LOOKING BELOWGROUND: THE ROLE OF SOIL SYMBIONTS IN TALLGRASS PRAIRIE INVASION AND RESTORATION

By

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LOOKING BELOWGROUND: THE ROLE OF SOIL SYMBIONTS IN TALLGRASS PRAIRIE INVASION AND RESTORATION

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Abstract: Tallgrass prairies of North America are under threat from a number of anthropogenic disturbances including the introduction and expansion of nonnative plant species. One potential mechanism for successful invasion by nonnative plants is alterations of native soil microbial communities, including symbiotic arbuscular mycorrhizal (AM) fungi. Restoration projects typically focus on aboveground processes with less focus on altered belowground aspects. However, to successfully restore prairies following invasion by non-native plants, a deeper understanding of belowground alterations facilitating invasive dominance and hindering native reestablishment is needed. A restoration project focused on repairing belowground processes following invasion by *Bothriochloa bladhii* was established at the Konza Prairie Biological Station near Manhattan, KS. This study evaluated the influence of native AM fungal communities on establishment and survival of reintroduced native plant species following eradication of the invasive. Seedlings received one of three soil microbial inoculants (whole prairie soil, selected AM fungi, or sterile soil), prior to introduction into the study site. Evaluations of species diversity, survival, and return of the invasive plant were conducted for five years following transplant. No significant differences in diversity were observed, which likely stemmed from a high rate of return of the invasive. Overall, whole soil and selected AM fungal inoculum significantly improved survival of native species, but survival was species-specific. A second project tapped into the plant-soil feedback (PSF) framework to determine the reciprocal effects of interactions between plants and root-associated microbes. Previous research indicates PSFs driven by invasive plants can be buffered by management practices. Therefore, I investigated the influence of herbivory as a potential restoration tool. In a greenhouse study, PSF responses of cool- and warm-season native and non-native grasses were examined to determine how herbivory (clipping) impacts PSF dynamics. My findings suggest herbivory alters the strength and direction of PSFs, and these changes may impact interactions of plants and associated soil microbes. Reintroducing herbivory into invaded areas may be a pathway to reset PSFs established by invasive grasses, potentially negating soil legacies by buffering the benefits established through soil microbial alterations.

TABLE OF CONTENTS

Chapter Page

I. TALLGRASS PRAIRIE PLANT RESPONSE TO INOCULATION WITH NATIVE MICROBES: IMPLICATIONS FOR RESTORATION SUCCESS 1

Abstract 1
Introduction 2
Materials and Methods 4
Results 7
Discussion 8
Tables 15
Figures 17

II. DIRECTION AND MAGNITUDE OF PLANT-SOIL FEEDBACKS FOR NATIVE AND INVASIVE PRAIRIE GRASSES SUBJECTED TO HERBIVORY 22

Abstract 22
Introduction 23
Materials and Methods 25
Results 29
Discussion 33
Tables 39
Figures 47

III. REFERENCES 53

LIST OF TABLES

Table Page

1. Survival calculated by Kaplan-Meier analysis for arbuscular mycorrhizal inoculum. Time is represented by the biannual sampling events, risk is the number of individuals at risk of death, death is the number of deaths recorded, and survival is the percent of individuals remaining. Sampling times not reported had no deaths recorded. Treatment sharing a superscript letter are not significantly different from one another ($p > 0.05$).....15

2. Linear mixed effects regression model performed on diversity produced by the Shannon-Wiener index (H'). "Whole" represents inoculation with whole prairie soil; "Selected" represents inoculation by beneficial AM fungal taxa microscopically isolated in the laboratory; "Sterile" represents sterilized soil inoculum. Means that share a letter are not different ($p \leq 0.05$). The "selected" inoculation group is the beta intercept for the model output.....16

3. Linear mixed effects regression model performed on *B. bladhii* cover of experimental plots following solarization. "Whole" represents inoculation with whole prairie soil; "Selected" represents inoculation by beneficial AM fungal taxa microscopically isolated in the laboratory; "Sterile" represents sterilized soil inoculum. Means that share a letter are not different ($p \leq 0.05$). The "selected" inoculation group is the beta intercept for the model output.....16

4. The effects of species, conditioning, and clipping on cool-season native and invasive biomass production and root colonization by AM fungi. Where "species" represents the plant species grown in each pot, "conditioning" represents the phase 1 species that conditioned the soil, and "clipping" represents simulated herbivory. All analyses were performed with a confidence interval of 0.95.....39

5. Interaction coefficients calculated for phase 2 (experimental) native and invasive cool-season grass species. Where "*P. smithii*" represents soil conditioned by the native, "*B. inermis*" represents soil conditioned by the non-native, "not clipped"

Table Page

represents soil conditioned in the absence of simulated herbivory, and "clipped" represents soil conditioned in the presence of herbivory.....40

6. The effects of species, conditioning, and clipping on native and invasive warm-season biomass production and root colonization by AM fungi. Where "species" represents the plant species grown in each pot, "conditioning" represents the phase 1 species that conditioned the soil, and "clipping" represents the simulated herbivory treatment. All analyses were performed with a confidence interval of 0.95.....41

7. Interaction coefficients calculated for phase 2 (experimental) native and invasive warm-season grass species. Where "*S. scoparium*" represents soil conditioned by the native, "*B. ischaemum*" represents soil conditioned by the non-native, "not clipped" represents soil conditioned in the absence of simulated herbivory, and "clipped" represents soil conditioned in the presence of herbivory.....42

8. The effects of species, conditioning, and clipping on AM fungal extra-radical hyphal (PLFA) and spore (NLFA) production associated with native and invasive cool-season plants. Where "species" represents the individual grown in each pot, "conditioning" represents phase 1 species that conditioned the soil, and "clipping" represents simulated herbivory. All analyses were performed with a confidence interval of 0.95. 43

9. The effects of species, conditioning, and clipping on AM fungal extra-radical hyphal (PLFA) and spore (NLFA) production associated with native and invasive warm-season plants. Where "species" represents the individual grown in each pot, "conditioning" represents phase 1 species that conditioned the soil, and "clipping" represents simulated herbivory. All analyses were performed with a confidence interval of 0.95. 44

10. Extra-radical AM fungal abundance of cool-season grasses following soil conditioning (phase 1). Phospholipid fatty acid (PLFA) represent hyphal abundance, neutral lipid fatty acid (NLFA) represents fungal spores associated with native and invasive cool-season grasses. Rows that share a superscript letter are not significantly different from one another ($p \leq 0.05$). 45

11. Extra-radical AM fungal abundance of warm-season grasses following soil conditioning (phase 1). Phospholipid fatty acid (PLFA) represent hyphal abundance, neutral lipid fatty acid (NLFA) represents fungal spores associated with native and invasive cool-season grasses. Rows that share a superscript letter are not significantly different from one another ($p < 0.05$). 46

LIST OF FIGURES

Figure Page

1. *Andropogon gerardii* survival across sampling periods. “Whole” represents inoculation with whole prairie soil; “Selected” represents inoculation by beneficial AM fungal taxa microscopically isolated in the laboratory; “Sterile” represents sterilized soil inoculum. In the legend, inoculation that share a letter are not different ($p \leq 0.05$).....17

2. *Pascopyrum smithii* survival across sampling periods. “Whole” represents inoculation with whole prairie soil; “Selected” represents inoculation by beneficial AM fungal taxa microscopically isolated in the laboratory; “Sterile” represents sterilized soil inoculum. In the legend, inoculation that share a letter are not different ($p \leq 0.05$). 18

3. *Asclepias syriaca* survival across sampling periods. “Whole” represents inoculation with whole prairie soil; “Selected” represents inoculation by beneficial AM fungal taxa microscopically isolated in the laboratory; “Sterile” represents sterilized soil inoculum. In the legend, inoculation that share a letter are not different ($p \leq 0.05$). 19

4. *Ratibida columnifera* survival across sampling periods. “Whole” represents inoculation with whole prairie soil; “Selected” represents inoculation by beneficial AM fungal taxa microscopically isolated in the laboratory; “Sterile” represents sterilized soil inoculum. In the legend, inoculation that share a letter are not different ($p \leq 0.05$).....20

5. *Lespedeza capitata* survival across sampling periods. “Whole” represents inoculation with whole prairie soil; “Selected” represents inoculation by beneficial AM fungal taxa microscopically isolated in the laboratory; “Sterile” represents sterilized soil inoculum. In the legend, inoculation that share a letter are not different ($p \leq 0.05$). 21

6. Schematic diagram for the experimental design of the conditioning and experimental testing phases of this research. The top line shows the phase 1 soil

conditioning: in this conditioning phase two warm-season grasses (*Schizachyrium scoparium, Bothriochloa ischaemum*) and two cool-season grasses (*Pascopyrum smithii, Bromus inermis*) were clipped or not clipped. The bottom half shows the conditioned soils collected from phase 1 being used to grow a new generation of plants. As shown by our schematic, each soil treatment from the conditioning phase was used as inoculum for native or invasive plant species of the same functional group. 47

7. On the left, the experimental design basis for the project. On the right, the equation used to determine the interaction coefficients (I_s) that represents the direction and magnitude of each PSF. 48

8. Percent arbuscular mycorrhizal fungal colonization response of phase 1 (conditioning) native and invasive cool-season grasses to the presence or absence of clipping. Violin shapes that share a letter are not significantly different from one another ($p \leq 0.05$).....48

9. 4A. Total biomass of cool-season phase 2 (experimental) native and invasive grasses in response to growth in soils conditioned by clipped or not clipped *P. smithii*. 4B. Total biomass of cool-season phase 2 (experimental) grasses in response to growth in soils conditioned by clipped or not clipped *B. inermis*. Bars within plant functional group that share a letter are not significantly different from one another ($p \leq 0.05$). 49

10. 5A. Arbuscular mycorrhizal fungal colonization (%) of phase 2 (experimental) native and invasive cool-season grasses to growth in soil conditioned by hetero- or conspecifics. Violin shapes that share a letter are not significantly different from one another. 5B. AM fungal colonization (%) of phase 2 (experimental) cool-season grasses to growth in soil conditioned by clipped or not clipped grasses. Violin shapes that share a letter are not significantly different from one another ($p \leq 0.05$).....50

11. Warm-season arbuscular mycorrhizal fungal colonization (%) of phase 1 (conditioning) native and invasive grasses following clipping. Violin shapes that share a letter are not significantly different from one another ($p \leq 0.05$).....51

12. 7A. Total biomass of phase 2 (experimental) native and invasive warm-season grasses following growth in soils conditioned by clipped or not clipped *S. scoparium*. 7B. Total biomass of phase 2 (experimental) warm-season grasses

Figure Page

following growth in soils conditioned by clipped or not clipped *B. ischaemum*. Bars within plant functional group that share a letter are not significantly different from one another ($p \leq 0.05$). 51

13. 8A. Arbuscular mycorrhizal fungal colonization (%) of phase 2 (experimental) native and invasive warm-season grasses in soil conditioned by hetero- or conspecifics. 8B. AM fungal colonization (%) of phase 2 (experimental) warm-season grasses in soil conditioned by clipped or not clipped grasses. Violin shapes that share a letter are not significantly different from one another ($p \leq 0.05$). 52

CHAPTER I

TALLGRASS PRAIRIE PLANT RESPONSE TO INOCULATION WITH NATIVE MICROBES: IMPLICATIONS FOR RESTORATION SUCCESS

ABSTRACT

Tallgrass prairies of North America are under threat from numerous anthropogenic disturbances including, but certainly not limited to, the introduction and expansion of non-native plant species. One potential mechanism for successful invasion by non-native plants is alterations of native soil microbial communities, including symbiotic arbuscular mycorrhizal (AM) fungi. *Bothriochloa bladhii* is an aggressive invasive known to alter soil microbial communities, likely aiding in its establishment and spread. Previous restoration projects have been met with little success, especially with successful reintroduction of late successional plant species. A restoration project with a focus on belowground processes following invasion by *B. bladhii* was established at the Konza Prairie Biological Station, Manhattan, KS (NSF LTER). In my study, I evaluated the influence of native AM fungal communities on establishment and survival of native plant species (C_4 grass, C_3 grass, and late successional prairie forbs) following eradication of the invasive grass. I inoculated seedlings of each functional group, established under greenhouse conditions prior to planting at KPBS, with AM fungal communities as

follows: 1) whole soil freshly collected from KPBS, including all soil microbial communities, 2) fungal spores specifically selected as beneficial and propagated under greenhouse conditions; or 3) inoculated with sterilized soil (control). I found significant increases in survival of legume and non-leguminous forb species inoculated with either whole prairie soil or selected AM fungal spores, compared to inoculation with sterile soil. This suggests successful restoration may be achieved through propagation of AM taxa specifically selected for target native species, with fewer disturbances to native grasslands. Therefore, restoration projects focusing on soil legacy effects following invasive grass species, it is not necessary to disrupt vast areas of adjacent grasslands, as propagated, beneficial AM fungal inoculum produce similar results with little grassland disturbance.

INTRODUCTION

Grasslands are one of the most endangered ecosystems on Earth, and this is especially evident in the prairies of central North America (Samson and Knopf 1994, Gibson 2009). The remaining grassland systems continue to be threatened by several anthropogenic disturbances, including invasion by non-native plant species (Wilcove et al. 1998, Powell et al. 2011, Downey and Richardson 2016), with resultant effects on ecosystem goods and services they provide. Invasion by non-native plants can disrupt carbon cycling both above- and belowground, as well as pollination services, and alter ecosystem productivity (Morales et al. 2017, Nie et al. 2017, Vila and Hulme 2017). Non-native, invasive grasses have been widely introduced as their rapid growth can benefit erosion control and produce livestock forages. However, non-native species frequently escape cultivation and spread into adjacent roadsides, pastures, and prairies, replacing historically diverse native plant communities with monocultures.

A suite of non-native grasses collectively known as the old-world bluestems (*Bothriochloa* spp.; *Dichanthium* spp.) have been widely planted and are invading into grasslands in the central and

southern Great Plains region of North America, with potentially damaging alterations to this ecosystem. For example, *Bothriochloa bladhii*, has been shown to alter soil microbial communities, including arbuscular mycorrhizal fungi (Wilson et al. 2012), which in turn, negatively affects native grassland plants. Due to these belowground alterations, coupled with functional similarity to native, dominant species, restoration of native grasses and forbs following eradication of *B. bladhii* has been met with little success.

Arbuscular mycorrhizal (AM) fungi are obligate symbionts that form relationships with over 90% of terrestrial plants (Smith and Read 2008). Arbuscular mycorrhizal fungi act as root extensions to increase host plant nutrient and water uptake, as well as pathogen defense, in exchange for carbohydrates produced during photosynthesis (Smith and Read 2008). Due to the prevalence and importance of this plant-fungal relationship, AM fungi play an important role in plant invasion success, as well as the outcomes of subsequent restorations. Non-native invasive plant species have been shown to alter the density and/or composition of the AM fungal communities, which may feedback on the spread of the invasion by the non-native plant species (Bever 2002, Bever 2003, Reinhart and Calloway 2006). Changes in AM fungal communities by *B. bladhii* can generate a positive feedback, stabilizing the plant communities now dominated by *B. bladhii*, while preventing re-establishment of a functional native plant community, thereby decreasing the success of restoration projects (Bever et al. 2010).

Historically, aboveground plant-plant interactions tended to receive the primary focus in restoration studies. However, belowground microbial communities have a tremendous impact on aboveground dynamics and can shape the structure and abundance of the native plant community (Klironomos 2002, Mangan et al. 2010, Bever et al. 2015a, Koziol et al. 2018). Recent research has begun to show ameliorations to altered belowground communities can aid in restoration success following invasion and anthropogenic disturbance (Koziol and Bever 2017, Koziol et al

2021). In fact, reintroducing native species without the native microbial counterparts, specifically AM fungi, greatly limits the outcome of restoration projects (Koziol et al. 2018).

To evaluate long-term species-specific responses to reintroducing native soil communities, including native AM fungi, on *B. bladhii* control, native plant survival, and restoration of a functioning native ecosystem, a field project was established at Konza Prairie Biological Station (KPBS; Long-Term Ecological Research Station [NSF LTER]) near Manhattan, KS, USA. The aim of this project was to reintroduce native plants inoculated with beneficial, locally-adapted microbial communities following eradication of the invasive plant species. Survival of introduced native plants, overall diversity, and re-establishment of *B. bladhii* was monitored over a five-year period. I hypothesized survival of the reintroduced native species would be greater when inoculated with beneficial microbes, relative to non-inoculated plants (hypothesis 1), and the overall effect of reintroducing beneficial native soil microbes and improving the success of native grasses and late-successional forb species would increase overall plant diversity (hypothesis 2). Finally, I hypothesized *B. bladhii* cover would decrease following inoculation with beneficial fungi, increases in native plant success, and increases in plant diversity would decrease re-invasion success of *B. bladhii* (hypothesis 3).

MATERIALS AND METHODS

Site description and experimental setup: Konza Prairie Biological Station (KPBS) is a 3,487 ha tallgrass prairie preserve in the Flint Hills of northeastern Kansas, USA (39 05’ N, 96 35’ W). This area is owned by The Nature Conservancy and managed by Kansas State University, Division of Biology and is a National Science Foundation Long-Term Ecological Research Station (KNZ). My study was conducted at the Belowground Plot experiment on KPBS, initiated in 1986 by the Konza Prairie Long-Term Ecological Research (LTER) program to assess plant-soil responses to fertilization and fire. The historic treatments of this location consisted of burning

(annually burned or non-burned), mowing (annually mowed or non-mowed), ammonium nitrate fertilization (10 g N m^{-2} annually or non-fertilized), and superphosphate fertilization (1 g P m^{-2} annually or non-fertilized), arranged in a split-split plot with burning as the whole plot treatment, mowing as the subplot treatment, and N and P amendments as factorial sub-subplot treatments. Whole plots were arranged in a randomized complete block design. Burning is conducted in April of each year and fertilizer amendments were applied starting in 1986 and ending in 2016, 7-10 days following burning. Mowing was conducted in June of each year starting from 1986 and ending in 2008, and mowed biomass was removed from plots. This study utilized a sub-set of the BGP (historically mowed), as mowing facilitated invasion by *Bothriochloa bladhii*. Prior to the initiation of the study, each of the 12 selected plots were delineated into nine 2 m^2 sub-plots with a 2 m buffer between plots. At the beginning of the growing season in 2016, plots were covered with a clear UV resistant polyethylene solarization tarp (16'x32'; poolsupplies.com, Tonawanda, NY) to eradicate *B. bladhii* through soil heating (solarization). In each of the 12 experimental invaded plots, one sub-plot remained uncovered to allow survival of *B. bladhii* as a control. Solarization tarps were removed at the end of the first growing season (October 2016).

Inoculum and plant species selection: In a field study adjacent to my plots, inoculation with native prairie soil benefited establishment of late-successional plant species (Duell et al. 2022a). However, inoculating with native prairie soil is not practical for large-scale restorations. Therefore, in my study, seedlings (i.e., nurse plants) were inoculated and grown in a greenhouse for 10 weeks. Plants were then transplanted into restoration plots, potentially allowing native AM fungi associated with each nurse plant to benefit adjacent non-inoculated plants. Nurse plants were inoculated with one of three mycorrhizal treatments: 1) whole "live" soil freshly collected from a non-invaded location at KPBS (hereafter referred to as 'whole'); 2) fungal spores selected microscopically in the laboratory, these spores were selected as beneficial for native late-successional prairie plants and propagated in the greenhouse on *Sorghum bicolor* (L.) (hereafter

referred to as ‘selected’); or 3) inoculated with sterile soil (control). Nurse plant species selected were: *Andropogon gerardii* Vitman (C_4 grass), *Pascopyrum smithii* (Rydb.) Á. Löve (C_3 grass), *Asclepias syriaca* L. (forb), *Ratibida columnifera* (Nutt.), Wooton & Stample. (forb), and *Lespedeza capitata* Michx. (legume).

Data collection: Following solarization of the experimental plots, nurse plants were transplanted into experimental plots in May 2017, following spring burning. Each of the six sub-plots within the 12 treatment plots received two nurse plants of each of the five plant species (10 seedlings per sub-plot). The three inoculum treatments were randomly assigned to two of the six sub-plots contained within each of the 12 experimental plots. Nurse plants were planted in the center of each sub-plot, in a scattered pattern. Data was collected for this project twice per growing season, once in the summer and once in the fall. However, due to the global pandemic of 2020, travel to this field site was restricted for the entirety of the growing season and sampling did not occur for this year. A total of nine data collection events occurred for the six years after transplanting (Summer 2017 – Summer 2022). Each nurse plant was introduced with a unique ID tag to accurately assess survival at each data collection event. Establishment of native grasses and forbs within the subplots were determined as percent species composition and used to assess and compare the overall diversity of each experimental plot. Percent coverage of *B. bladhii* was also assessed from each subplot to determine if inoculation with native soil or AM fungal communities influenced re-invasion.

Statistics: *Survival:* Data for assessing survival of the nurse plants was collected as absence and presence data. For each data collection event, either the plant was present (1) or absent (0) from the plot. If absence was recorded, the plant was presumed dead, and an event occurrence (death) was recorded. Further, the time to death was recorded. These data were assessed using the “Survival” package in R statistical software (Therneau 2021). Due to the categorical nature of the treatment groups as predictor variables, a univariate Kaplan-Meier (KM) model was employed

for each species to analyze the survival trend over time. The differences between species-specific survival curves were produced using log-rank pairwise comparisons with Bonferroni-Holm adjustment.

Diversity: The Shannon-Wiener diversity index (Shannon 1948) was used to convert species composition from a qualitative percent coverage estimate to a quantitative value (H') that considers both the species richness and evenness of the plots. This conversion was performed using the "Vegan" package in R (Oksanen et al. 2020). After conversion, the data were assessed using a linear mixed effects regression (LMER) model with the subplot-plot structure as a hierarchical random factor (due to the minor differences between the plot locations), time as a random factor, the diversity values as the response variable, and the inoculation treatment groups as the predictor. Post hoc analyses were performed using the estimated marginal means with the "emmeans" package in R (Lenth 2022).

Invasive cover: Cover of *B. bladhii* within the plots was assessed using a similar approach as that of the diversity values. An LMER model with the subplot-plot structure as a hierarchical random factor, time as a random factor, the coverage estimates as the response variable, and the inoculation treatment groups as the predictor. The estimated marginal means were used to perform the pairwise comparisons and post hoc analysis (Lenth 2022).

RESULTS

Survival: The univariate KM model was built with the soil inoculum treatments as the only predictors of survival. Survival following inoculation with selected AM fungal inoculum (selected) and native soil (whole) did not differ from one another (p value = .61). However, inoculation with sterile soil resulted in significantly lower survival, relative to selected and whole soil inoculum (Table 1; selected-sterile p-value = > 0.001; whole-sterile p-value = > 0.001).

Survival of individual species was variable. The two grass species, *A. gerardii* and *P. smithii*, each maintained high survival regardless of inoculum (Figures 1 and 2). Survival of *A. syriaca* was not influenced by AM fungal inoculation and performed poorly regardless of inoculum (Figure 3). Inoculation with selected or native whole soil significantly increased survival of *R. columnifera*, compared to plants inoculated with sterile soil (Figure 4; selected-sterile p-value = > 0.001; whole-sterile p-value = > .001), until 2021 when survival of all *R. columnifera* plants was extremely low. Survival of the leguminous species, *L. capitata*, did not differ between inoculation with either selected AM taxa or whole native soil, however, both were significantly greater than plant survival following inoculation with sterile soil (Figure 5; selected-sterile p-value = > 0.001; whole-sterile p-value = > 0.001).

Diversity: No differences in Shannon diversity (H') were detected among treatments (Table 2). The confidence intervals produced by the three treatment groups are wide and completely overlap one another. (Table 2; selected-sterile p= 0.86; sterile-whole p = 0.59; selected-whole p = 0.89). Finally, there are no significant effects between inoculation when analyzing data within sampling time or after revaluating results with *B. bladhii* removed from the diversity index.

Invasive coverage: Cover of *B. bladhii* did not differ between inoculation treatments, with consistent beta values across all treatment groups, and confidence intervals are wide and overlapping (Table 3; selected-sterile p = 0.94; whole-sterile p = 0.41; selected-whole p = 0.61).

DISCUSSION

Plant-microbial interactions have gained much attention recently in the field of ecological restoration. Benefits a given plant receives can depend on the identity of its AM fungal associates (e.g., Johnson et al. 2010a, Hoeksema et al. 2010), and non-native invasive plant species have been shown to alter the density and/or composition of the AM fungal communities, which may feedback on the subsequent spread of the introduced plant species (Reinhart and Calloway 2006,

Koziol et al. 2021). It is possible that invasive plants alter AM fungal communities to promote their own success, as plants can allocate preferentially to the most beneficial fungal partner (Bever et al. 2009, Kiers et al. 2011). In my study, I examined the role of AM fungal reintroduction on survival of native grassland plant species, and reduction in *B. bladhii*. Influence of AM fungal introduction was species-specific, with little influence on either warm- or cool-season grasses, yet significant increase in survival of *L. capitata* and *R. columnifera*. However, my results indicate *A. syriaca* will require further information and possibly specialized solutions to increase establishment and survival. My research provides evidence that locally adapted AM fungi can provide critical benefits to native legumes and non-leguminous forbs and can be utilized to enhance successful establishment and persistence in prairie restorations. Given these sub-dominant late successional forb species often fail to establish with conventional (no microbiome amendment) restoration practices (McCain et al. 2010, Grman et al. 2015, Koziol and Bever 2017), my study indicates native microbiome amendments may be useful in restorations to improve establishment of late successional plants.

Importantly, including only native AM fungal communities selected and propagated (selected) produced similar plant survival as was found with live (whole) native prairie soil inoculum. That the propagated (selected) inoculum was derived from small amounts of live soil suggests native plant success is possible with minimal disturbance of the remaining areas of intact tallgrass prairie. The whole native soil included a suite of native prairie bacterial and fungal communities, likely including pathogens, Rhizobia, mycorrhizal fungi, soil bacteria, and nematodes as well as other invertebrates. However, nurse plants generally responded positively to whole soil inoculation, suggesting that the benefit from the addition of native AM fungi is greater to native forbs than the harm from native pathogens. While negative feedbacks, which have been shown to be commonly driven by host-specific pathogens (Klironomos 2002, Bauer et al. 2015, Bever et al. 2015b), may increase with introduced native pathogens in future years, my results

indicate that plant benefits from whole soil inoculations are largely driven by the presence of beneficial mycorrhizal species over a 6-year period.

Previous studies have compared locally collected whole soil inocula and commercial mycorrhizal inocula, typically of non-local origin (Rowe et al. 2007, Emam 2016) and found commercial inocula to be less effective. Recently, Duell et al. (2022b) reported no effectiveness in improving establishment and growth of native prairie grasses and forbs following inoculation with commercial AM fungi. As AM fungi can be adapted to specific soil conditions (Johnson et al. 2010b, Rúa et al. 2016), the use of non-local inoculum may lead to fungal and soil mismatches between soil characteristics and fungal capabilities. Therefore, it should be cautioned against using commercial AM fungal inoculum in native grassland restorations. However, my results suggest that native, locally adapted mycorrhizal mixtures may improve establishment and survival of native plant species with high conservation value in grassland restorations.

Associations with AM fungi have many benefits that improve individual plant health and productivity, as well as population and community outcomes. The primary function of AM fungi is to aid the plant with nutrient uptake, especially with less mobile nutrients such as phosphorous. In fact, AM fungi have been credited with as much as 80% of a plant's total phosphorous needs (Marschner and Dell 1994, Miller, Wilson, and Johnson 2012). For leguminous species such as *L. capitata*, trading photosynthetic products for assistance with phosphorous uptake is vital, as N_2-fixation is typically phosphorus-demanding (Azcon-Aguilar and Barea 1992, Lazali and Bargaz 2017). Prairie legumes typically have a high reliance on mycorrhizal symbiosis (Wilson and Hartnett 1998), indicating the significant increase in survival by AM inoculated *L. capitata*, compared to inoculation with sterile soil, was likely facilitated by beneficial native AM fungal species. As a large number of perennial prairie legumes are typically highly mycotrophic (Wilson and Hartnett 1998, Hoeksema et al. 2010), increased survival of *L. capitata* may be representative of functionally similar leguminous plants. Survival results of *L. capitata* warrant further

investigation into the restoration benefits of reintroducing legumes inoculated with native AM fungal species into degraded grassland landscapes.

Despite the dramatic increased mortality in 2020, survival of *R. columnifera* was significantly greater following inoculation with native whole soil or selected AM fungal spores, compared to sterile inoculum. While it is not known what drove mortality of *R. columnifera*, my results indicate that for four years following initiation of this restoration study, AM fungal inoculation was important for success of this forb species. However, the substantial loss of this late-season forb during the fifth year of my study reinforces the importance of long-term restoration studies, both in identifying loss of native species over time, as well as identifying benefits of inoculation that may improve over time (Neuenkamp et al. 2019). The perennial forb *A. syriaca* exhibited poor survival regardless of inoculation. In fact, after three years the majority of *A. syriaca* plants had died, with only approximately 5% of the individuals surviving after the sixth year of this study. Recent research by Koziol et al. (2022) suggests *A. syriaca* is not responsive to the presence or composition of inoculated AM fungal communities, suggesting the lack of survival is not linked to an unsuccessful matching of AM fungal taxa in my study.

Both species of grass exhibited high survival regardless of mycorrhizal inoculum. It was not unexpected that survival of *P. smithii* was not dependent on inoculation with native AM fungal taxa, as this cool-season grass is facultatively mycotrophic, and therefore less dependent on the symbiosis for establishment and survival (Wilson and Hartnett 1998, Hoeksema et al. 2010). These grasses are characterized by finely branched root systems, and the extraradical hyphal network of AM fungi is likely functionally redundant (Miller et al. 1997, Miller et al. 2012). Conversely, warm-season grass root systems typically have a relatively large diameter with less secondary and tertiary branching, making them more reliant on the mycorrhizal relationship (Hetrick et al, 1991; Wilson and Hartnett 1998). In fact, *Andropogon gerardii* is considered an obligate symbiont with an extremely high reliance on AM fungi, when grown in

low nutrient native prairie soils (Wilson and Hartnett 1998). The high rate of survival for *A. gerardii* regardless of inoculation, including high survival when inoculated with sterile soil, was unexpected as previous research has found native soil inoculation increased establishment and growth of warm-season grasses (Koziol et al. 2021, Duell et al. 2022a). The lack of *A. gerardii* preference for native AM fungal taxa in my study suggests that this grass may be able to associate with similar AM fungal taxa as that of *B. bladhii*, but more information is needed. However, the lack of dependence for native soil inoculation may indicate *A. gerardii* is a preferred choice for establishing a native warm-season grass in soils degraded by *B. bladhii* and may have potential to serve as a nurse plant for reintroducing native AM fungal species via inoculation.

Increasing restoration success by enhancing native plant community diversity is critical for reversing the extensive loss of grasslands worldwide and I hypothesized inoculation with native AM fungal taxa would increase survival of native prairie plant species, with a concomitant increase in overall plant diversity. However, plant diversity, as measured by Shannon diversity index, was not influenced by inoculum treatments. Previous research has shown that reintroducing native AM fungi to degraded soil can improve native plant species diversity (Middleton and Bever 2012, Koziol and Bever 2017) and inoculation with native soil can reduce *B. bladhii* cover, likely through increased native plant diversity (Duell et al. 2022a). However, I found no effect of inoculation on *B. bladhii* cover throughout the duration of my experiment, allowing the invasive to continue to alter the AM fungal communities and disrupt plant-soil feedbacks. While *B. bladhii* expanded its dominance each year of my study, this species did not appear to drive the lack of overall native plant diversity, as removal of *B. bladhii* from diversity calculations did not influence native plant species diversity and the restoration plots remained considerably lower in diversity compared to adjacent non-invaded prairie. *Bothriochloa bladhii* composed approximately 50% cover for all plots at the completion of my study, indicating the

soil legacy of the invasive grass continues to persist and impose negative effects on the native plant community.

The inability to remove *B. bladhii* from our study site reflects the high invasibility of this non-native grass (Reed et al. 2005). However, the continued presence and expansion of this non-native is also very likely an artifact of the selected methodology used in this study, and this methodology should be reviewed before initiating future restoration projects in the wake of *B. bladhii*. Solarization in the first year of the study was selected for *B. bladhii* eradication, and this technique also eradicated native plant species, likely reduced soil microbial communities, as well as native and invasive species seed banks. However, little is known about the dormancy capabilities of *B. bladhii* seed, and an established seed bank will allow the invasive to survive and re-establish after solarization. Furthermore, in each of the 12 experimental invaded plots, the solarization tarp was removed from one sub-plot to allow survival of *B. bladhii* as a control and areas surrounding the experimental plots were heavily invaded by established stands of *B. bladhii*. In future studies, these established stands of the invasive should be carefully eradicated prior to establishment of inoculated nurse plants and effective and continual long-term control methods of the returning invasives is needed during the restoration process.

For North American tallgrass prairie, my results suggest that adding locally derived AM fungal taxa may provide the same restoration benefits as whole soil collected from native prairies. Additions of AM fungi without other whole soil microbiomes is comparable to whole soil amendments provides evidence that AM fungi are keystone components of the soil microbiome for native prairie plants and that inoculation with AM fungi alone can improve plant establishment and survival for later successional plant species. This is important knowledge as harvesting whole soil inocula from endangered tallgrass prairie soils could decimate the native soil communities of the tallgrass prairie system very quickly. Based on my results, I suggest that native mycorrhizal fungi can be propagated from small quantities of native soil and used as a tool

for the restoration of difficult to establish late-successional plants and improve restoration quality. The species-specific responses to soil inoculation highlights one of the many complications of successful restorations and gives insight as to why restorations tend to have lower diversity than undisturbed locations (Middleton et al. 2010). Knowledge gained from my study may allow selection of effective inoculant, possibly with fewer disturbances to native grasslands through propagation of selected AM taxa and lays a baseline that indicates successful reintroduction of forb and legume species can be improved by determining the specific AM fungal taxa that will benefit specific forb species.

TABLES

Table 1: Survival calculated by Kaplan-Meier analysis for arbuscular mycorrhizal inoculum. Time is represented by the biannual sampling events, risk is the number of individuals at risk of death, death is the number of deaths recorded, and survival is the percent of individuals remaining. Sampling times not reported had no deaths recorded. Treatment sharing a superscript letter are not significantly different from one another ($p > 0.05$).

Treatment	Sample Event	At Risk	Death	Survival %	Std. Error	Lower 95% CI	Upper 95% CI
Whole[a]	Fall 2017	240	24	90.0	0.0194	0.863	0.939
	Summer 2018	216	30	77.5	0.0270	0.724	0.830
	Fall 2018	186	6	75.0	0.0280	0.697	0.807
	Summer 2019	180	1	74.6	0.0281	0.693	0.803
	Fall 2019	179	6	72.1	0.0290	0.666	0.780
	Summer 2021	167	49	50.9	0.0326	0.449	0.577
	Fall 2021	118	4	49.2	0.0326	0.432	0.560
	Summer 2022	114	5	47.0	0.0326	0.411	0.539
Selected[a]	Fall 2017	240	34	85.8	0.0225	0.815	0.904
	Summer 2018	206	27	74.6	0.0281	0.693	0.803
	Fall 2018	179	4	72.9	0.0287	0.675	0.788
	Fall 2019	175	9	69.2	0.0298	0.636	0.753
	Summer 2021	162	48	48.7	0.0325	0.427	0.555
	Fall 2021	114	4	47.0	0.0325	0.410	0.538
	Summer 2022	110	3	45.7	0.0324	0.398	0.525
Sterile[b]	Fall 2017	240	79	67.1	0.0303	0.614	0.733
	Summer 2018	161	33	53.3	0.0322	0.474	0.600
	Fall 2018	128	5	51.2	0.0323	0.453	0.580
	Summer 2021	122	25	40.7	0.0318	0.350	0.475
	Fall 2021	97	8	37.4	0.0313	0.317	0.441
	Summer 2022	89	10	33.2	0.0305	0.277	0.397

Table 2. Linear mixed effects regression model performed on diversity produced by the Shannon-Wiener index (H'). "Whole" represents inoculation with whole prairie soil; "Selected" represents inoculation by beneficial AM fungal taxa microscopically isolated in the laboratory; "Sterile" represents sterilized soil inoculum. Means that share a letter are not different ($p \leq 0.05$). The "selected" inoculation group is the beta intercept for the model output.

Inoculum	Beta	EM Mean	Lower 95% CI	Upper 95% CI
Whole	0.08	1.68[a]	1.06	2.31
Selected	1.67	1.66[a]	1.04	2.28
Sterile	0.05	1.63[a]	1.01	2.25

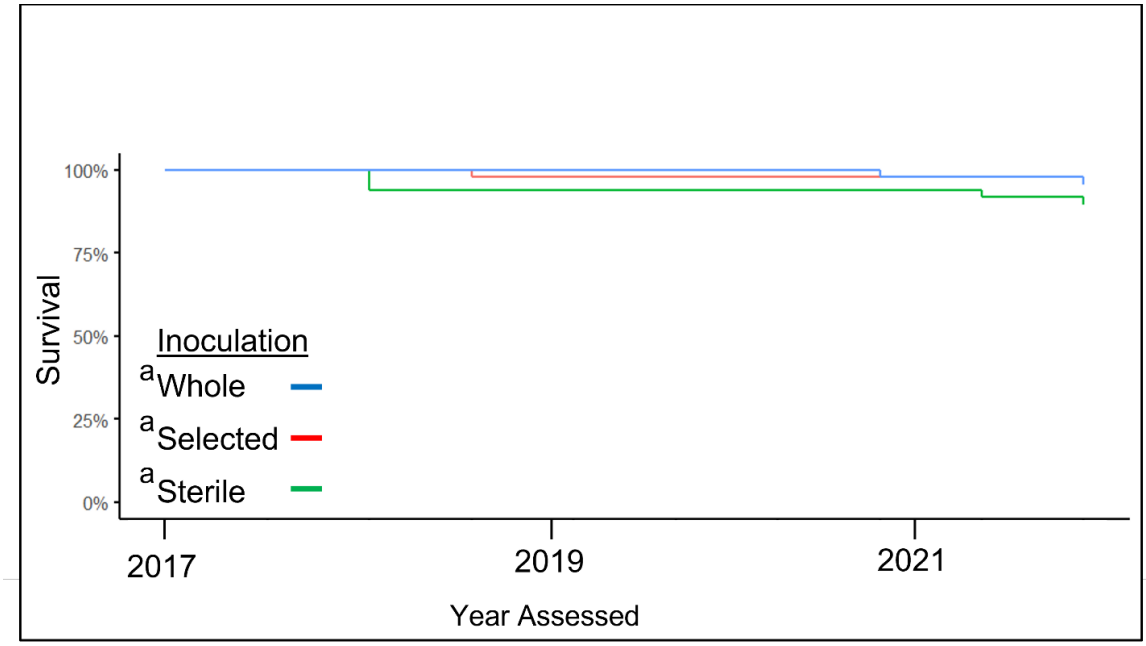
Random Effects	Std. Deviation
Plot	0.497
Subplot	0.069
Sampling Event	0.406
Residual	0.356

Table 3. Linear mixed effects regression model performed on *B. bladhii* cover of experimental plots following solarization. "Whole" represents inoculation with whole prairie soil; "Selected" represents inoculation by beneficial AM fungal taxa microscopically isolated in the laboratory; "Sterile" represents sterilized soil inoculum. Means that share a letter are not different ($p \leq 0.05$). The "selected" inoculation group is the beta intercept for the model output.

Inoculum	Beta	EM Mean	Lower 95% CI	Upper 95% CI
Whole	-2.356	44.7[a]	19.8	69.6
Selected	47.09	47.1[a]	22.2	72.0
Sterile	0.803	47.9[a]	23.0	72.8

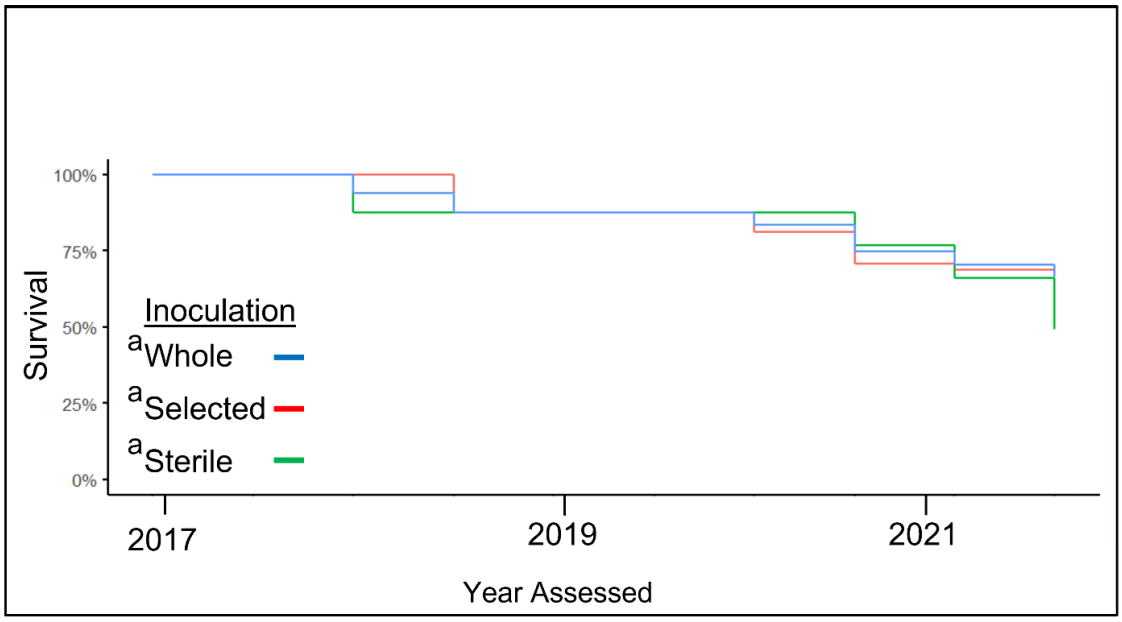
Random Effects	Std. Deviation
Plot	14.94
Subplot	2.296
Sampling Event	16.31
Residual	16.08

FIGURES



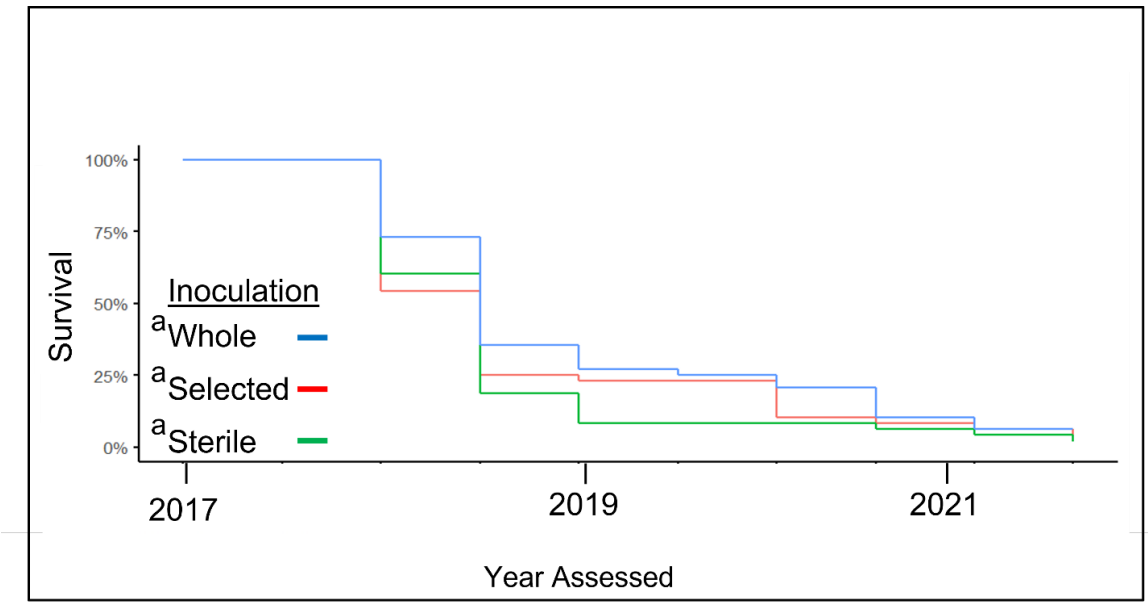
Andropogon gerardii

Figure 1. *Andropogon gerardii* survival across sampling periods. “Whole” represents inoculation with whole prairie soil; “Selected” represents inoculation by beneficial AM fungal taxa microscopically isolated in the laboratory; “Sterile” represents sterilized soil inoculum. In the legend, inoculation that share a letter are not different ($p \leq 0.05$).



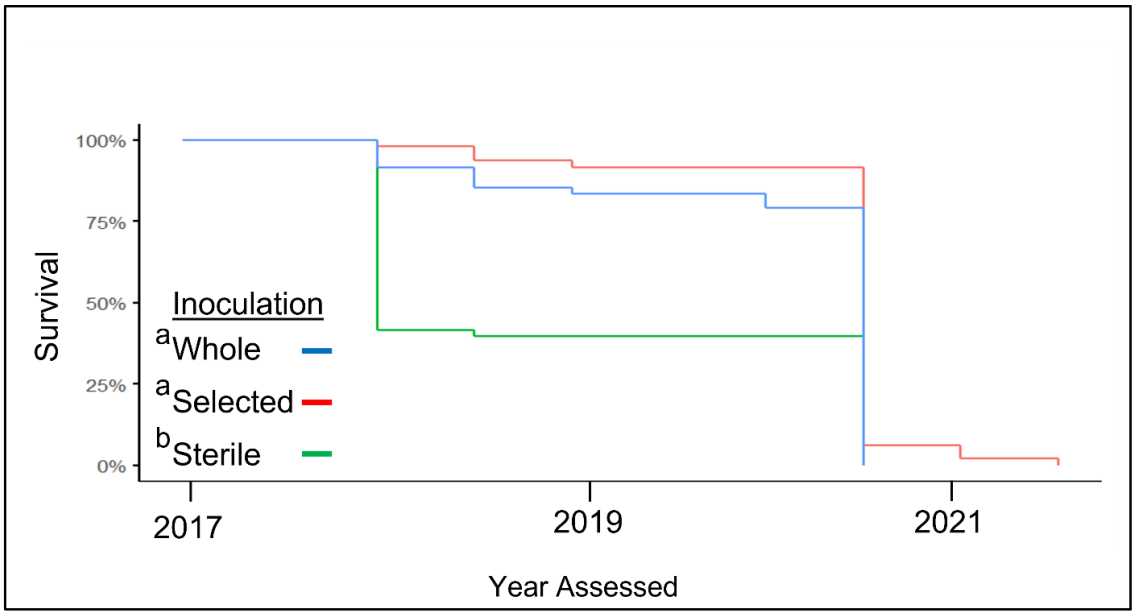
Pascopyrum smithii

Figure 2. *Pascopyrum smithii* survival across sampling periods. “Whole” represents inoculation with whole prairie soil; “Selected” represents inoculation by beneficial AM fungal taxa microscopically isolated in the laboratory; “Sterile” represents sterilized soil inoculum. In the legend, inoculation that share a letter are not different ($p \leq 0.05$).



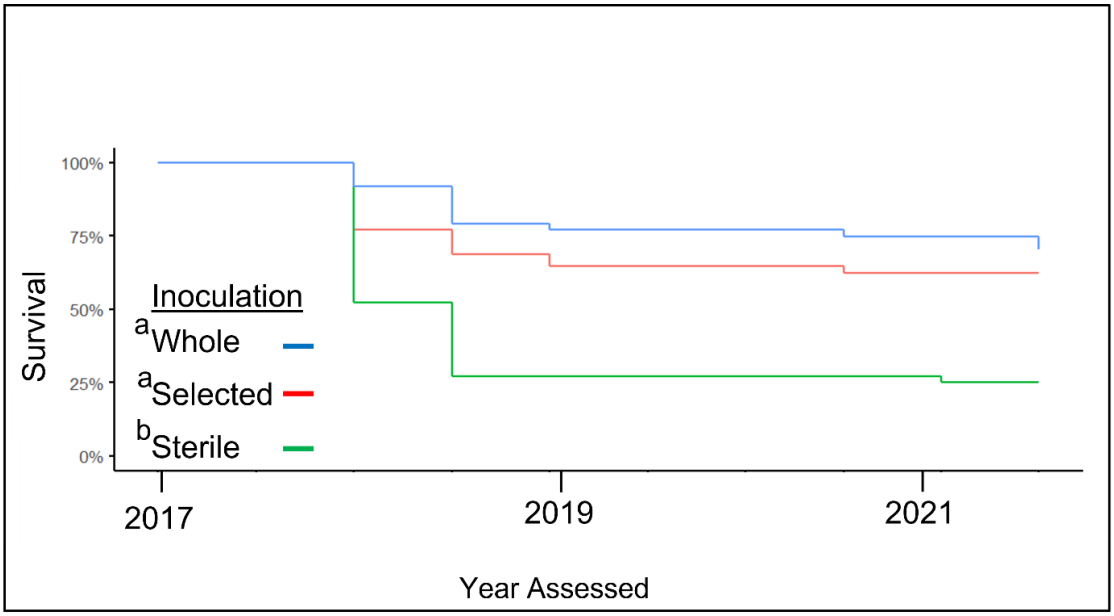
Asclepias syriaca

Figure 3. *Asclepias syriaca* survival across sampling periods. "Whole" represents inoculation with whole prairie soil; "Selected" represents inoculation by beneficial AM fungal taxa microscopically isolated in the laboratory; "Sterile" represents sterilized soil inoculum. In the legend, inoculation that share a letter are not different ($p \leq 0.05$).



Ratibida columnifera

Figure 4. *Ratibida columnifera* survival across sampling periods. “Whole” represents inoculation with whole prairie soil; “Selected” represents inoculation by beneficial AM fungal taxa microscopically isolated in the laboratory; “Sterile” represents sterilized soil inoculum. In the legend, inoculation that share a letter are not different ($p \leq 0.05$).



Lespedeza capitata

Figure 5. *Lespedeza capitata* survival across sampling periods. “Whole” represents inoculation with whole prairie soil; “Selected” represents inoculation by beneficial AM fungal taxa microscopically isolated in the laboratory; “Sterile” represents sterilized soil inoculum. In the legend, inoculation that share a letter are not different ($p \leq 0.05$).

CHAPTER II

DIRECTION AND MAGNITUDE OF PLANT-SOIL FEEDBACKS FOR NATIVE AND INVASIVE PRAIRIE GRASSES SUBJECTED TO HERBIVORY

ABSTRACT

The plant-soil feedback (PSF) framework allows researchers to target the interplay of plants and root-associated microbes and to determine the reciprocal effects of these interactions. PSFs in terrestrial ecology are well documented, but the strength and direction of feedbacks as influenced by management practices, such as herbivory or mowing, has not been fully explored. The replacement of native plant communities by monocultures of nonnative, invasive plant species is degrading biodiversity, functionality, and productivity of remaining remnant tallgrass prairies. Restoration projects typically focus on aboveground processes with a lack of focus on potentially altered belowground aspects, specifically the arbuscular mycorrhizal (AM) fungal community. To restore grasslands following invasion by non-native grasses, a deeper understanding of the belowground mechanisms facilitating invasive dominance is needed. As research has shown that alterations to PSFs can be buffered by management practices, such as herbivory, I investigated the influence of herbivory (clipping) as a potential restoration tool. In my greenhouse study, I examined PSF responses of both cool- and warm-season native and non-native grasses and determined how herbivory plays into this dynamic. My findings suggest that changes in management practices (herbivory) impact the strength and direction of PSFs, and these changes may also impact the interactions of plants and their associated soil microbial communities.

Clipping resulted in a neutralizing effect on the PSF direction, for both warm- and cool-season grasses, as the interaction coefficients were pushed from the established direction towards neutrality. My research suggests alterations in soil microbial communities by invasive grasses likely increase invasion success. However, reintroducing herbivory into invaded areas may be a pathway to reset the PSFs established by invasive plant species, potentially negating soil legacies by buffering the benefits invasive species establish through alterations in soil microbiomes.

INTRODUCTION

As little as 1% of the historic range of North American tallgrass prairies remains intact (Samson and Knopf 1994). Historically, the largest loss of North American grasslands was due to conversion to row-crop agriculture. Currently, alterations of fire regime, urban sprawl, and the introduction of non-native invasive species are largely responsible for further losses of this ecosystem. In fact, alterations due to non-native invasions is considered a primary driver of biodiversity loss in grasslands (Wilcove et al. 1998, Powell et al. 2011, Downey and Richardson 2016). While mechanisms for invasion success of non-native species is complex, biodiversity loss resulting from non-native plant invasion is a global concern and, therefore, an improved understanding of underlying mechanisms facilitating non-native species is needed. Often, the primary focus of invasion and restoration projects is assessing aboveground parameters, with less focus placed on the potentially altered belowground processes. However, above- and belowground communities are inextricably linked, and it is well documented that soil organisms play important roles in regulating ecosystem-level processes in native systems.

Plants can alter soil characteristics in ways that feed back to affect the performance of that species or other plant species (Bever et al. 1997). While these plant-soil feedbacks (PSFs) may alter the success of native or non-native species, with individual studies showing both positive and negative feedbacks during invasion (Reinhart and Callaway 2006, Bever et al. 2010,

Duell et al. 2019), research has shown that interactions between non-native plants and belowground microorganisms typically play fundamental roles in invasion success of non-native plant species (Vogelsang and Bever 2009, Zubek et al. 2016, Moyano et al. 2020). Therefore, recovery of altered soil microbial communities, particularly arbuscular mycorrhizal (AM) fungal communities, may be critical for restoration success following eradication of non-native grasses (Koziol and Bever 2017).

Mycorrhizal fungi form symbiotic associations with plant roots and aid in plant uptake of limiting soil resources such as phosphorus, nitrogen, and water. In exchange, plants deliver carbon to their obligate symbionts in the form of sugars (Smith and Read 2008). Fungal species richness and composition influence both functionality and productivity of ecosystems (Vogelsang et al. 2006, Wagg et al. 2011, Bainard et al. 2017), and disruption of this symbiotic association can reduce carbon sequestration and soil aggregate stability, thereby potentially contributing to soil erosion and the overall degradation of suitable habitat (Wilson et al. 2009). Mycorrhizal fungi can contribute to PSF, as AM fungal taxa can exhibit host-specific growth responses (Bever 2002a, Kiers et al. 2011), and the benefits a given plant receives can depend on the identity of associated AM fungal taxa (e.g., Johnson et al. 2010, Hoeksema et al. 2010). Non-native invasive plant species have been shown to alter the density and/or composition of the AM fungal communities, which may feedback on the subsequent spread of the non-native species (Bever 2002b, 2003, Reinhart and Callaway 2006, Lau and Suwa 2016, Zubek et al. 2016). Duell et al. (2019) suggested that abiotic factors, such as drought, can undermine forces that stabilize beneficial negative PSFs, which may further promote monotypic stands of invasive plants. While invasive plants have been shown to alter the direction of PSFs, management factors, such as herbivory, may also influence the direction of feedbacks, and may be exasperated in areas invaded by non-native plant species. Heinze et al. (2019, 2020) reported aboveground herbivory drives the direction of the PSF to a neutral state, and this effect increases as the intensity of

herbivory increases. However, previous research has not examined PSF dynamics in the context of invasive plant species combined with common management practices such as herbivory. A deeper understanding of the influence of herbivory on the direction of PSFs in grasslands invaded by nonnative grasses may add to our understanding of potential indirect methods utilized by invasive plant species to establish and maintain dominance. Therefore, I conducted a greenhouse study that included both above- and belowground measurements to assess contrasting PSFs of native and non-native plant species, providing key insights to improve restoration success following eradication of monotypic stands of invasive plant species.

I hypothesized that in the absence of herbivory, native grasses would generate a negative PSF that would promote coexistence and diversity while the invasive grasses would generate a positive PSF to further facilitate dominance (hypothesis 1). Also, for both native and invasive grasses, I hypothesized the presence of herbivory (clipping) would reduce PSF effects, driving PSFs to a more neutral state (hypothesis 2).

MATERIALS AND METHODS

Experimental setup: Two native and two non-native grasses were selected as model species for this greenhouse experiment. *Pascopyrum smithii* (Rydb.) Á. Löve, a native cool-season perennial grass and sub-dominant species of North American grasslands was paired with *Bromus inermis* Leyss., a functionally similar non-native cool-season perennial grass that often grows in dense monocultures, replacing native grass species throughout North America. *Schizachyrium scoparium* (Michx.) Nash, a dominant warm-season prairie grass native to North America was paired with *Bothriochloa ischaemum* (L.) Keng, an aggressive invader of North American prairie ecosystems with similar functionality. This experiment was conducted in two phases: 1) conditioning phase and 2) experimental phase (Figure 6). The conditioning phase was set up as a randomized block design consisting of 8 treatment combinations (4 plant species x 2 clipping

treatments) with 25 replications for a total of 200 soil conditioning pots. The cool-season grass seeds were sourced from Sharp Brothers (Healy, KS) and the warm-season grass seed from Johnston seed company (Enid, OK). The seeds were planted in trays of vermiculite until germination occurred and seedlings reached the two-leaf stage. Seedlings of each species were then individually transplanted into plastic pots (6 cm dia. X 25 cm depth) containing approximately 600 g (dry wt) of native prairie soil collected from the Oklahoma State University Range Research Station, Stillwater, OK. Soil had a pH of 7.94, contained 11.75 mg kg^{-1} NO_3, 6.81 mg kg^{-1} NH_4, 12.6 mg kg^{-1} plant-available P, and 281 mg kg^{-1} plant-available K, as determined by the Soil, Water, and Forage Analytical Laboratory at Oklahoma State University. Soil pH was quantified using a pH electrode in a 1:1 soil to water suspension. Soil NO_3-N and NH_4-N were extracted with 1 M KCl solution and measured by a Lachat Quickchem 8000 Flow Injection Autoanalyzer. Plant-available P and K were extracted using Mehlich 3 solution (Mehlich 1984), and P and K in the extract were measured by inductively coupled plasma emission spectroscopy (ICP). Seedlings were grown for 8 weeks, at which time simulated herbivory (clipped with scissors to a height of 4 cm, based on Pfeiffer and Hartnett 1995) was imposed every other week. Plants conditioned the soil for a total of three months, and plants were clipped twice in the final month of conditioning. After the conditioning period, the second phase (experimental phase) of the experiment was initiated using the conditioned soil as microbial inoculum for the next generation of seedlings. The experimental phase was set up in a similar randomized block design but consisted of a total of 16 treatment combinations [4 plant species x 4 soil conditioning treatments (clipped or unclipped, soil conditioned by the native or nonnative)] with 8 reps each for both cool- and warm-season grasses for a total of 128 pots. Grasses in the experimental phase were grown in “home” (conditioned by conspecifics) or “away” (conditioned by heterospecifics) soil for 16 weeks, at which time signs of senescence became apparent and the project takedown for final data collection occurred. To minimize the possible impacts of season on functional group, the cool-season species (*P. smithii* and *B. inermis*) were grown during their

favored growing conditions (April - July) and the experiment assessing warm-season plants was conducted during the summer (May - August). Plants were fertilized with ammonium nitrate on a biweekly basis at a rate representative of the natural conditions of a tallgrass prairie ecosystem (Seastedt et al. 1991).

Intra-radical AM fungal assessments: For harvesting and data collection of each phase, roots were washed to remove soil, and above- and belowground biomass were separated and oven-dried for 48 hours at 60℃ and weighed immediately upon removal from the oven. Approximately 0.25 g of representative root was sub-sampled from each individual root system and stained with trypan blue to assess percent root colonization by AM fungi. Percent intra-radical AM fungal colonization was determined using the magnified gridline intersect method (McGonigle et al. 1990). A digital microscope (Hirox KH 7700) at 100x magnification was used to assess total intra-radical hyphal abundance, including all AM fungal structures (intra-radical hyphae, arbuscules, vesicles, and coils) of the stained root samples. Three representative strands from each subsampled root biomass were assessed at 150 reads per strand for a total of 450 per sample.

Extra-radical AM fungal assessments: Relative abundances of extra-radical AM fungi were assessed using signature fatty acids (Olsson et al. 1995). Phospholipid fatty acids (PLFAs) are constituents of biological membranes that can be used to estimate active biomass of bacteria and fungi as biovolume, and cell surface area are well-correlated (Frostegard et al. 2011). Neutral lipid fatty acids (NLFAs) are basic storage products of many fungi and serve as the primary energy reserve in AM fungi, representing spore abundance (Sharma and Buyer 2015). Soil samples were freeze-dried and finely ground with a mortar and pestle, and 5 g of soil was mixed with 200 ml of 50 mM phosphate buffer, 500 ml methanol, and 250 ml chloroform. The soil-solvent mixture was separated by centrifugation and then decanted with a 1:2 mix of chloroform and methanol. Phosphate buffer was added and left for phase separation to occur overnight (12 hours), and then the chloroform layer containing the lipids was recovered and reduced by

nitrogen flow at 50°C. Lipids were separated into neutral lipids, glycolipids, and phospholipids by solid-phase extraction by eluting with chloroform, acetone and methanol, respectively. Lipids were hydrolyzed and methylated. The methylated fatty acids were extracted with 75 µl hexane and evaporated under nitrogen at 37°C (Buyer and Sasser 2012).

The PLFA/NLFA analyses were performed using an Agilent 7890A gas chromatograph with an Agilent 5975C series mass selective detector. We utilized c:19 as an internal standard. For AM fungal extra-radical hyphal (ERH) biomass, selected biomarkers were 16:1ω5c, 20:1ω9, and 22:1ω13 for both PLFA and NLFA determination (Sakamoto et al. 2004). The abundances associated with these biomarkers were used to calculate a total nmol per gram of soil.

PSF assessments: Plant-soil feedbacks were calculated for warm- and cool-season grass pairs separately. The direction and magnitude of each PSF was determined as a function of biomass production or AM fungal colonization. Interaction coefficients (I_s) were used to quantify the direction and magnitude of the PSF and were calculated for all treatments (native and nonnative plants grown in soil conditioned by clipped and not clipped hetero- or conspecifics) using the following equation: $I_s = G(A)\alpha - G(A)\beta - G(B)\alpha + G(B)\beta$ (Bever 1997, Duell et al. 2019). For this equation, A represents the native grass, B represents the non-native, α represents soil conditioned by the native or absence of clipping, β represents soil conditioned by the non-native or presence of clipping, and G was interchanged with biomass, intra-radical root colonization, extra-radical hyphal abundance (PLFA), or relative spore abundance (NLFA) as needed to calculate the interaction coefficient (Figure 7).

Statistics: Warm- and cool-season grass data were analyzed separately due to functional trait differences and differing levels of AM fungal dependency (Miller 2012). All data were tested for normality and homogeneity of variance by viewing distribution plots and performing Shapiro-Wilk and Levene's tests prior to performing any analyses. AM fungal root colonization data was

recorded as proportion data but log-transformed and fit to a general linear model (GLM) (Duell et al. 2019, 2021). For both the cool- and warm-season data, global models were created that considered the full effects of the explanatory variables and their interactions.

The impact of species and clipping on phase 1 AM fungal root colonization and species, clipping, and soil conditioning by species on the phase 2 colonization was performed with Gaussian error distributed GLM's due to the inherent normal nature of the data. Analyses of the impacts of species, clipping, and soil conditioning on phase 2 grass biomass were performed using similar methods when normality was confirmed. When right-skewed data were found in the belowground biomass results of the warm season grasses, the data were assessed using a GLM with gamma error distribution (log-link) to achieve approximate normality. All post-hoc analyses of established GLM's were performed with the "emmeans" (Lenth 2022) package that utilizes the estimated marginal means ($\alpha = .05$). All statistical methods above were performed using R-software version 4.0.4 (R Core Team 2021).

Extra-radical fungal abundance (NLFA and PLFA) was assessed using a GLM. The response variable was AM fungal abundance in nmol/g soil and the predictors were the species at experimental conclusion, the species that conditioned the soil in phase 1, and the presence or absence of simulated herbivory in phase 1. All data collected for AM fungal abundance were assessed with a Gaussian error GLM due to inherent normality.

RESULTS

Cool-season grasses

AM Fungal Colonization: Simulated herbivory had no effect on AM fungal colonization of cool-season grasses (Figure 8). Colonization of AM fungi in cool-season grasses was consistent across all treatments, despite differing levels of mycorrhizal dependency between the two species, with *P. smithii* generally having a higher dependency (Wilson and Hartnett 1998). At completion of

the experimental phase (phase 2), significant effects were detected for experimental species ($p < 0.01$; $F = 22.191$; df=1) and the three-way interaction of species, treatment, and clipping ($p < 0.01$; $F = 15.926$; df = 1) had a strong influence over the colonization levels (Table 4). Colonization in phase 2 fluxed more than that of phase 1 (Figures 9a, and 9b). Growth of *P. smithii* in soil conditioned by conspecifics led to significantly higher levels of colonization compared to growth in soil conditioned by heterospecifics, while no difference was detected for *B. inermis* roots following growth in soil conditioned by conspecific or heterospecific species (Figure 10a). Soil conditioned by clipped or unclipped phase 1 plants did not impact the phase 2 species' AM fungal colonization (Figure 10b). For either species, the clipped and not clipped treatment groups were not significantly different from one another (Table 4).

Biomass Production: Aboveground (shoot) and belowground (root) biomass production were not statistically different from one another, nor total biomass production regardless of clipping treatment (Table 4). Therefore, results will be presented only as total biomass production. There was a significant main effect of conditioning species identity on above-, belowground, and total plant biomass production of experimental plants (Table 4). In phase 2, both cool-season grass species (*P. smithii* or *B. inermis*) produced similar biomass production regardless of clipping treatment among their respective treatment groups (Figures 9a and 9b). AM fungal colonization for *P. smithii* was reduced when plants were grown in soils conditioned by its invasive counterpart, *B. inermis* (Figure 10).

PSF Interaction Coefficients: For above, below, and total biomass, native *P. smithii* maintained a negative interaction coefficient (I_S) ($I_s = -0.123, -0.180$; Table 5) while non-native *B. inermis* maintained a positive I_S ($I_s = 0.058, 0.244$; Table 5). However, when AM fungal colonization was used as the primary variable in the coefficient calculation (G, Figure 7), the opposite trend was observed. Following clipping, a strong alteration to the PSF, determined by I_S value, was apparent, as the reported coefficients responded by moving in the opposite direction of the not

clipped PSFs towards neutrality (Table 5). In response to clipping, the total biomass focused PSF moved from negative to neutral to weakly positive (not clipped I_s = -0.112, Clipped I_s = 0.069; Table 5). A neutralizing effect was also apparent for belowground biomass of cool-season plants, with an overall trend of interaction coefficients shifting from negative to positive in response to simulated herbivory (Table 5). The PSF coefficients calculated with extra-radical hyphal abundance (PLFA), or relative spore abundance (NLFA) produced no discernible patterns and were therefore not included.

AM Extra-radical Fungal Abundance: No clear effects occurred due to any variables or their interactions on AM spore abundance (NLFA) or extra-radical hyphal abundance (PLFA) (Table 10).

Warm-season grasses

Intra-radical AM fungal abundance: Similar to the cool-season grasses, there was no effect of clipping on percent AM fungal colonization of warm-season grasses; following phase 1 neither *S. scoparium* nor *B. ischaemum* were significantly different in intra-radical fungal colonization (Figures 13a and 13b). However, intra-radical AM fungal colonization of *S. scoparium* was greater than *B. ischaemum* when not subjected to clipping (Figure 13b).

Following growth in phase 2, soils conditioned by clipping produced no differences in intra-radical fungal colonization of warm-season grasses despite the statistical significance of the variable ($p < 0.01$; $F = 15.928$; $df = 1$; Table 6). The plant species which conditioned the soil in phase 1 also significantly affected intra-radical AM hyphal abundance ($p < 0.01$; $F = 7.703$; $df = 1$; Table 6). When grown in soil conditioned by conspecifics, *S. scoparium* exhibited greater levels of colonization than when grown in heterospecific soil (Figure 8a). While growth in any of the conditioned soils produced no differences for the AM fungal root colonization of *B. ischaemum* (Figure 13b).

Extra-radical AM Fungal Abundance: Extra-radical fungal spore and hyphal abundance (PLFA and NLFA) for warm-season grasses was significantly influenced by the plant species that conditioned the soils (Table 9). According to the PLFA results, soil conditioned by clipping increased AM hyphal abundance, compared to the invasive *B. ischaemum* (Table 11). Similarly, when grown in soils conditioned by clipped invasives, the native *S. scoparium* responded with increased AM hyphal abundance (Table 11). AM spore abundance (NLFA) associated with either *S. scoparium* or *B. inermis* was generally not significantly different across species or clipping (Table 11).

Biomass Production: Aboveground (shoot) and belowground (root) biomass production were not statistically different from one another, nor total biomass production regardless of clipping treatment (Table 4). Therefore, results will be presented only as total biomass production. Total biomass production was influenced by the interaction of the plant species used to condition the soil in phase 1 and by clipping ($p < 0.01$; $F = 10.089$; $df = 1$; Table 6). When soil was conditioned by not clipped *S. scoparium*, the total biomass of both *S. scoparium* and *B. ischaemum* tended to be greater than when grown in soils conditioned by clipped *S. scoparium* (Figure 12a). Conversely, when the soil was conditioned by clipped *B. ischaemum*, the phase 2 plants responded with a tendency for greater biomass production than when grown in soils conditioned by not clipped *B. ischaemum* (Figure 12b). However, while these patterns were observed, there was no significant difference in biomass production in response to the plant identity or clipping treatments that conditioned the soils in phase 1 (Figures 12a and 12b).

PSF Interaction Coefficients: For both colonization and biomass interaction coefficients, *S. scoparium* produced a positive PSF ($I_s = 0.147, 0.052$; Table 7). Conversely, *B. ischaemum* maintained a negative PSF for total biomass and intra-radical AM fungal colonization ($I_s = -0.059, -0.092$; Table 7). The PSF altering effect of clipping can be seen in the interaction coefficients calculated, as the PSF coefficients shifted in the opposite direction in response to

clipping (Table 7). A strong neutralizing effect was reported for the biomass calculated coefficients (Not clipped I_s = -0.213, Clipped I_s = -0.006; Table 7) while the intra-radical AM fungal colonization coefficients shifted from weakly negative to a weakly positive value (Not clipped I_s = -0.047, Clipped I_s = -0.096; Table 7). No discernible pattern was produced by calculating the PSF with warm-season extra-radical hyphal abundance (PLFA) or relative spore abundance (NLFA) and are therefore not included.

DISCUSSION

When assessing PSF results, it helps to think of the interaction coefficient as a number that fits on a sliding scale and can fall into categories of strongly to weakly negative, moving into a neutral interaction coefficient, and continuing into weakly to strongly positive. The positive to negative direction of the feedback is largely driven by the presence and abundance of soil microorganisms. Negative PSFs occur when a plant performs better in soils conditioned by different species (heterospecific) and are primarily self-limiting, thus promoting community diversity, typical of native plant communities. Positive PSFs occur when a plant's growth is increased when grown in soils conditioned by itself (conspecific), often resulting in monotypic stands typical of invasive species (Bever et al. 1997, van der Putten et al. 2013, Crawford et al. 2017). While there is increasing evidence suggesting that abiotic factors, such as light availability or drought, can impact soil microbial communities and influence the strength and direction of feedback interactions (Rillig et al. 2002, Johnson et al. 2013, Smith & Reynolds 2015, Schmidt et al. 2018, Duell et al. 2019), the effects of management practices, such as herbivory, on the strength and direction of PSF interactions is much less known. My study is the first of its kind to test PSF theory with combined herbivory and non-native invasive plant species.

My findings suggest that changes in management practices (herbivory) impact the strength and direction of PSFs, and these changes may also impact the interactions of plants and

their associated soil microbial communities. In agreement with my first hypothesis, clipping resulted in a neutralizing effect on the PSF direction, for both warm- and cool-season grasses, as the interaction coefficients were pushed from the established direction towards neutrality. However, there was no effect of simulated herbivory on biomass production or intra-radical hyphal abundance for either functional group. The lack of substantial effect on biomass production following clipping plants was not unexpected, as plant growth responses to herbivory is variable and AM fungal colonization has been reported to increase, decrease, or exhibit no change in response to herbivory (Barto and Rillig 2010, Faghihinia 2020, Frew 2021). While there were no obvious aboveground effects of clipping in phase 1, the alteration in PSF indicates there were alterations in microbial communities associated with the grasses. This became apparent in phase 2, as microbial alterations likely influenced the greater biomass production of the nonnative grass species, *B. inermis*, when grown in soils conditioned by not clipped heterospecific species (*P. smithii)*, as compared to growth in soils conditioned by the conspecific species (*B. inermis)*.

With the production of a negative PSF, it is possible the invasive warm-season grass *B. ischaemum* was investing in contrasting AM fungal species, suggesting an alteration to the microbial community. That herbivory influenced the direction of PSFs in the absence of apparent aboveground differences may provide useful information for restoration following non-native plant species establishment. However, the effectiveness of herbivory as a restoration strategy to buffer belowground feedbacks established by nonnative plants appears to be species specific and the impacts of herbivory on the PSF should not be generalized. Using herbivory (or mowing) to buffer PSF alterations are best approached by taking into consideration what plant species has invaded the area and what species a restoration manager is considering for reintroduction.

I also hypothesized that the native species (*P. smithii* or *S. scoparium)* would tend towards a PSF in the negative direction, while the non-native, invasive species (*B. inermis* or *B.*

ischaemum) would tend towards a PSF in the positive direction. This hypothesis was supported for the cool-season grasses grown in soil conditioned by non-clipped *P. smithii* plants, as the PSF was negative. Previous research has shown the presence of invasive non-native plant species can alter the density and composition of AM fungal communities, potentially influencing feedback interactions and effecting subsequent growth and establishment of both native and invasive species (Reinhart and Callaway 2006, Vogelsang and Bever 2009, Allen et al. 2018), as was observed in my study. The alteration to a positive I_s observed when *B. inermis* was conditioning the soils likely reflects that this non-native species takes advantage, thrives, and inherently begins imposing selective pressures on soil microbial communities following introduction into a new environment. Soil microbial communities are composed of a rich suite of AM fungal taxa that perform different variations of nutrient uptake, pathogenic defense, or environmental stress resistance (Kiers et al. 2011). The preferred symbiotic partners of one plant species typically differ between competing species (Kiers and Denison 2008, Bever et al. 2009, Miller et al. 2012). Soil microbial alterations allow non-native species to establish a positive PSF for the system, as plant invasion can disrupt mutualistic interactions between native plants and soil microbial communities, further increasing ecosystem susceptibility to invasion (Bever et al. 1997, Bever et al. 2010, Duell et al. 2019).

Despite the hypothesis that the native species (*S. scoparium)* would tend towards a PSF in the negative direction, and non-native (*B. ischaemum)* would tend toward a positive PSF, the opposite was found. Native species *S. scoparium* tended towards a positive direction, while the non-native, invasive species *B. ischaemum* tended towards a PSF in the negative direction. These responses to soil conditioning (phase 1) followed similar trends for both intra-radical AM fungal colonization and biomass production. The unexpected positive PSF of *S. scoparium* may reflect that the dominant native grass formed a beneficial relationship with the same AM fungal taxa preferred, and therefore selected, by the invasive *B. ischaemum*. Previous research has proposed

non-native invasive species tend to form positive PSF, as a mechanism for developing and sustaining a monotypic stand. A negative PSF is generated by the differing selective pressures on the soil microbial community (Bever 2002b). *Bothrichloa ischaemum* has been documented as highly invasive, establishing monotypic stands described as a moving front (Reed et al 2005, Greer et al. 2014). The unexpected negative PSF of *B. ischaemum* may reflect changes in the AM fungal community in phase 1 (Bever 2002a), as warm-season grasses, such as *B. ischaemum*, readily associate with AM fungi (Wilson & Hartnett 1998, Wilson et al. 2012) and can alter the soil community. In my study, it is clear *B. ischaemum* is altering the soil microbial community as it produced an overall negative PSF coefficient, likely from sharing a different selection of mutualistic partners as native species *S. scoparium* (Bever 2002a). In phase 2 of my study, the native *S. scoparium* may have taken advantage of the changes in the fungal community composition more effectively than the non-native species. We can speculate based on these results that *S. scoparium* may be forming stable symbioses with the same fungal partners as *B. ischaemum,* but the nonnative prefers to select for separate AM fungal taxa, reinforcing the monoculture as it expands. Due to a lack of clarity provided by AM fungal abundance results, proper confirmation will require molecular assessments to define the specific AM fungal taxa selected by each plant species.

Consistent differences in biomass and AM fungal abundance were observed between the cool- and warm-season grass species, as warm-season grasses produced greater biomass and intra-radical hyphal abundance, compared to cool-season grasses. Cool-season grasses typically exhibit a facultative dependence on the symbiosis, whereas warm-season grasses generally have a high dependency on AM fungal symbiosis, and typically have greater fungal abundance to support the high dependence (Wilson and Hartnett 1998, Hoeksema et al. 2010, Miller, Wilson, and Johnson 2012). In fact, both warm-season grasses used in this study (*S. scoparium* and *B. ischaemum*) have been previously found to be highly dependent, whereas both cool-season

grasses (*P. smithii* and *B. inermis*) were reported as facultative, not requiring the symbiosis for biomass production in low nutrient soil (Wilson and Hartnett 1998). Non-native plant species are also often less dependent on native AM fungi, compared to native species, decreasing AM fungal densities that leads to a decrease of native plant growth (Vogelsang and Bever 2009, Pringle et al. 2009, Zubek et al. 2016, Grove et al. 2017). However, in my study, neither invasive species were characterized by lower AM fungal abundance, compared to their corresponding native species. Alternatively, invasive species can be highly dependent on AM fungal associations yet alter local soil microbial community composition (Wilson et al. 2012, Zubek et al 2016, Ba et al. 2018, Zhang et al. 2019). Importantly, the lack of difference in AM fungal abundance in this study does not negate the possibility that microbial communities were altered in response to the plant species selected for soil conditioning in phase 1, or in response to clipping in phase 1. Identification of the AM fungal partners of the two species will provide more insight into the indirect relationship between native and non-native species, will allow for the confirmation of a partner taxa preference, and determine the shifts in AM fungal taxa following aboveground biomass removal by simulated herbivory or management practices such as grazing or mowing. As molecular identification of AM fungal taxa continues to improve for both efficiency and availability, identification of specific taxa is becoming more dependable. Confirmation of AM fungal partner preference at the species level is important for successful restoration projects, as it is clear belowground communities are inextricably linked to the diversity and productivity of aboveground biomass production and plant species diversity. Proper management of the belowground communities is tightly linked to aboveground dynamics, and restoration following eradication of non-native invasive plant species requires careful assessments of soil microbial dynamics to increase the longevity and success of native plant reintroduction.

My findings are directly applicable to managing land and planning restorations to achieve biodiversity goals. Understanding plant-soil feedbacks is also germane to improving prediction of

species, community, and ecosystem response following establishment of invasive plant species, with important broader impacts for ecological restoration. My research indicates herbivory may be an effective restoration strategy to reduce the positive PSFs observed for invasive species *B. inermis*. However, the effectiveness of herbivory as a restoration strategy to buffer plant soil feedbacks established by nonnative plants is likely highly species specific, the influence of herbivory on the PSF cannot be generalized, and the invasive species and the desired native plant community should be taken into consideration for management decisions. More research is required to confirm the extent to which soil environmental legacy affects the following year's PSF, especially in the context of management such as aboveground plant removal through herbivory or mowing. Furthermore, my study consisted of two pairings of functionally-similar native and non-native invasive prairie grasses, and additional species should be assessed to further our knowledge of the role of soil conditioning on invasive species PSF dynamics. Previous research has indicated the effect of aboveground herbivory on the direction of plant-soil feedbacks increases as the intensity of herbivory increases (Heinze et al. 2019, 2020), therefore, future studies should also include a wide range of herbivory intensities. There are still many questions surrounding plant invasion dynamics, and research such as my current study provide key insight into plant-soil-microbial interactions following plant invasion and herbivory reintroduction in a grassland system; understanding of the importance of plant-microbiome interactions may motivate interest in the management of host microbiomes to benefit restoration success.

TABLES

Table 4. The effects of species, conditioning, and clipping on cool-season native and invasive biomass production and root colonization by AM fungi. Where "species" represents the plant species grown in each pot, "conditioning" represents the phase 1 species that conditioned the soil, and "clipping" represents simulated herbivory. All analyses were performed with a confidence interval of 0.95.

Cool-season			
Source	df	F	P
Total biomass			
Species	1	10.365	< **0.01**
Conditioning	1	28.924	< **0.01**
Clipping	1	0.102	0.75
Species x Conditioning	1	0.407	0.53
Species x Clipping	1	0.486	0.49
Conditioning x Clipping	1	0.179	0.67
Species x Conditioning x Clipping	1	2.811	0.09
Aboveground biomass			
Species	1	2.904	0.09
Conditioning	1	19.394	< **0.01**
Clipping	1	0.221	0.64
Species x Conditioning	1	0.460	0.50
Species x Clipping	1	0.139	0.71
Conditioning x Clipping	1	1.222	0.27
Species x Conditioning x Clipping	1	0.828	0.37
Belowground biomass			
Species	1	48.770	< **0.01**
Conditioning	1	19.701	< **0.01**
Clipping	1	0.985	0.33
Species x Conditioning	1	2.969	0.09
Species x Clipping	1	0.598	0.44
Conditioning x Clipping	1	0.166	0.68
Species x Conditioning x Clipping	1	3.403	0.07
AM fungal colonization			
Species	1	22.191	< **0.01**
Conditioning	1	2.091	0.15
Clipping	1	0.010	0.92
Species x Conditioning	1	20.740	< **0.01**
Species x Clipping	1	0.480	0.49
Conditioning x Clipping	1	0.527	0.47
Species x Conditioning x Clipping	1	15.926	< **0.01**

Table 5. Interaction coefficients calculated for phase 2 (experimental) native and invasive cool-season grass species. Where "*P. smithii*" represents soil conditioned by the native, "*B. inermis*" represents soil conditioned by the non-native, "not clipped" represents soil conditioned in the absence of simulated herbivory, and "clipped" represents soil conditioned in the presence of herbivory.

Soil Training	I_s
Total biomass	
P. smithii	-0.123
B. inermis	0.058
Not Clipped	-0.112
Clipped	0.069
Aboveground biomass	
P. smithii	-0.063
B. inermis	0.030
Not Clipped	-0.018
Clipped	0.075
Belowground biomass	
P. smithii	-0.135
B. inermis	0.056
Not Clipped	-0.158
Clipped	0.033
Colonization	
P. smithii	0.180
B. inermis	-0.244
Not Clipped	0.452
Clipped	0.028

Table 6. The effects of species, conditioning, and clipping on native and invasive warm-season biomass production and root colonization by AM fungi. Where "species" represents the plant species grown in each pot, "conditioning" represents the phase 1 species that conditioned the soil, and "clipping" represents the simulated herbivory treatment. All analyses were performed with a confidence interval of 0.95.

	Warm-season		
Source	df	F	P
Total biomass			
Species	1	0.123	0.73
Conditioning	1	0.009	0.92
Clipping	1	0.121	0.73
Species x Conditioning	1	1.899	0.17
Species x Clipping	1	0.577	0.45
Conditioning x Clipping	1	10.089	< **0.01**
Species x Conditioning x Clipping	1	1.665	0.20
Aboveground biomass			
Species	1	86.438	< **0.01**
Conditioning	1	0.117	0.73
Clipping	1	0.200	0.66
Species x Conditioning	1	2.182	0.15
Species x Clipping	1	0.360	0.55
Conditioning x Clipping	1	6.297	0.02
Species x Conditioning x Clipping	1	6.776	0.01
Belowground biomass			
Species	1	91.140	< **0.01**
Conditioning	1	0.302	0.59
Clipping	1	0.019	0.89
Species x Conditioning	1	0.741	0.39
Species x Clipping	1	0.503	0.48
Conditioning x Clipping	1	8.797	< **0.01**
Species x Conditioning x Clipping	1	0.279	0.60
AM fungal colonization			
Species	1	7.357	< **0.01**
Conditioning	1	7.703	< **0.01**
Clipping	1	15.928	< **0.01**
Species x Conditioning	1	0.225	0.64
Species x Clipping	1	3.329	0.07
Conditioning x Clipping	1	0.363	0.55
Species x Conditioning x Clipping	1	2.647	0.11

Table 7. Interaction coefficients calculated for phase 2 (experimental) native and invasive warm-season grass species. Where "*S. scoparium*" represents soil conditioned by the native, "*B. ischaemum*" represents soil conditioned by the non-native, "not clipped" represents soil conditioned in the absence of simulated herbivory, and "clipped" represents soil conditioned in the presence of herbivory.

Soil Training	I_s
Total biomass	
S. scoparium	0.147
B. ischaemum	-0.059
Not Clipped	-0.213
Clipped	-0.006
Aboveground biomass	
S. scoparium	0.198
B. ischaemum	-0.147
Not Clipped	-0.286
Clipped	0.059
Belowground biomass	
S. scoparium	0.033
B. ischaemum	0.045
Not Clipped	-0.043
Clipped	-0.022
Colonization	
S. scoparium	0.052
B. ischaemum	-0.092
Not Clipped	-0.047
Clipped	0.096

Table 8. The effects of species, conditioning, and clipping on AM fungal extra-radical hyphal (PLFA) and spore (NLFA) production associated with native and invasive cool-season plants. Where "species" represents the individual grown in each pot, "conditioning" represents phase 1 species that conditioned the soil, and "clipping" represents simulated herbivory. All analyses were performed with a confidence interval of 0.95.

Cool-season NLFA & PLFA			
NLFA	df	F	P
Species	1	0.303	0.59
Conditioning	1	0.001	0.98
Clipping	1	0.045	0.83
Species x Conditioning	1	0.047	0.83
Species x Clipping	1	0.402	0.53
Conditioning x Clipping	1	1.533	0.23
Species x Conditioning x Clipping	1	4.522	**0.04**
PLFA			
Species	1	1.673	0.21
Conditioning	1	0.162	0.69
Clipping	1	0.127	0.73
Species x Conditioning	1	1.401	0.25
Species x Clipping	1	2.005	0.17
Conditioning x Clipping	1	0.045	0.84
Species x Conditioning x Clipping	1	0.786	0.38

Table 9. The effects of species, conditioning, and clipping on AM fungal extra-radical hyphal (PLFA) and spore (NLFA) production associated with native and invasive warm-season plants. Where "species" represents the individual grown in each pot, "conditioning" represents phase 1 species that conditioned the soil, and "clipping" represents simulated herbivory. All analyses were performed with a confidence interval of 0.95.

Warm-season NLFA & PLFA			
NLFA	df	F	P
Species	1	11.126	< **0.01**
Conditioning	1	0.045	0.83
Clipping	1	2.231	0.15
Species x Conditioning	1	1.478	0.24
Species x Clipping	1	0.056	0.82
Conditioning x Clipping	1	1.998	0.17
Species x Conditioning x Clipping	1	1.665	< **0.01**
PLFA			
Species	1	26.233	< **0.01**
Conditioning	1	0.485	0.49
Clipping	1	54.599	< **0.01**
Species x Conditioning	1	3.818	0.06
Species x Clipping	1	4.396	**0.05**
Conditioning x Clipping	1	0.028	0.87
Species x Conditioning x Clipping	1	1.272	0.27

Table 10. Extra-radical AM fungal abundance of cool-season grasses following soil conditioning (phase 1). Phospholipid fatty acid (PLFA) represent hyphal abundance, neutral lipid fatty acid (NLFA) represents fungal spores associated with native and invasive cool-season grasses. Rows that share a superscript letter are not significantly different from one another ($p \leq 0.05$).

Cool-season		
Species Growing	Soil Conditioning	AM Fungal Abundance (nmol/g soil)
PLFA		
P. smithii	Not clipped *P. smithii*	0.71[a]
P. smithii	Clipped *P. smithii*	1.55 [a]
P. smithii	Not clipped *B. inermis*	0.63 [a]
P. smithii	Clipped *B. inermis*	0.90 [a]
B. inermis	Not clipped *P. smithii*	1.74 [a]
B. inermis	Clipped *P. smithii*	0.29 [a]
B. inermis	Not clipped *B. inermis*	1.87 [a]
B. inermis	Clipped *B. inermis*	1.69 [a]
NLFA		
P. smithii	Not clipped *P. smithii*	4.02 [a]
P. smithii	Clipped *P. smithii*	6.79 [a]
P. smithii	Not clipped *B. inermis*	3.29 [a]
P. smithii	Clipped *B. inermis*	2.62 [a]
B. inermis	Not clipped *P. smithii*	6.51 [a]
B. inermis	Clipped *P. smithii*	5.99 [a]
B. inermis	Not clipped *B. inermis*	5.26 [a]
B. inermis	Clipped *B. inermis*	6.71 [a]

Table 11. Extra-radical AM fungal abundance of warm-season grasses following soil conditioning (phase 1). Phospholipid fatty acid (PLFA) represent hyphal abundance, neutral lipid fatty acid (NLFA) represents fungal spores associated with native and invasive cool-season grasses. Rows that share a superscript letter are not significantly different from one another ($p \leq 0.05$).

Warm-season		
Species Growing	Soil Conditioning	AM Fungal Abundance (nmol/g soil)
PLFA		
S. scoparium	Not clipped *S. scoparium*	0.94[d]
S. scoparium	Clipped *S. scoparium*	1.85 [cd]
S. scoparium	Not clipped *B. ischaemum*	1.27 [cd]
S. scoparium	Clipped *B. ischaemum*	2.62 [bc]
B. ischaemum	Not clipped *S. scoparium*	0.94 [cd]
B. ischaemum	Clipped *S. scoparium*	4.12 [a]
B. ischaemum	Not clipped *B. ischaemum*	1.78 [cd]
B. ischaemum	Clipped *B. ischaemum*	3.53 [ab]
NLFA		
S. scoparium	Not clipped *S. scoparium*	7.86 [ab]
S. scoparium	Clipped *S. scoparium*	12.2 [ab]
S. scoparium	Not clipped *B. ischaemum*	18.2 [a]
S. scoparium	Clipped *B. ischaemum*	7.03 [ab]
B. ischaemum	Not clipped *S. scoparium*	8.91 [ab]
B. ischaemum	Clipped *S. scoparium*	3.56 [b]
B. ischaemum	Not clipped *B. ischaemum*	3.98 [b]
B. ischaemum	Clipped *B. ischaemum*	3.90 [b]

FIGURES

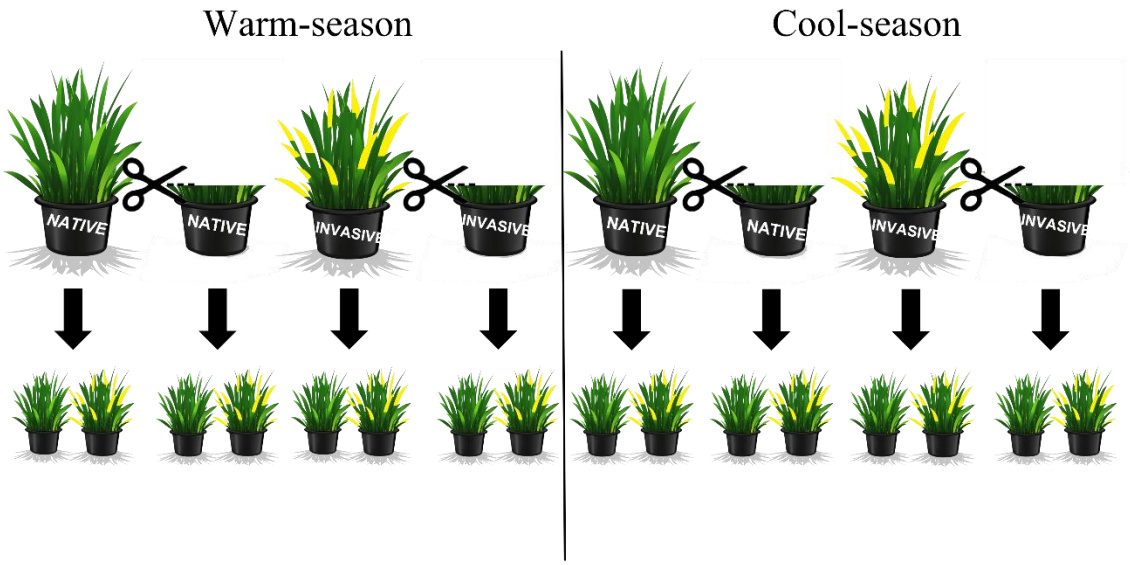


Figure 6. Schematic diagram for the experimental design of the conditioning and experimental testing phases of this research. The top line shows the phase 1 soil conditioning; in this conditioning phase two warm-season grasses (*Schizachyrium scoparium*, *Bothriochloa ischaemum*) and two cool-season grasses (*Pascopyrum smithii*, *Bromus inermis*) were clipped or not clipped. The bottom half shows the conditioned soils collected from phase 1 being used to grow a new generation of plants. As shown by our schematic, each soil treatment from the conditioning phase was used as inoculum for native or invasive plant species of the same functional group.

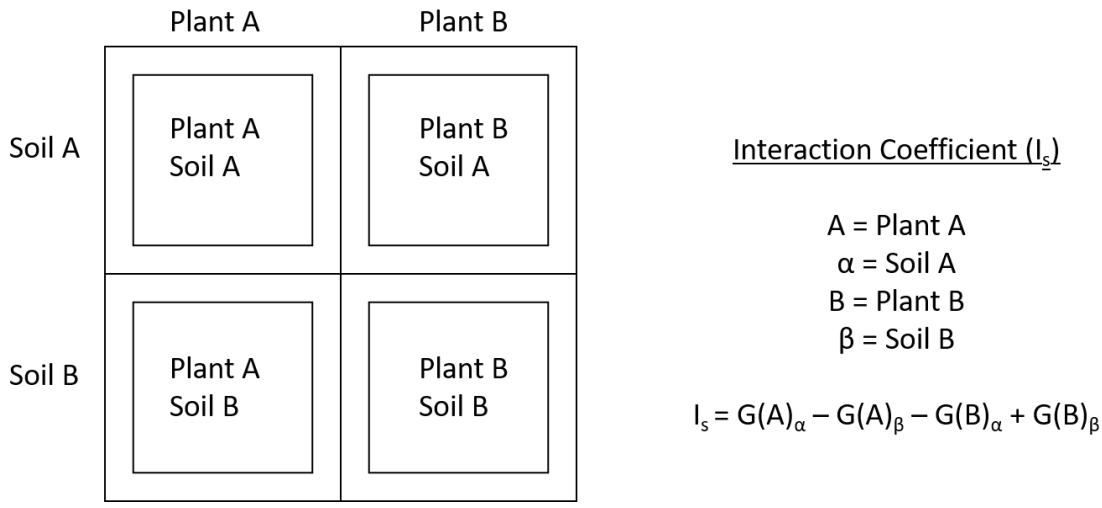


Figure 7. On the left, the experimental design basis for the project. On the right, the equation used to determine the interaction coefficients (I_s) that represents the direction and magnitude of each PSF.

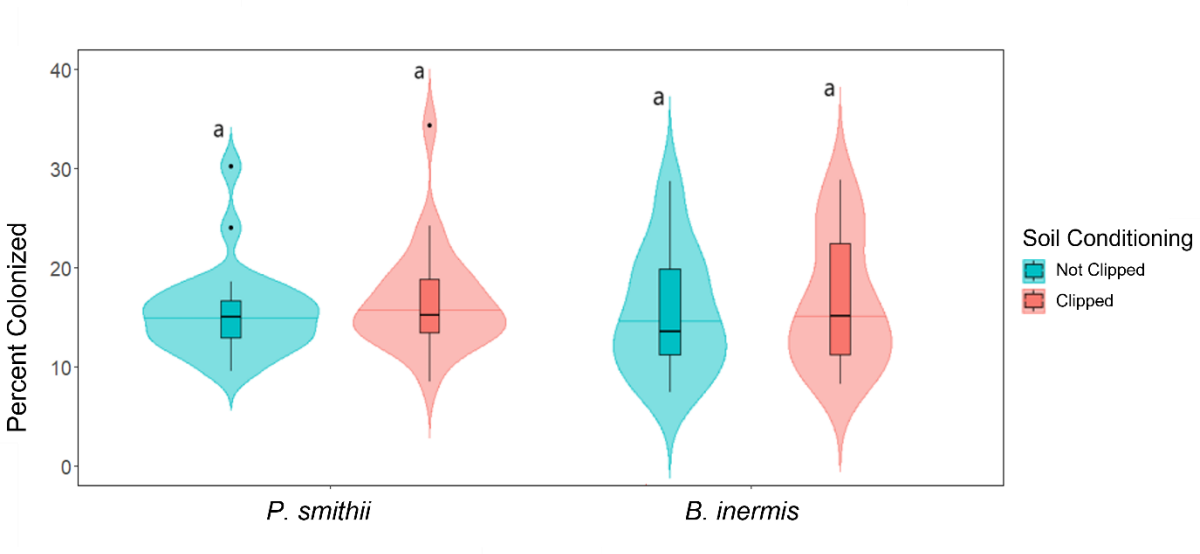


Figure 8. Percent arbuscular mycorrhizal fungal colonization response of phase 1 (conditioning) native and invasive cool-season grasses to the presence or absence of clipping. Violin shapes that share a letter are not significantly different from one another ($p \leq 0.05$).

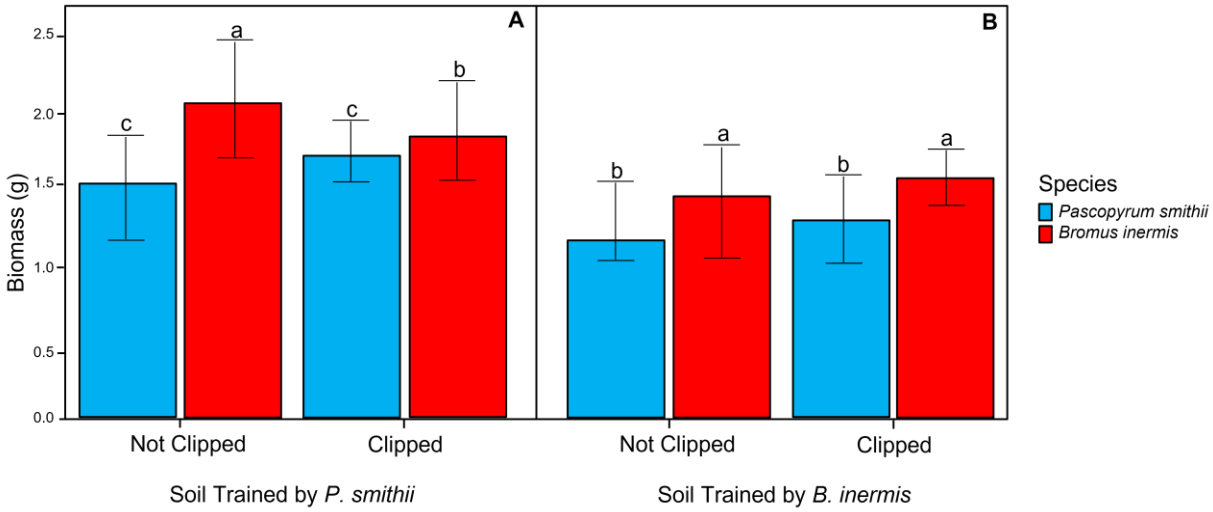
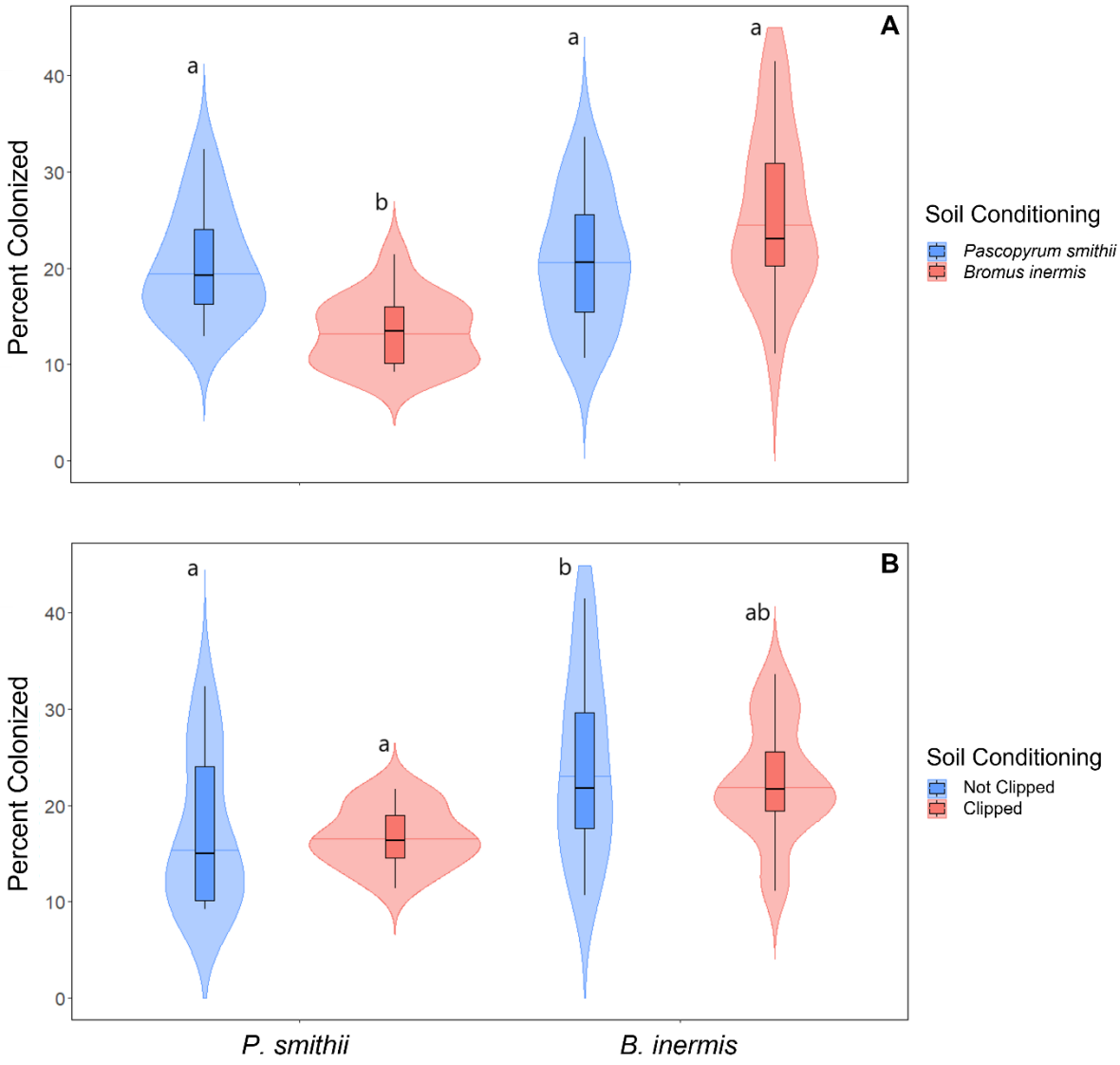


Figure 9A. Total biomass of cool-season phase 2 (experimental) native and invasive grasses in response to growth in soils conditioned by clipped or not clipped *P. smithii*. Figure 9B. Total biomass of cool-season phase 2 (experimental) grasses in response to growth in soils conditioned by clipped or not clipped *B. inermis*. Bars within plant functional group that share a letter are not significantly different from one another ($p \leq 0.05$).



Figures 10A. Arbuscular mycorrhizal fungal colonization (%) of phase 2 (experimental) native and invasive cool-season grasses to growth in soil conditioned by hetero- or conspecifics. Violin shapes that share a letter are not significantly different from one another. Figure 10B. AM fungal colonization (%) of phase 2 (experimental) cool-season grasses to growth in soil conditioned by clipped or not clipped grasses. Violin shapes that share a letter are not significantly different from one another ($p \leq 0.05$).

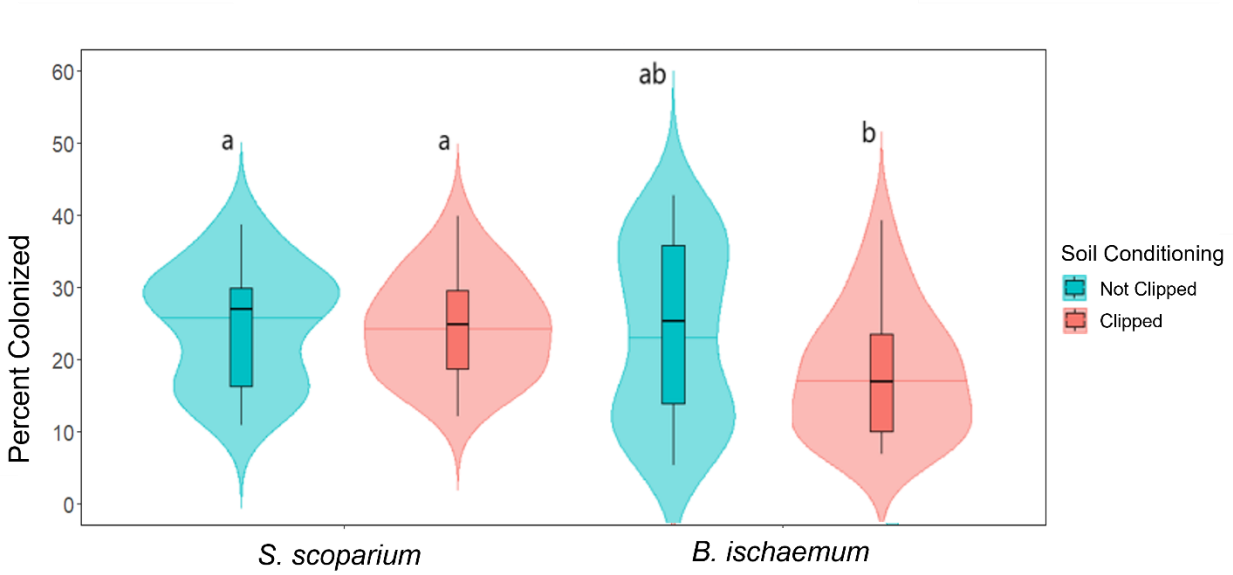


Figure 11. Warm-season arbuscular mycorrhizal fungal colonization (%) of phase 1 (conditioning) native and invasive grasses following clipping. Violin shapes that share a letter are not significantly different from one another ($p \leq 0.05$).

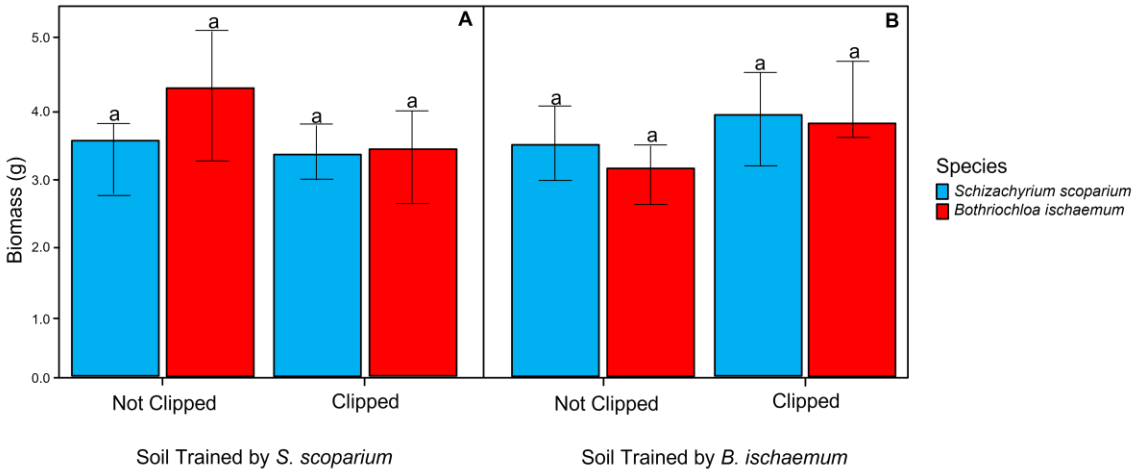


Figure 12A. Total biomass of phase 2 (experimental) native and invasive warm-season grasses following growth in soils conditioned by clipped or not clipped *S. scoparium*. Figure 12B. Total biomass of phase 2 (experimental) warm-season grasses following growth in soils conditioned by clipped or not clipped *B. ischaemum*. Bars within plant functional group that share a letter are not significantly different from one another ($p \leq 0.05$).

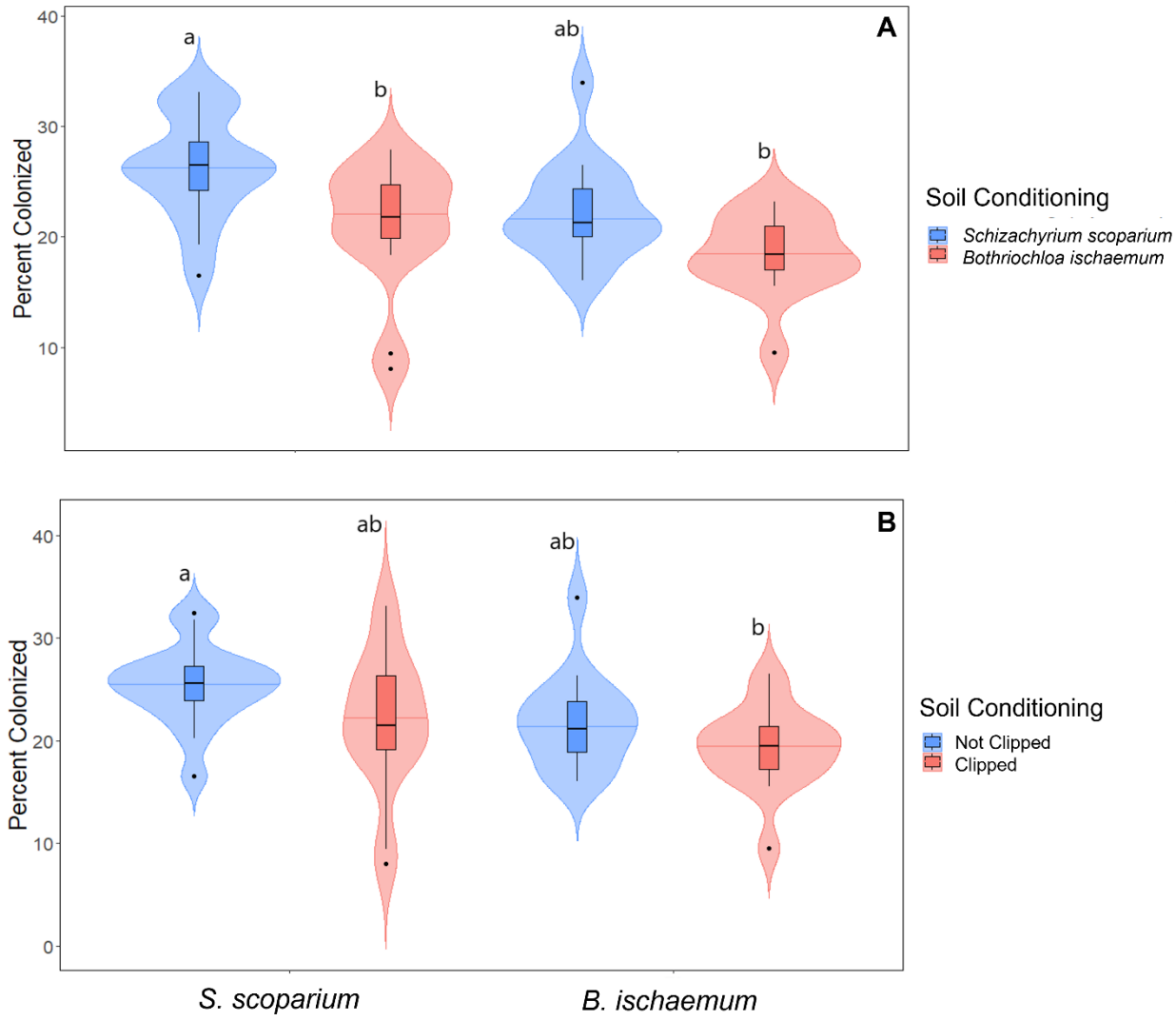


Figure 13A. Arbuscular mycorrhizal fungal colonization (%) of phase 2 (experimental) native and invasive warm-season grasses in soil conditioned by hetero- or conspecifics. Figure 13B. AM fungal colonization (%) of phase 2 (experimental) warm-season grasses in soil conditioned by clipped or not clipped grasses. Violin shapes that share a letter are not significantly different from one another ($p \leq 0.05$).

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