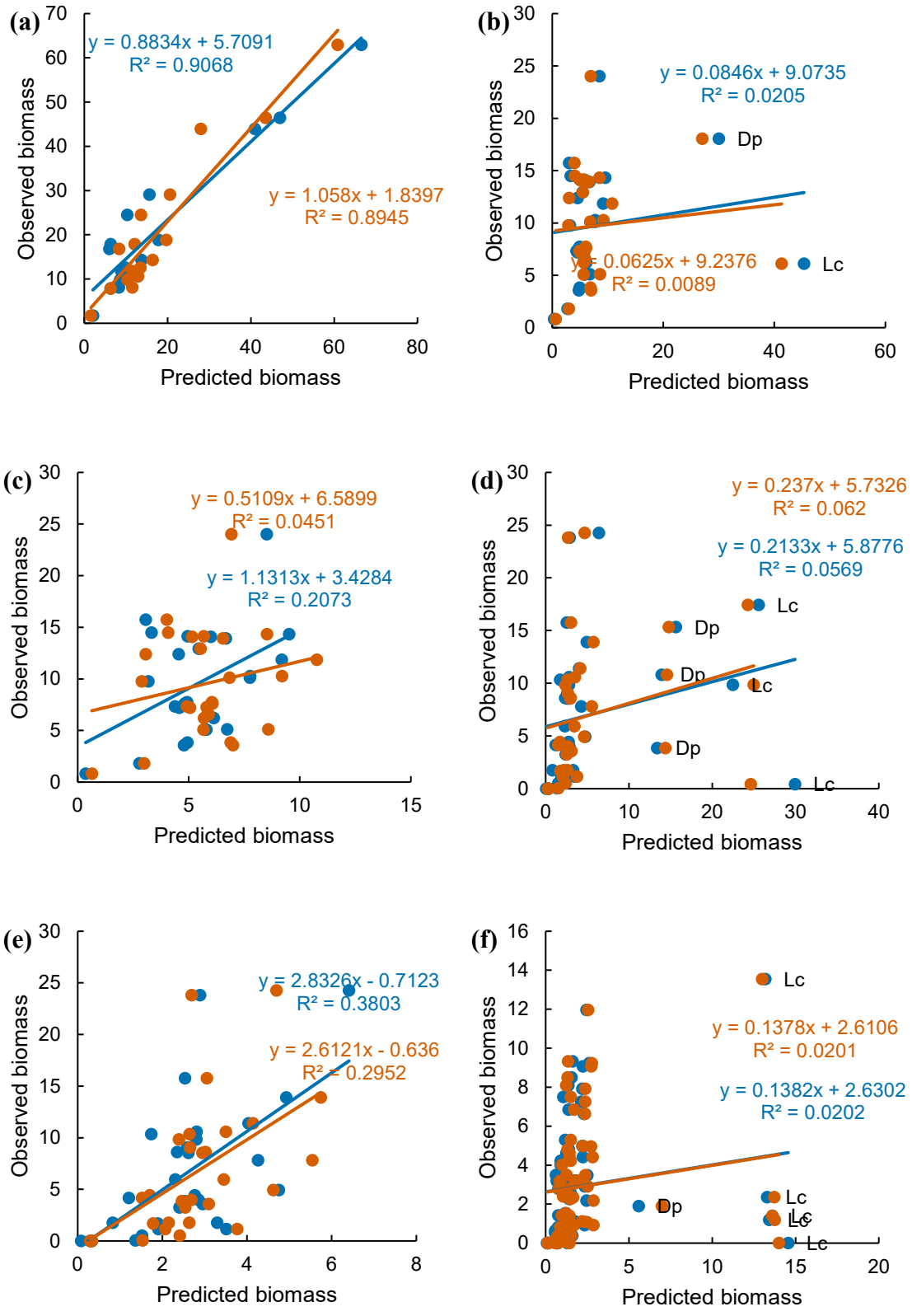


Figure A-1. An example of the PSF model in a community with two species.


(a)
y = 0.8834x + 5.7091
R² = 0.9068
y = 1.058x + 1.8397
R² = 0.8945
Observed biomass
Predicted biomass
(b)
y = 0.0846x + 9.0735
R² = 0.0205
Dp
y = 0.0625x + 9.2376
R² = 0.0089
Lc
Observed biomass
Predicted biomass
(c)
y = 0.5109x + 6.5899
R² = 0.0451
y = 1.1313x + 3.4284
R² = 0.2073
Observed biomass
Predicted biomass
(d)
y = 0.237x + 5.7326
R² = 0.062
y = 0.2133x + 5.8776
R² = 0.0569
Lc
Dp
Dp
Lc
Dp
Lc
Observed biomass
Predicted biomass
(e)
y = 2.8326x - 0.7123
R² = 0.3803
y = 2.6121x - 0.636
R² = 0.2952
Observed biomass
Predicted biomass
(f)
Lc
y = 0.1378x + 2.6106
R² = 0.0201
y = 0.1382x + 2.6302
R² = 0.0202
Dp
Lc
Lc
Lc
Observed biomass
Predicted biomass

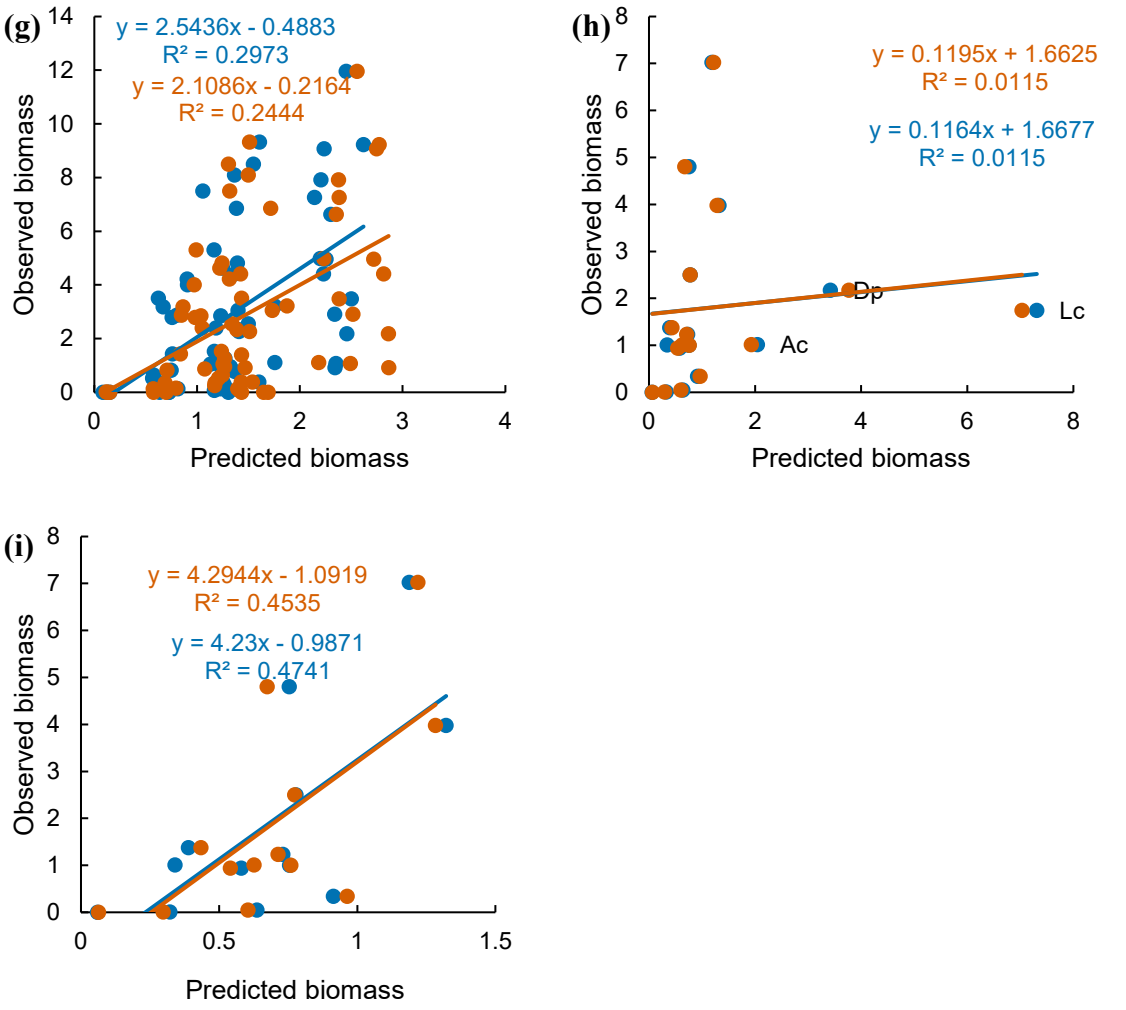


Figure A-2. Predicted species biomass (g per 12 L pot) from a PSF-informed model (blue) and models without PSF effects (orange) versus observed species biomass (g per 12 L pot) for a) monocultures, b) two-species communities and all species, c) two-species communities without *Dalea purpurea* and *Lespedeza capitata*, d) four-species communities and all species, e) four-species communities without *D. purpurea* and *L. capitata*, f) 8-species communities and all species, g) eight-species communities without *D. purpurea* and *L. capitata*, h) 16-species communities and all species, and i) 16-species communities without *D. purpurea*, *L. capitata*, and *A. canescens*. Note that *A. canescens* did not occur in two-, four-, and eight-species communities.

APPENDIX B

CHAPTER 3 SUPPLEMENTAL INFORMATION

SUPPLEMENTARY METHODS

Cedar Creek, Minnesota

At Cedar Creek Ecosystem Science Reserve, four C_3 grasses, four C_4 grasses, four leguminous forbs, and four non-leguminous forbs were selected to investigate based on functional group and abundance in plant communities at Cedar Creek: *Pascopyrum smithii* Rydb., *Poa pratensis* L., *Koeleria macrantha* (Ledeb.) Schultes, *Elymus canadensis* L., *Andropogon gerardii* Vitman, *Panicum virgatum* L., *Schizachyrium scoparium* (Michx.) Nash, *Sorghastrum nutans* (L.) Nash, *Amorpha canescens Pursh*, *Lupinus perennis* L., *Lespedeza capitata Michx.*, *Dalea purpurea* (Vent) Rydb., *Achillea millefolium* L., *Liatris aspera* Michx., *Solidago rigida* L., and *Monarda fistulosa* L. (Ownbey and Morley, 1991).

Field research was conducted at Cedar Creek Ecosystem Science Reserve in East Bethel, Minnesota (45.403290, -93.187411), on the Nymore series (mixed, frigid, Typic Udipsamment). Mean annual precipitation was 788.07 mm and mean annual temperature is 6.7° C. A 1750 m^2 fallow area adjacent to a large, long-term biodiversity experiment at Cedar Creek was sprayed with glyphosate and disked to thoroughly remove vegetation and homogenize soils in the top 15 cm. 0.75 mm thick HDPE root barrier was inserted to 35 cm deep between each plot. 2,720 0.75 by 0.35 m PSF plots were established. In spring 2015, all plots were seeded with 10 g pure live seed per m^2. During the first year, plots were watered weekly to ensure germination. To establish the phase 1 treatment,

each of the 16 target species were planted as monocultures and grown from 2015 to 2016. Non-target species were removed by hand-weeding. In 2016, fall PSF plots were sprayed with glyphosate and hand-tilled using a garden claw. Plots with exceptionally thick roots were tilled using a rototiller. In spring 2017, any living plants were sprayed again with glyphosate. 2,608 plots realized growth in phase I. Plots were replanted with either the same ("self" treatment) or a different ("other" treatment) species. Plots that did not realize growth were replanted randomly as a control soil treatment. Growth was estimated using percent cover in summer 2017, and twice in summer 2018. Plots were clipped, dried, and weighed in fall 2018.

The greenhouse experiment was implemented at the Utah State University Crop Physiology Lab in Logan, UT. Soil from an area adjacent to the field experiment was dried in a 31° C room, and shipped to Logan, UT. A 6:1 mixture of loamy sand and sphagnum peat from Miller Companies, LLC in Hyrum UT was steam sterilized, and inoculated with 10% field soil. 2,720 1-L pots were planted with four seedlings, and then thinned down two seedlings after a one-month period. Plants were grown for a 6 month period, then killed by clipping. 2,466 pots had growth in phase I, and all other pots were discarded. Pots that realized growth were replanted with either the same ("self" treatment) or a different ("other" treatment) species, and grown for a 6 month period. At the end of the experiment, aboveground biomass was clipped, dried, and weighed.

Jena, Germany

At Jena, Germany, nine plants were selected based on dominance in the local biodiversity experiment: *Arrhenatherum elatius* (L.) P.Beauv., *Anthriscus sylvestris* (L.)

Hoffm., *Dactylis glomerata* L., *Poa trivialis* L., *Geranium pratense* L., *Trifolium pratense* L., *Trifolium repens*. Of the nine, only five were successful in the greenhouse: *Arrhenatherum elatius*, *Dactylis glomerata*, *Poa trivialis*, *Trifolium pratense*, and *Trifolium repens* L. (Tutin et al., 1972).

Field research was conducted at the Jena Experiment in Jena, Germany on a eutric fluvisol on the bank of the Saale River (50.951951, 11.623832). Mean annual precipitation was 559 mm and mean annual temperature is 9.5° C. A 730 m^2 area in a fallowed field was sprayed with glyphosate and disked to thoroughly remove vegetation and homogenize soils in the top 15 cm. 0.75 mm thick HDPE root barrier was inserted to 35 cm deep between each plot. 1251 0.75 by 0.35 m PSF plots were established in the area. In spring 2015, all plots were seeded with 2000 pure live seeds per m^2. During the first year, plots received water twice weekly to ensure germination. To establish the phase 1 treatment, each of the nine target species were planted as monocultures and grown from 2015 to 2016. Non-target species were removed by hand-weeding. In 2016, fall PSF plots were sprayed with glyphosate and hand-tilled using a garden claw. To prevent regrowth roots of phase 1 species were removed. Plots were replanted with 2000 pure live seeds per square meter with either the same ("self" treatment) or a different ("other" treatment) species. Growth was estimated using percent cover in fall 2017 and fall 2018. Plots were clipped, dried, sorted, and weighed in summer 2018.

The greenhouse experiment was implemented at the Halle Institute of Biology/Geobotany and Botanical Garden. Soil from an area adjacent to the field experiment was dried and transported to Halle. A 3:1 mixture of compost and sand from SWH Stadtwerke Halle in Halle DE was inoculated with 10% field soil. 650 1-L pots

were planted with 1 seedling each and grown for an eight month period. Phase 1 plants were then killed by clipping, and hand-weeding if needed. 475 pots had growth in phase I, and all other pots were discarded. Pots that realized growth were replanted with either the same ("self" treatment) or a different ("other" treatment) species. At the end of the experiment, aboveground biomass was clipped, dried, and weighed.

REFERENCES

Ownbey, G. B., and Morley, T. (1991). *Vascular Plants of Minnesota: A Checklist and Atlas*. Minneapolis, UNITED STATES: University of Minnesota Press Available at: http://ebookcentral.proquest.com/lib/usu/detail.action?docID=3039160 [Accessed February 27, 2019].

Tutin, T. G., Heywood, V. H., Burges, N. A., Valentine, D. H., Ball, P. W., Walters, S. M., et al. (1972). *Flora Europaea*. Cambridge University Press.

Supplementary Tables

Table B-1. Species for paired experiments.

Species	Growth Form	Origin	Experiment
Achillea millefolium	Forb	native	Berlin, DE
			Cedar Creek, MN, US
Amorpha canescens	C3	native	Cedar Creek, MN, US
Andropogon gerardii	Legume	native	Cedar Creek, MN, US
Anthoxanthum odoratum	C3	native	Potsdam, DE
Arrhenatherum elatius	C3	native	Jena, DE
			Potsdam, BB, DE
Bromus tectorum	C3	invasive	Winthrop, WA, US
Capsella bursa-pastoris	Forb	native	Berlin, DE
Centaurea diffusa	Forb	invasive	Winthrop, WA, US
Cichorium intybus	Forb	native	Berlin, DE
Dactylis glomerata	C3	native	Jena, DE
Dalea purpurea	C4	native	Cedar Creek, MN, US
Elymus canadensis	C3	native	Cedar Creek, MN, US
Festuca idahoensis	C3	native	Winthrop, WA, USA
Holcus lanatus	C3	native	Potsdam, DE
Koeleria macrantha	C3	native	Cedar Creek, MN, US
			Winthrop, WA, US
Lactuca serriola	Forb	invasive	Winthrop, WA, US
Lespedeza capitata	Legume	native	Cedar Creek, MN, US
Liatris aspera	Forb	native	Cedar Creek, MN, US
Lupinus perennis	Legume	native	Cedar Creek, MN, US
Medicago x varia	Legume	invasive	Berlin, DE
Monarda fistulosa	Forb	native	Cedar Creek, MN, US
Panicum virgatum	C4	native	Cedar Creek, MN, US
Pascopyrum smithii	Legume	native	Cedar Creek, MN, US
Poa pratensis	C3	invasive	Cedar Creek, MN, US
Poa trivialis	C3	native	Jena, DE
Pseudoregneria spicata	C3	native	Winthrop, WA, US
Schizachyrium scoparium	C4	native	Cedar Creek, MN, US
Sisymbrium loeselii	Forb	native	Berlin, DE
		invasive	Winthrop, WA, US
Solidago canadensis	Forb	invasive	Berlin, DE
Solidago rigida	Forb	native	Cedar Creek, MN, US
Sorghastrum nutans	C4	native	Cedar Creek, MN, US
Tanacetum vulgare	Forb	native	Berlin, DE
Tragopogon dubius	Forb	invasive	Winthrop, WA, US
Trifolium pratense	Legume	native	Jena, DE

Species	Growth Form	Origin	Experiment
Trifolium repens	Legume	native	Berlin, DE Jena, DE

Table B-2. Locales, climate conditions, soil information, and site-use history for the field experiments.

	Berlin	Cedar Creek	Jena	Potsdam	Winthrop
Species	8	16	5	3	4
Location	52.475° N, 13.362° E	45.403° N, 93.187°	50.952° N, 11.623° E	52.408° N, 13.020°	48.481° N, 120.117° W
Köppen Climate Classification	Oceanic climate (Cfb)	Hot summer continental (Dfa)	Oceanic climate (Cfb)	Oceanic climate (Cfb)	Warm summer continental (Dfb)
MAT	9.1 °C	6.5° C	8.6 °C	9.2 °C	6.9 °C
MAP	570 mm	753 mm	565 mm	566 mm	395 mm
Soil	loamy sand	loamy sand	silty clay	slightly loamy sand	Gravelly loam
Field site history	Abandoned urban site	Managed tallgrass prairie	Managed meadow	Managed meadow	ex-arable alfalfa field
Greenhouse experiment location	Freie Universität Berlin, Germany	Crop Physiology Laboratory in Logan, UT, USA	Botanical Gardens of Martin-Luther University in Halle (Saale), Germany.	University of Potsdam, Germany	USDA Forage and Range Research greenhouse in Logan, UT, USA

APPENDIX C

CHAPTER 3 SUPPLEMENTAL INFORMATION

Model Details

Plant species biomass in communities was predicted using logistic growth models (Table C-1; Kulmatiski et al. 2011). Species growth rates were derived from a) growth on control soils (control Null model), b) growth on 'self' soils (self Null model), or c) growth on all soil types (PSF model). Competition coefficients were assigned a value of '1', but each species could affect the growth of other species due to community-level carrying capacities. Each of these three models was run with five different carrying capacities: 1) the maximum observed growth in any plot in the community experiment, 2) the maximum mean observed growth in any community, 3) the maximum species-specific growth in community plots, 4) the maximum observed growth in any PSF plot, and 5) the maximum species-specific growth in any PSF plot. Mean Null model predictions of community biomass were calculated from the 10 model simulations (Control Null, Self Null each with five carrying capacities. Mean PSF model predictions were calculated from the five simulations with different carrying capacities.

Plant growth rates were calculated from the initial seed mass (0.002 g) and final observed biomass of each species on each soil. For example, for a model with 52 time steps, the growth rate of species A on soil α, $\Gamma_{A\alpha} = \sqrt[52]{A\alpha/I}$, where Aα = the final biomass of plant A on soil α, and I = initial seed mass (Table C-1). A different growth rate is calculated for each plant species on each species-conditioned soil (i.e., soil type):

$$\Gamma_{A_t} = \Gamma_{A\alpha}P\alpha + \Gamma_{A\beta}P\beta + \cdots + \Gamma_{A\iota}P$$

$$\Gamma_{B_t} = \Gamma_{B\alpha} P\alpha + \Gamma_{B\beta} P\beta + \cdots + \Gamma_{B\iota} P$$

$$\vdots$$

$$\Gamma_{I_t} = \Gamma_{I\alpha} P\alpha + \Gamma_{I\beta} P\beta + \cdots + \Gamma_{I\iota} P$$

Species-conditioned soils 'grow' as a function of plant biomass, plant species growth rates, and a conversion factor μ (Table C-1). Conversion factor μ was set to 5 to reflect the assumption that microbial communities grow faster than plants (Kulmatiski et al. 2011). Species-conditioned soil growth was modeled as $\alpha_{t+1} = \left(1 + \mu\Gamma_{A_t} A_t\right)\alpha_t$, $\beta_{t+1} = \left(1 + \mu\Gamma_{B_t} B_t\right)\beta_t$, …, $\iota_{t+1} = \left(1 + \mu\Gamma_{I_t} I_t\right)\iota_t$.

The proportion each conditioned soil type comprises of the total soil community can be described by $P_{\alpha_t} = \alpha_t/(\alpha_t + \beta_t + \cdots + \iota_t)$ (Table C-1). Plant growth rates are a function of the proportion of different conditioned soil types present. To prevent run-away growth, biomass is limited by a carrying capacity κ, which can be either unique to a species or to the community (Table 1). Changes in each plant's biomass can be described as $A_{t+1} = A_t + \Gamma_{A_t}\left((\kappa - A_t)/\kappa\right)$, $B_{t+1} = B_t + \Gamma_{B_t}\left((\kappa - B_t)/\kappa\right)$, …, $I_{t+1} = I_t + \Gamma_{I_t}\left((\kappa - I_t)/\kappa\right)$.

Although the Null models are similar in their implementation, they do not incorporate growth on all conditioned soil types. For the self Null model, plant species biomass is a function of observed plant biomass on "self" soil only, i.e. $A = f(\Gamma_{A\alpha})$, $B = f\left(\Gamma_{B\beta}\right)$, …, $I = f(\Gamma_{I\iota})$ (Table 1). For the control Null model, plant species biomass is a function of observed plant biomass on unconditioned control soils only, i.e. $A = f\left(\Gamma_{A_{cntl}}\right)$, $B = f\left(\Gamma_{B_{cntl}}\right)$, ..., $I = f\left(\Gamma_{I_{cntl}}\right)$ (Table C-1).

Because growth rates were derived from the second year of growth, we assumed

that growth rates represented two years of growth. To simulate the four years of growth in the biodiversity-productivity experiment, model simulations were executed for 52 timesteps, after which plant biomass was reduced to 1% of the previous timestep and allowed to run for another 52 timesteps. Mean model output for the sum of species growth from the suite of Null or PSF model simulations are reported.

References

Kulmatiski, A., Heavilin, J. & Beard, K.H. (2011). Testing predictions of a three-species plant–soil feedback model. *J. Ecol.*, 99, 542–550.

Supplementary Tables

Table C-1. Parameter definitions for the PSF and Null models.

Parameter	Definition
A, B, …, I	Plant A through I
A_t, B_t, …, I_t	Biomass of plant A through I at time t
α, β, …, ι	Conditioned soil types α through ι, cultivated by plants A through I
Γ_{At}, Γ_{Bt}, … , Γ_{It}	Growth rate of plant A through I at time t
P_α, P_β,…,P_ι	Proportion of conditioned soil type α through ι
μ	Conversion factor
κ	Carrying capacity
A_{cntl}, B_{cntl}, …, I_{cntl}	Plant A through I's biomass on unconditioned soil
Aα, Aβ, …, Aι	Plant A through I's biomass on
Bα, Bβ, …, Bι	conditioned soil types α through ι
⋮	
Iα, Iβ, …, Iι	
$\Gamma_{A\alpha}$, $\Gamma_{A\beta}$, … , $\Gamma_{A\iota}$	Growth rates of species A through I on
$\Gamma_{B\alpha}$, $\Gamma_{B\beta}$, … , $\Gamma_{B\iota}$	conditioned soil types α through ι
⋮	
$\Gamma_{I\alpha}$, $\Gamma_{I\beta}$, … , $\Gamma_{I\iota}$	

Table C-2. Treatments and sample sizes for the plant-soil feedback experiment. Each Phase I conditioned soil type had between five and nine 'control' plots, between 27 and 30 'self' plots, and between five and nine 'other' plots per Phase II species for a total of between 75 and 135 'other' plots. 112 plots that did not have seeded species growth in Phase I, i.e. Phase I control treatment, were seeded with either one of the sixteen target species (N = 96) or left unseeded (N = 16).

Phase I	Phase II	N
Ac	Control	5
Ac	Self	34
Ac	Other	120
Ag	Control	5
Ag	Self	30
Ag	Other	135
Am	Control	5
Am	Self	30
Am	Other	135
Control	Self	16
Control	Other	96
Dp	Control	5
Dp	Self	30
Dp	Other	135
Ec	Control	5
Ec	Self	30
Ec	Other	135
Km	Control	5
Km	Self	30
Km	Other	135
La	Control	5
La	Self	34
La	Other	120
Lc	Control	5
Lc	Self	30
Lc	Other	135
Lp	Control	5
Lp	Self	30
Lp	Other	120
Mf	Control	5
Mf	Self	34
Mf	Other	120
Pp	Control	5
Pp	Self	28
Pp	Other	135

Phase I	Phase II	N
Ps	Control	5
Ps	Self	31
Ps	Other	135
Pv	Control	5
Pv	Self	30
Pv	Other	135
Sn	Control	5
Sn	Self	30
Sn	Other	135
Sr	Control	5
Sr	Self	27
Sr	Other	75
Ss	Control	5
Ss	Self	30
Ss	Other	135

Table C-3. Planting treatments for BP_{1997} and the BP experiment. The first digit indicates whether the species is planted (1) or not planted (0) in the BP_{1997} community. The second digit indicate whether a species is planted (1) or not planted (0) in the BP community. Thus, a 1 1 indicates that the species occurs in the mixture in both experiments, a 0 0 indicates that the species occurs in the mixture in neither experiment, a 1 0 indicates it was planted in the mixture in BP_{1997}but not in BP, and a 0 1 indicates it was planted in BP but not in BP_{1997}. Codes indicate the reason the community was changed from the original community in BP1997. Code A indicates no difference between the BP_{1997} and BP communities. Code B indicates that *Dalea candida* and *Dalea villosa* were removed from the community when seeding BP plots. Code C indicates that woody species were removed from the community when seeding BP plots. Code D indicates *A. tuberosa* was removed from the community when seeding BP plots. For one community, after woody species and *A. tuberosa* were removed, the diversity would be 7 species, so *S. scoparium* was randomly selected to create a diversity of 8 species; this community is indicated by code CDE. Two novel communities, community A and community B were created specifically for the BP study because *M. fistulosa* and *S. rigida* co-occur in the intended monoculture plots in BP_{1997} due to a seeding error. Changed planting treatments are bolded and occur in red.

Com	Div. BP_{1997}	Div. BP	Am	Ac	Ag	At	Dc	Dv	Dp	Ec	Km	Lc	La	Lp	Mf	Pp	Ps	Pv	Qe	Qm	Ss	Sn	Sr	Code
2	1	1	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	1 1	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	A
5	1	1	0 0	0 0	1 1	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	A
6	2	2	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	1 1	0 0	0 0	1 1	0 0	0 0	A
11	1	1	1 1	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	A
12	10	8	1 1	0 0	0 0	0 0	**1 0**	**1 0**	0 0	0 0	1 1	0 0	0 0	1 1	1 1	1 1	0 0	0 0	0 0	0 0	1 1	1 1	1 1	B
20	1	1	0 0	1 1	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	A
26	4	4	0 0	0 0	0 0	0 0	0 0	0 0	1 1	1 1	0 0	0 0	0 0	0 0	0 0	0 0	1 1	0 0	0 0	0 0	0 0	1 1	0 0	A
30	17	14	1 1	1 1	1 1	**1 0**	0 0	0 0	0 0	1 1	1 1	0 0	1 1	1 1	1 1	1 1	1 1	1 1	**1 0**	**1 0**	1 1	1 1	1 1	CD
31	1	1	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	1 1	0 0	0 0	A
34	17	14	1 1	1 1	1 1	**1 0**	0 0	0 0	1 1	1 1	1 1	0 0	0 0	1 1	1 1	1 1	1 1	1 1	**1 0**	**1 0**	1 1	1 1	1 1	CD
35	17	14	0 0	1 1	1 1	**1 0**	0 0	0 0	1 1	1 1	1 1	1 1	1 1	1 1	1 1	0 0	1 1	1 1	**1 0**	**1 0**	1 1	1 1	1 1	CD
46	17	14	1 1	1 1	1 1	**1 0**	0 0	0 0	1 1	1 1	1 1	0 0	1 1	1 1	1 1	1 1	1 1	1 1	**1 0**	**1 0**	1 1	0 0	1 1	CD
48	2	2	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	1 1	1 1	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	A
56	2	2	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	1 1	0 0	0 0	0 0	0 0	0 0	0 0	1 1	0 0	0 0	A
57	10	8	1 1	0 0	0 0	0 0	**1 0**	**1 0**	0 0	0 0	1 1	1 1	0 0	0 0	1 1	1 1	1 1	0 0	0 0	0 0	1 1	0 0	1 1	B

Com	Div. BP_{1997}	Div. BP	Am	Ac	Ag	At	Dc	Dv	Dp	Ec	Km	Lc	La	Lp	Mf	Pp	Ps	Pv	Qe	Qm	Ss	Sn	Sr	Code
68	17	14	1 1	0 0	0 0	**1 0**	0 0	0 0	1 1	1 1	1 1	1 1	1 1	1 1	1 1	1 1	1 1	1 1	**1 0**	**1 0**	1 1	1 1	1 1	CD
81	8	8	1 1	0 0	0 0	0 0	0 0	0 0	1 1	1 1	0 0	1 1	1 1	0 0	0 0	1 1	1 1	0 0	0 0	0 0	1 1	0 0	0 0	A
83	1	1	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	1 1	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	A
87	1	**1**	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	1 1	0 0	A
93	4	4	0 0	0 0	0 0	0 0	0 0	0 0	1 1	0 0	1 1	0 0	0 0	1 1	0 0	0 0	1 1	0 0	0 0	0 0	0 0	0 0	0 0	A
107	17	14	0 0	1 1	1 1	**1 0**	0 0	0 0	1 1	1 1	1 1	1 1	1 1	1 1	1 1	1 1	1 1	1 1	**1 0**	**1 0**	1 1	0 0	1 1	CD
110	4	4	0 0	0 0	0 0	0 0	0 0	0 0	0 0	1 1	0 0	1 1	0 0	1 1	0 0	0 0	0 0	1 1	0 0	0 0	0 0	0 0	0 0	A
115	9	8	0 0	0 0	1 1	**1 0**	0 0	0 0	0 0	1 1	1 1	0 0	0 0	0 0	1 1	1 1	1 1	0 0	**1 0**	0 0	**0 1**	0 0	1 1	CDE
125	2	2	0 0	0 0	0 0	0 0	0 0	0 0	1 1	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	1 1	0 0	0 0	A
129	1	1	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	1 1	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	A
136	17	14	1 1	1 1	1 1	**1 0**	0 0	0 0	1 1	1 1	0 0	1 1	1 1	1 1	1 1	0 0	1 1	1 1	**1 0**	**1 0**	1 1	1 1	1 1	CD
138	4	4	1 1	0 0	0 0	0 0	0 0	0 0	0 0	1 1	1 1	0 0	1 1	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	A
139	4	4	0 0	0 0	0 0	0 0	0 0	0 0	0 0	1 1	1 1	1 1	0 0	0 0	0 0	0 0	1 1	0 0	0 0	0 0	0 0	0 0	0 0	A
142	1	1	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	1 1	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	A
156	17	14	1 1	1 1	1 1	**1 0**	0 0	0 0	1 1	1 1	1 1	1 1	1 1	0 0	1 1	0 0	1 1	1 1	**1 0**	**1 0**	1 1	1 1	1 1	CD
160	17	14	1 1	1 1	1 1	**1 0**	0 0	0 0	1 1	0 0	1 1	1 1	1 1	1 1	1 1	0 0	1 1	1 1	**1 0**	**1 0**	1 1	1 1	1 1	CD
168	2	2	0 0	0 0	1 1	0 0	0 0	0 0	0 0	0 0	1 1	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	A
171	2	2	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	1 1	0 0	0 0	1 1	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	A
175	2	2	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	1 1	0 0	0 0	0 0	0 0	0 0	0 0	0 0	1 1	0 0	A
176	4	4	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	1 1	0 0	0 0	1 1	1 1	1 1	0 0	0 0	0 0	0 0	0 0	A
178	9	8	1 1	0 0	0 0	0 0	0 0	0 0	0 0	1 1	1 1	0 0	10	0 0	1 1	0 0	1 1	1 1	0 0	0 0	1 1	0 0	1 1	A
185	1	1	0 0	0 0	0 0	0 0	0 0	0 0	1 1	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	A
193	2	2	0 0	0 0	1 1	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	1 1	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	A
202	17	16	1 1	1 1	1 1	**1 0**	0 0	0 0	1 1	1 1	1 1	1 1	1 1	1 1	1 1	1 1	1 1	1 1	0 0	0 0	1 1	1 1	1 1	D
206	9	8	0 0	0 0	1 1	**1 0**	0 0	0 0	0 0	0 0	0 0	1 1	0 0	1 1	1 1	1 1	1 1	0 0	0 0	0 0	0 0	1 1	1 1	D

Com	Div. BP_{1997}	Div. BP	Am	Ac	Ag	At	Dc	Dv	Dp	Ec	Km	Lc	La	Lp	Mf	Pp	Ps	Pv	Qe	Qm	Ss	Sn	Sr	Code
208	8	8	1 1	0 0	0 0	0 0	0 0	0 0	0 0	0 0	1 1	1 1	0 0	1 1	0 0	1 1	1 1	1 1	0 0	0 0	1 1	0 0	0 0	A
210	8	8	1 1	0 0	0 0	0 0	0 0	0 0	0 0	1 1	1 1	1 1	1 1	1 1	0 0	1 1	0 0	0 0	0 0	0 0	1 1	0 0	0 0	A
220	17	14	1 1	1 1	1 1	**1 0**	0 0	0 0	1 1	0 0	1 1	1 1	0 0	1 1	1 1	1 1	1 1	1 1	**1 0**	**1 0**	1 1	1 1	1 1	CD
224	2	2	1 1	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	1 1	0 0	A
229	4	4	0 0	0 0	1 1	0 0	0 0	0 0	1 1	0 0	0 0	0 0	0 0	0 0	0 0	1 1	0 0	0 0	0 0	0 0	1 1	0 0	0 0	A
230	1	1	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	1 1	0 0	0 0	0 0	0 0	0 0	A
234	2	2	0 0	0 0	0 0	0 0	0 0	0 0	0 0	1 1	0 0	0 0	0 0	1 1	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	A
237	1	1	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	1 1	0 0	0 0	0 0	0 0	0 0	0 0	0 0	A
239	17	14	1 1	1 1	1 1	**1 0**	0 0	0 0	1 1	1 1	1 1	1 1	0 0	1 1	1 1	1 1	1 1	0 0	**1 0**	**1 0**	1 1	1 1	1 1	CD
242	17	14	0 0	1 1	1 1	**1 0**	0 0	0 0	1 1	1 1	1 1	0 0	1 1	1 1	1 1	1 1	1 1	1 1	**1 0**	**1 0**	1 1	1 1	1 1	CD
256	1	1	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	1 1	0 0	0 0	0 0	0 0	0 0	0 0	A
273	17	14	1 1	0 0	1 1	**1 0**	0 0	0 0	1 1	1 1	1 1	1 1	1 1	1 1	1 1	1 1	0 0	1 1	**1 0**	**1 0**	1 1	1 1	1 1	CD
286	4	4	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	1 1	0 0	0 0	0 0	1 1	0 0	0 0	0 0	0 0	1 1	1 1	0 0	A
290	2	2	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	1 1	0 0	0 0	0 0	0 0	0 0	0 0	0 0	1 1	A
300	2	2	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	1 1	0 0	0 0	0 0	1 1	0 0	0 0	0 0	0 0	0 0	A
302	5	4	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	1 1	1 1	1 1	0 0	0 0	0 0	0 0	**1 0**	0 0	0 0	1 1	C
303	10	8	1 1	0 0	0 0	0 0	**1 0**	**1 0**	0 0	0 0	1 1	0 0	1 1	1 1	1 1	1 1	1 1	0 0	0 0	0 0	0 0	0 0	1 1	A
311	2	2	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	1 1	0 0	0 0	0 0	0 0	0 0	0 0	1 1	0 0	0 0	0 0	0 0	0 0	D
330	2	2	0 0	0 0	1 1	0 0	0 0	0 0	0 0	0 0	0 0	0 0	1 1	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	A
333	1	1	0 0	0 0	0 0	0 0	0 0	0 0	0 0	1 1	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	A
336	17	14	1 1	1 1	1 1	**1 0**	0 0	0 0	0 0	1 1	1 1	1 1	1 1	1 1	1 1	0 0	1 1	1 1	**1 0**	**1 0**	1 1	1 1	1 1	CD
A	None	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	
B	None	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	

Supplementary Figures

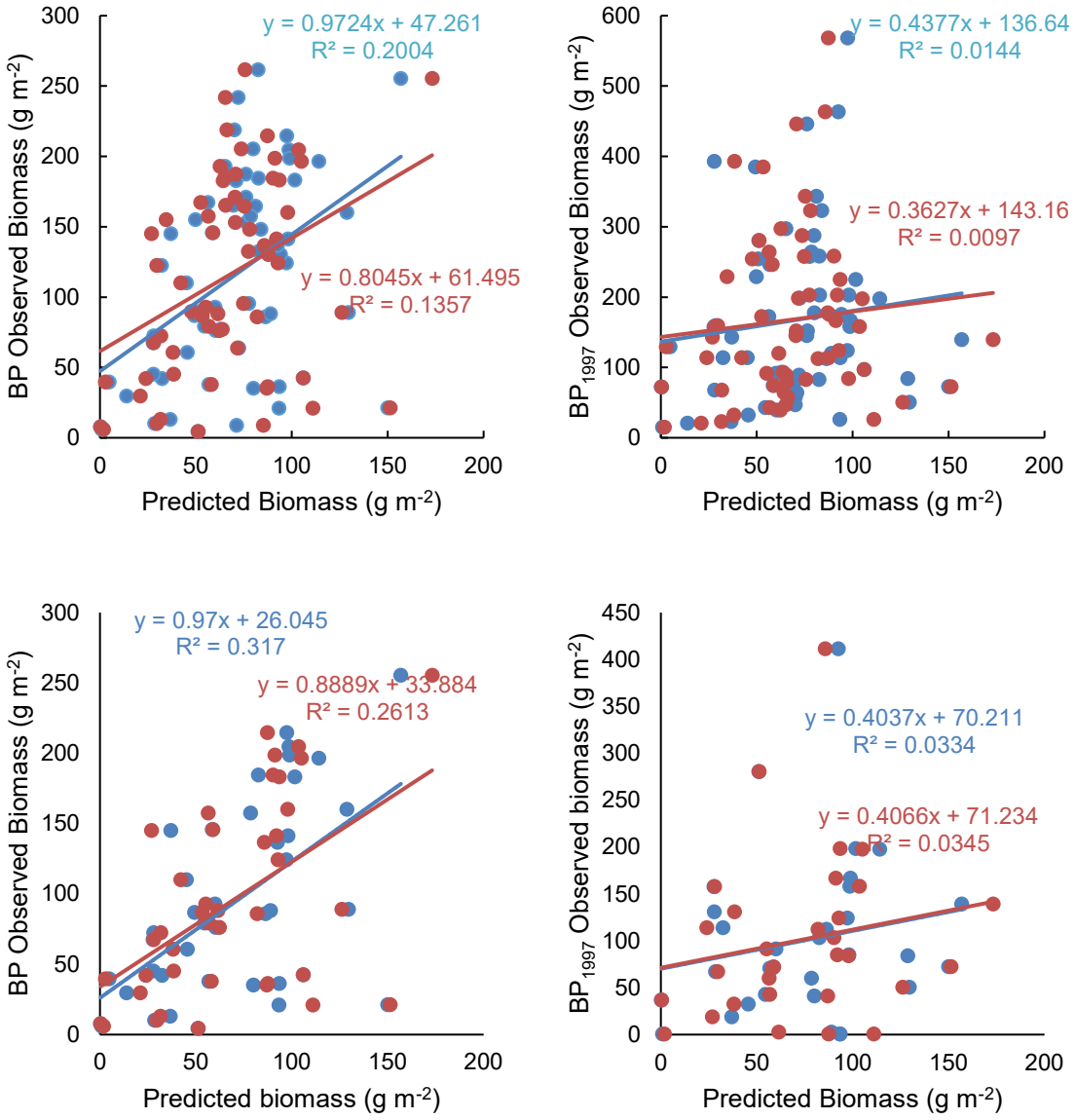


Figure C-1. Observed versus predicted biomasses between the two biodiversity-productivity experiments. The BP experiment (a, c) showed a stronger correlation between PSF-informed model predictions (blue) and observed biomass than null models (orange). Model predictions did not correlate with BP_{1997} experiment (b, d) observations. Results did not differ between models run with all 63 communities (a, b), and models run with only the 40 communities with identical composition in BP and BP_{1997} (c, d).

APPENDIX D

CHAPTER 4 SUPPLEMENTAL INFORMATION

Supplementary Data

Generalized Additive Mixed Models

To approximate the continuous soil profiles of root biomass and tracer uptake with depth we fit generalized additive mixed effects models (GAMMs) for water and ^{15}N uptake (Wood 2012; Kulmatiski et al. 2017). We let the GAMMs have four "knots" to allow for a smooth interpolation between the five sample depths. We fit nested subsets of the mixed models with different groupings of species-level tracer uptake, which define each model's random effects structure. Groupings included 1) an ungrouped "global" model, 2) a model grouped by C3 grasses, C4 grasses, forbs, and ferns, 3) a model grouped by grasses, forbs, and ferns, 4) a model grouped by grasses and forbs/ferns, and 5) a model grouped by species. We fit models with group level intercepts and slopes (the "effect" of soil depth). All models were fit in R (R Core Research Team 2004) using the gam function from the mgcv package (Metadata S1; Data S1; Wood 2012). We used Akaikie's Information Criterion (AIC) to rank models in terms of their support by the data. The model with the lowest AIC is the best model in terms of predictive ability and in terms of support from the data. Likewise, for any given hypothesis, we can compare two of the models and assess their relative support. Simple regressions were used to compare observed vs. predicted plant absolute and rank abundance.

References

Grigal, D. F., L. M. Chamberlain, H. R. Finney, D. V. Wroblewski, and E. R. Gross. 1974. Soils of the Cedar Creek Natural History Area. University of Minnesota, Agricultural Experiment Station.

Supplementary tables and figures

Table D-1. Parameters and initial simulations for Hydrus 1-D runs.

Parameter	**Initial simulation**
Number of soil materials	10 (Appendix D Table 2)
Number of layers for mass balance	5
Decline from vertical axis (0 = no slope)	0.01
Soil depth (cm)	178
Minimum time step (days)	0.00024
Maximum time step (days)	72
Water content tolerance	0.001
Critical Stress Index for Water Uptake	1
S-Shape parameters (P50, P3)	-15296, 3
HcritA (cm)	500000
Latitude (degrees)	45.397
Crop height (cm)	Appendix D Table 4
Leaf area index	1
Root depth (cm)	178
Radiation extinction	0.463
Meterological conditions	Hourly from 3/1/2016 to 9/30/2016 from on-site measurements
Soil profile	Appendix D Figure 2

Table D-2. Proportion sand, silt, and clay and bulk density in grams cm^{-3} for the Zimmmerman series at Cedar Creek, taken from Grigal et al. 1974.

Depth (cm)	Sand	Silt	Clay	Db (g cm^{-3})
0-18	93	3	4	1.3
18-71	95.5	2	2.5	1.55
71-91	90	4.5	5.5	1.65
91-115	93	4	3	1.6
115-120	88	2	10	1.7
120-132	95	2	3	1.6
132-137	91	1	8	1.7
137-155	98	0	2	1.6
155-160	94	0	6	1.6
160-178	97	1	2	1.6

Table D-3. Water Flow parameters.

Material	Qr	Qs	α (cm^{-1})	N	Ks (cm/hr)	I
1	0.0536	0.451	0.0366	2.6596	28.2792	0.5
2	0.0529	0.3723	0.0313	3.5442	31.4137	0.5
3	0.0496	0.3453	0.0338	2.4319	9.52708	0.5
4	0.0499	0.3574	0.0333	3.0469	19.4613	0.5
5	0.0537	0.3349	0.0303	1.9815	4.52417	0.5
6	0.0522	0.3575	0.0314	3.3392	25.5608	0.5
7	0.0538	0.3327	0.0299	2.3184	7.80833	0.5
8	0.0538	0.3561	0.0299	3.927	39.7817	0.5
9	0.0564	0.3611	0.0289	2.9432	18.7746	0.5
10	0.0527	0.3561	0.0308	3.7585	35.0737	0.5

Table D-4. Plant heights used to parameterize the Hydrus 1-D model.

Species	Height (cm)
Achmi	20
Andge	100
Ambco	30
Artlu	33
Elyre	40
Equsp	20
Erica	20
Panol	20
Poapr	40
Schsc	100
Sornu	100

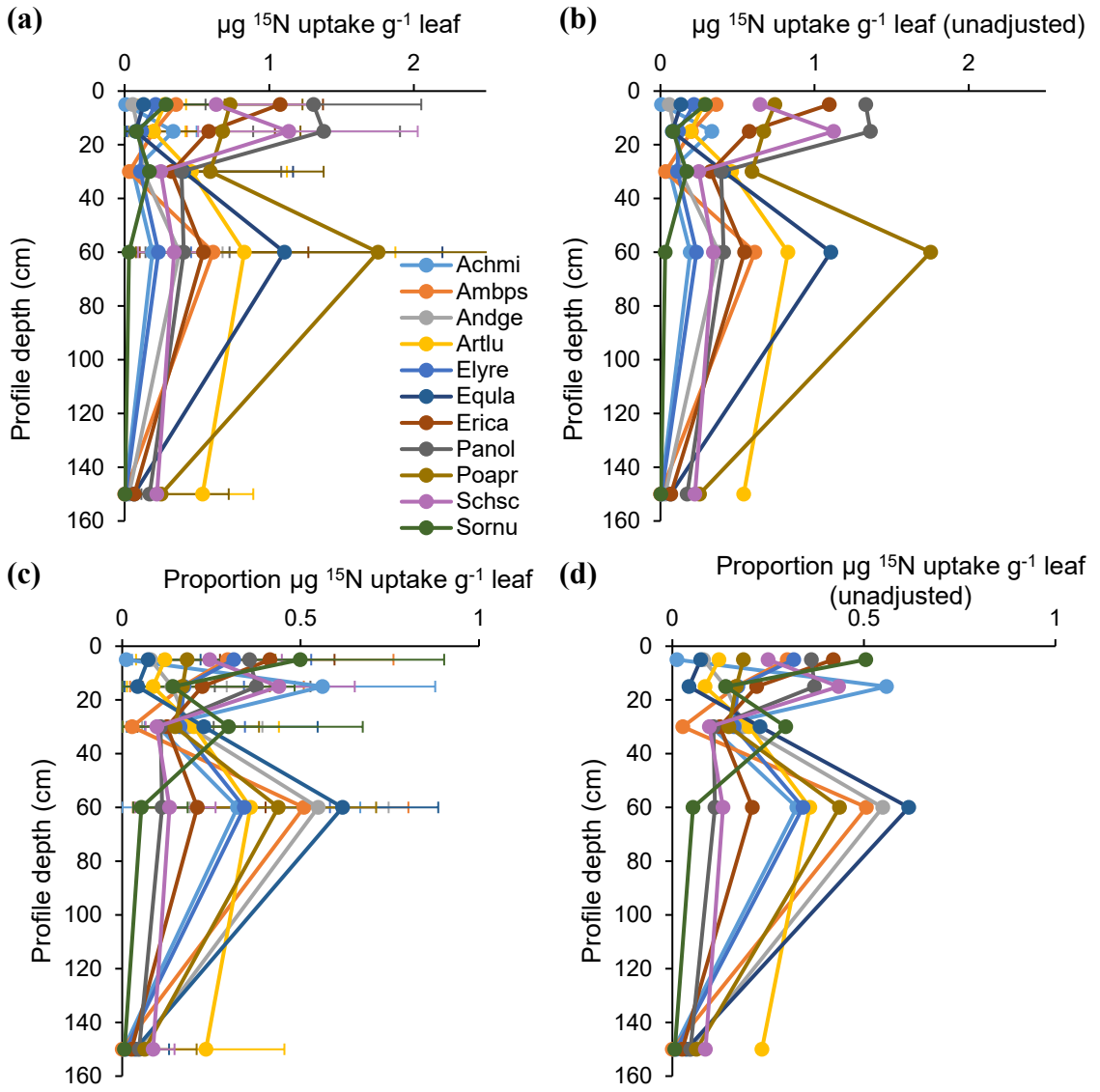


Figure D-1. Micrograms of ^{15}N uptake per gram of leaf matter (a, b) and proportion of ^{15}N taken up per gram of leaf matter for each depth for each species (c, d), either adjusted by background pool of nitrogen (a, c), or unadjusted by background pool of nitrogen (b, d).

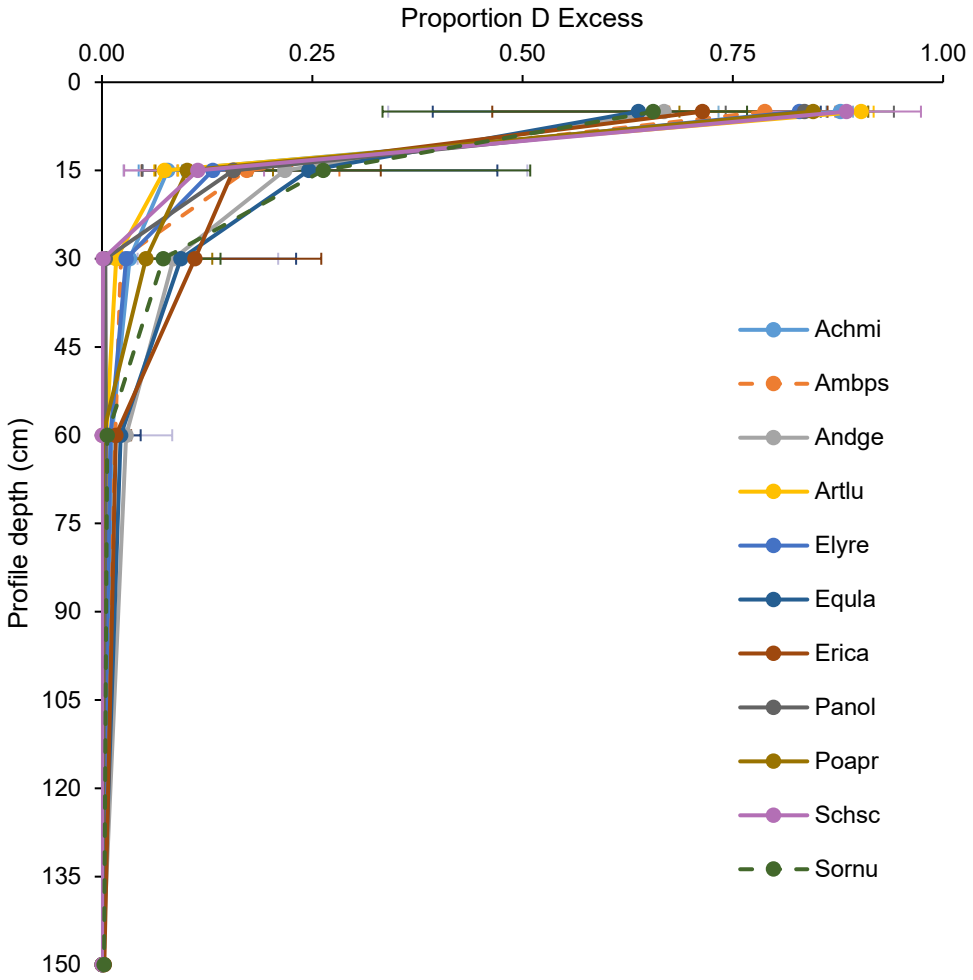


Figure D-2. Proportion of deuterium uptake by depth adjusted by centimeters in the profile depth range was used to parameterize Hydrus 1-D soil profiles.

APPENDIX E

AMPLICON ITS AND 16S RNA SEQ OF SOIL METAGENOME: QUANTIFYING PLANT-SOIL FEEDBACK EFFECTS IN CLASSIC DIVERSITY-PRODUCTIVITY EXPERIMENTS

ABSTRACT

Two-way interactions between plants and soil microbiota, also known as plant-soil feedbacks, have major effects on the productivity of plant species. This project tested the role of plant-soil feedbacks in plant community productivity across two sites, one at Cedar Creek Ecosystem Science Reserve in East Bethel, Minnesota, USA, and the other at the Jena Experiment in Jena, Thuringia, Germany. Soil metagenomic analyses were undertaken to help identify the specific microbes that drive feedbacks.

1. MATERIALS AND METHODS

1.1 Cedar Creek Plant-soil feedback experiment

A 1750 m^2 fallow area adjacent to a large, long-term biodiversity experiment at Cedar Creek was sprayed with glyphosate and disked to thoroughly remove vegetation and homogenize soils in the top 15 cm. 0.75 mm thick HDPE root barrier was inserted to 35 cm deep between each plot. 2,720 0.75 by 0.35 m PSF plots were established. In spring 2015, all plots were seeded with 10 g pure live seed per m^2. During the first year, plots were watered weekly to ensure germination. To establish the phase 1 treatment, each of the 16 target species were planted as monocultures and grown from 2015 to 2016. Non-target species were removed by hand-weeding. In 2016, fall PSF plots were sprayed with glyphosate and hand-tilled using a garden claw. Plots with exceptionally thick roots

were tilled using a rototiller. In spring 2017, any living plants were sprayed again with glyphosate. 2,608 plots realized growth in phase I. Plots were replanted with either the same ("self" treatment) or a different ("other" treatment) species. Plots that did not realize growth were replanted randomly as a control soil treatment. Yearly growth was recorded as ocular estimate of percent cover prior to soil sampling for metagenomic analyses.

1.2 Cedar Creek Plant-soil feedback experiment

On 7/20/2015, 7/15/2016, 10/30/2017, and 10/7/2018, a 15cm x 4cm core consisting of approximately 200 g of soil was taken from the center of three randomly-selected "self" plots and stored on ice. Soil corers were cleaned with 90% ethanol between samples. Samples were immediately transported to the University of Minnesota campus and stored at -80° C until DNA could be extracted. Soils were sieved and DNA was extracted using a MoBio Power Soil DNA kit.

"Self" plots were re-sampled whenever growth on such plots was above zero, however, failed growth in some plots necessitated changes in plots being sampled in 2016, 2017, and 2018.

1.3 Cedar Creek Rhizosphere Soil Metagenomic Sampling

On 10/7/2018, three individuals from the bulk soil plots sampled in 2018 were removed from the plot and stored on ice. Trowels used to remove individuals were cleaned with 90% ethanol between samples. Samples were immediately transported to the University of Minnesota campus and stored at -80° C until DNA could be extracted. Soils were dusted from the roots of the stored individuals and DNA was extracted using a

MoBio Power Soil DNA kit.

1.4 Jena Plant-Soil Feedback Experiment

A 730 m^2 area in a fallowed field was sprayed with glyphosate to remove vegetation. 0.75 mm thick HDPE root barrier was inserted to 35 cm deep between each plot. 1251 0.75 by 0.35 m PSF plots were established in the area. In spring 2015, all plots were seeded with 2000 pure live seeds per m^2. During the first year, plots received water twice weekly to ensure germination. To establish the phase 1 treatment, each of the nine target species were planted as monocultures and grown from 2015 to 2016. Non-target species were removed by hand-weeding. In 2016, fall PSF plots were sprayed with glyphosate and hand-tilled using a garden claw. To prevent regrowth roots of phase 1 species were removed. Plots were replanted with 2000 pure live seeds per square meter with either the same ("self" treatment) or a different ("other" treatment) species. Yearly growth was recorded as ocular estimate of percent cover prior to soil sampling for metagenomic analyses.

1.5 Jena Bulk Soil Metagenomic Sampling

On 9/1/2015, 1/1/2017, 10/1/2017, and 9/1/2018, a 15cm x 4cm core consisting of approximately 200 g of soil was taken from the center of nine randomly-selected "self" plots and stored on ice. Soil corers were cleaned with 90% ethanol between samples. Samples were transported to the Friedrich-Schiller-Universität Jena campus and stored at -80 ° C until DNA could be extracted. Soils for three "self" plots were pooled and sieved through a 2mm sieve to create three pooled samples from three plots for each species. DNA was extracted using a MoBio Power Soil DNA kit.

1.6 Jena Rhizosphere Soil Metagenomic Sampling

On 9/1/2018, a minimum of three individuals from the bulk soil plots sampled in 2018 were removed from the plot and stored on ice. Trowels used to remove individuals were cleaned with 90% ethanol between samples. Samples were transported to the Friedrich-Schiller-Universität Jena campus and stored at -80° C until DNA could be extracted. Soils were dusted from the roots of the stored individuals and pooled to create three pooled samples from three plots for each species. DNA was extracted using a MoBio Power Soil DNA kit.

1.7 Metagenomic Processing

DNA concentrations were checked using PicoGreen assay on a Modulus Microplate reader. Purified DNA was diluted to a maximum concentration of 6.0 ng/?l for bulk samples and 50.0 ng/µl for rhizosphere samples and stored at -80 °C until sequencing. Fungal ITS and bacterial 16S rRNA genes in the rhizosphere and bulk soil samples were amplified by Argonne National Laboratory using the primer sets ITS1f-ITS2 (ITS) and 515F-806R (bacterial). The amplified genes were subsequently sequenced by Argonne National Laboratory on the Illumina MiSeq platform (Novogene Corporation, Beijing China) using the Earth Microbiome Protocol.

2. RESULTS

2.1 Description of files:

MIMARKS.survey.soil.5.xlsx

Description of MIMARKS.survey.soil.5.1.xlsx: This file broadly describes the collection dates, elevations, environmental contexts, and agricultural additives for the

microbial data collected.

SRA_metadata.xlsx

Description of SRA_metadata_vers2.xlsx: This file broadly describes the library strategy, library source, library selection, library layout, platform, instrument, and design description for the microbial data collected.

2.2 Descriptions of parameters/variables

2.2.1 Temporal (beginning and end dates of data collection):

The Cedar Creek plant-soil feedback field experiment took place between May 2015 and October 2018. Soil samples were collected on 7/20/2015, 7/15/2016, 10/30/2017, and 10/7/2018.

The Jena plant-soil feedback experiment took place between May 2015 and October 2018. Soil samples were collected on 9/1/2015, 1/1/2017, 10/1/2017, and 9/1/2018.

2.2.2 Instruments used and units of measurements:

PicoGreen assay on a Modulus Microplate reader (ng DNA/µl) was used to measure DNA concentrations.

2.2.3 Column headings of data files for tabular data:

The file MIMARKS.survey.soil.5.xlsx consists of the following columns:

Sample title

Organism

Collection date

Depth (centimeters)

Elevation (meters)

Broad-scale environmental context (ENVO type)

Local-scale environmental context

Environmental medium

Geographic location

Agrochemical additions

The file SRA_metadata.xlsx consists of the following columns:

Library ID

Title

Library strategy

Library source

Library selection

Library layout

Platform

Instrument model

Design description

Filetype

Filename

Filename2

Filename3

Filename4

Filename5

Filename6

Filename7

Filename8

2.2.4 Location

Cedar Creek Field Site: 45.403290 N, 93.187411 W

Jena Field Site: 50.951951 N, 11.623832 E

2.2.5 Symbol used for missing data:

Empty cells represent missing data.

2.2.6 Recommended software

QIIME2 is recommended for processing the fastq data.

2.4 Data Archive

Data can be found archived at the National Center for Biotechnology Information Sequence Read Archive and USU Digital Commons at http://www.ncbi.nlm.nih.gov/bioproject/683074 and https://digitalcommons.usu.edu/all_datasets/127/.

CURRICULUM VITAE

Leslie E. Forero
(December 2020)

EDUCATION

Ph.D. in Ecology, G.P.A. 3.88 August 2014 to December 2020
Utah State University, Logan, Utah, USA; Dept. of Wildland Resources and the Ecology Center.
Advisor: Dr. Andrew Kulmatiski

- Awarded the Presidential Doctoral Research Fellowship in 2014, the Gary Smith Scholarship in 2018, the Ecology Center Research Grant in 2018, the USUSA Graduate Enhancement Award in 2018, and the Quinney College of Natural Resources Doctoral Student Researcher of the Year in 2021.
- My dissertation research focused on plant-soil feedbacks and spatial niche partitioning as mechanisms of coexistence and the biodiversity-productivity relationship in the tallgrass prairie at Cedar Creek.
- Skills: programming and statistical analyses, stable isotope techniques, genomic techniques, field research, greenhouse research, experiment management, professional presentations, grant proposals, and publication writing.
- Software: MATLAB, R, QIIME2, Hydrus

B.S. in Molecular Environmental Biology, GPA: 3.336 August 2009 to May 2013
University of California Berkeley, Berkeley, USA; College of Natural Resources.

- Minor Program: Conservation and Resource Studies

PUBLICATIONS

- **Forero, L. E**., J. Grenzer, J. Heinze, C. Schittko, and A. Kulmatiski. 2019. Greenhouse- and Field-Measured Plant-Soil Feedbacks Are Not Correlated. Frontiers in Environmental Science 7. DOI:10.3389/fenvs.2019.00184

- Kulmatiski, A., K. H. Beard, J. M. Norton, J. E. Heavilin, J. Grenzer, **L. E. Forero**. 2017. Live long and prosper: plant–soil feedback, lifespan, and landscape abundance covary. Ecology 98:3063-3073. DOI:10.1002/ecy.2011

- Saitone, T. L., L. C. Forero, G. A. Nader, and **L. E. Forero**. 2016. Calf and yearling prices in California and the western United States. California Agriculture 70:179-186.

- Kulmatiski, A., K. H. Beard, J. Grenzer, **L. E. Forero**, and Justin Heavilin. 2016. Using plant-soil feedbacks to predict plant biomass in diverse communities. Using plant-soil feedbacks to predict plant biomass in diverse communities. Ecology 97:2064-2073. DOI:10.1890/15-2037.1

- **Forero, L. E**., A. Kulmatiski, J. Grenzer, and J. M. Norton. Plant-soil feedbacks help explain the biodiversity-productivity relationship. In review.

- Kulmatiski, A., and **L. E. Forero**. Bagging: a cheaper, faster, non-destructive transpiration water sampling method for tracer studies. In review.

- **Forero, L. E**., and A. Kulmatiski. Niche differentiation in nitrogen and water use in an ex-arable field. In preparation.

- **Forero, L. E**., J. Grenzer, and A. Kulmatiski. Plant-soil feedbacks help explain biomass production in communities. In preparation.

- **Forero, L. E.**, J. Grenzer, and A. Kulmatiski. Metagenomic function in soil bacteria and fungi in a large plant-soil feedback experiment. In preparation.

PROFESSIONAL EXPERIENCE

Science Correspondent December 2017 – August 2020
Utah Public Radio
Pitched scientific story ideas to editors at UPR, scheduled and conducted interviews, edited audio and recorded narration, created online content, covered Utah Legislative Session, produced news and politics pieces when needed. Awarded Utah Society of Professional Journalism 2nd Place for Radio Business and Consumer Stories for *Wondering Why Logan City No Longer Accepts #3 Through #7 Plastics? It's Too Contaminated*.

Lead Instructor August 2019 – December 2019
Utah State University
Lead instructor for Range Plant Taxonomy and Function (WILD 3830). Planned course content, gave lectures, proctored quizzes, graded assignments. Managed a team of two undergraduate teaching fellows.

Teaching Assistant 2015, 2017, 2019, 2020
Utah State University
Assisted in teaching Microbiology (BIOL 3300), Range Plant Taxonomy and Function (WILD 3830), Genetics in Conservation and Management (WILD 4880), Conservation

Biology (WILD 4600). Graded assignments, tutored students, organized study groups, and gave lectures when needed.

Graduate Fellow May 2015 – August 2015
Cedar Creek Ecosystem Science Reserve
Mentored undergraduate researchers' independent research projects, presented at seminars and symposia, developed and implemented a weekly seminar series.

Field Technician December 2011 to July 2014
California Department of Fish and Wildlife
Performed fieldwork deploying automated bird recorders, completed detailed vegetation surveys, and set up baited camera stations on randomized plots throughout northern California's national forests for a long-term monitoring project. Performed surveys for mountain pine beetle/whitebark pine interaction project in the Warner Wilderness. Seveloped trainings and mentored new employees, created a gigapixel panoramic photo pilot for the long-term monitoring project.

Laboratory Technician August 2012 to May 2013
UC Berkeley Bruns Laboratory
Aided with DNA extractions, PCR amplification and QUBIT quantification, collected soil and fungal samples in the field.

PRESENTATIONS
Forero, L., A. Kulmatiski, J. M. Norton, and J. Grenzer. 2018. Using Plant-Soil Feedbacks to Predict Community Composition and Productivity in the context of the diversity-productivity relationship. Oral presentation at the Wildland Resources Graduate Research Symposium.

Forero, L. 2016, 2017, and 2018. Collecting Fungal Specimens for Scientific Research. Oral Presentation at Quinney College of Natural Resources Ecolunch.

Forero, L., A. Kulmatiski, J. M. Norton, and J Grenzer. 2017. Soil microbial communities of a field plant-soil feedback experiment. Oral presentation at an organized oral session at the Ecological Society of America Annual meeting and at the Utah State University Student Research Symposium.

Forero, L., J. Grenzer, and A. Kulmatiski. 2017. The role of plant-soil feedbacks in the diversity productivity relationship as observed at Cedar Creek. Oral presentation at the Cedar Creek Ecosystem Science Reserve Intern Symposium.

Forero, L., J. Grenzer and A. Kulmatiski. 2016. Using Plant-Soil Feedbacks to Predict Community Composition in the context of the diversity-productivity relationship. Oral presentation at the Cedar Creek Ecosystem Science Reserve Intern Symposium.

Kulmatiski, A., J. M. Norton, J. Grenzer, L. Forero and J. Heavilin. 2015 and 2016. Modeling Plant Biomass Using Plant-Soil Feedbacks. Poster presented at the Long-Term

Ecological Research All Scientists Meeting and at the Utah State University Student Research Symposium.

Forero, L. 2015. The importance of transpiration to global water budgets. Oral Presentation at the Cedar Creek Ecosystem Science Reserve Seminar Series.

Forero, L., J. Grenzer and A. Kulmatiski. 2015. Why do monocultures produce less biomass than polycultures? Poster presented at the Utah State University Student Research Symposium.

SERVICE

University Contributions

Ecology Seminar Committee Co-Chair August 2017 – May 2018
Ecology Center Utah State University
Planned meetings to select ecology experts around the world.

Ecology Seminar Committee Member August 2016 – May 2018
Ecology Center Utah State University
Chose and invited ecology experts from around the world to give two presentations to Utah State University Ecology students. Hosted ecology experts during their visits to Utah State University.

Graduate Student Council Department Representative August 2017 – May 2018
Graduate Student Council Quinney College of Natural Resources
Brought issues from graduate students to faculty. Worked with faculty to implement a stipend offer letter and revise the graduate student orientation.

Graduate Student Council Member August 2015 – May 2018
Graduate Student Council Quinney College of Natural Resources
Discussed and gave input on university policies, seminars, emergency medical fund, and first aid training.

Graduate Student Council Seminar Coordinator August 2016 – December 2016
Graduate Student Council Quinney College of Natural Resources
Planned and implemented weekly paper discussions or scientific talks.

Graduate Student Council Emergency Medical Fundraiser Planner January 2015
Graduate Student Council Quinney College of Natural Resources
Developed and implemented a fundraiser for the graduate student emergency medical fund. We raised $1400 for the emergency medical fund (more than double the previous years' funds raised).

Press Coverage

- Utah Public Radio: Undisciplined: The Sports Media Analyst And The Research Ecologist
- USU Today: Get Outside: Plant-Soil Research in Greenhouses Differs Strongly From Field Studies

Membership in Professional Organizations
Mushroom Society of Utah
Ecological Society of America

Personal
Volunteer Mobile Crisis Team Advocate August 2015 to May 2018
Citizens Against Physical And Sexual Abuse (CAPSA)
Provided on-call assistance to victims of domestic violence and sexual assault. Met with victims of abuse at CAPSA, the police station, or the hospital and provided information and assistance on restraining orders, crime victim reparations, and other topics.