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Exploring soil ecology gradient concepts: sensitivity of ecosystem properties to symbiotic fungi in semiarid prairie

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Abstract

In resource-limited environments, plants are predicted to increase root mining or collaboration with nutritional mutualists. Little is known about arbuscular mycorrhizal fungi (AMF) effects on grasslands with low soil phosphorus (P) solubility. We evaluated AMF effects on plants, plant–plant interactions, plant nutrient limitation, and nutrient acquisition strategy in a series of experiments. A 5-year field experiment tested how AMF suppression affected the plant community; lichen and moss abundance; nutrient limitation and P acquisition; and soil structure and function. Fungi suppression eliminated lichens, increased mosses 410%, increased plant biomass 13% and shifted grass dominance. AMF suppression increased shoot N: P (indicator of P limitation) of *Koeleria macrantha* and had no effect on *Hesperostipa comata* N: P. Pot bioassays tested how grass coexistence was affected by three pairs of soil treatments (1) field soil inoculant (FSI) versus FSI with AMF suppression, (2) FSI versus sterilized FSI, and (3) AMF inoculant versus sterilized AMF inoculant. *Koeleria* outperformed *Hesperostipa* across treatments. *Hesperostipa* was likely P-limited, with 2.5 times greater N: P. *Koeleria* had 2.2 to 4.1 times greater shoot [Mn] (indicator of root exudation to mobilize calcium-bound P) than *Hesperostipa*. FSI sterilization increased shoot [Mn] of both grasses, suggesting plasticity for root mining. AMF had small effects on some grasses, but plant community composition, soil structure, and soil function were resistant (or resilient) to AMF suppression. Results provide insight to plant P acquisition strategies where soils with low organic matter and P solubility promote autonomous P-acquisition while moderate soil fertility and P lability favor AMF collaboration.

Keywords Apparent competition, Calcareous soil, Drought, Dryland, Grassland, Plant–soil biota interactions, Phosphorus-acquisition strategy

1 Introduction

Plant co-existence is impacted by negative frequency dependence and resource competition [1, 2]. Plants may struggle to acquire phosphorus (P) which is often unavailable to roots and mycorrhizal fungi due to slow diffusion and strong sorption (poor solubility) in soils [3, 4]. When P is not available, plants utilize various modes of acquisition



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including: modification of root morphology (e.g. length, architecture), exudates that mobilize insoluble P forms (e.g. secretion of carboxylate, organic acids), and investment in mycorrhizal associations [4, 5].

A plant's primary mode of P acquisition likely affects whether it is accessing strongly sorbed (less labile) sources of P via root mining or more labile forms of P via mycorrhizal scavenging [6, 7]. Conversely, a popular generalization for arbuscular mycorrhizal fungi (AMF) is that they are most important to plants in P-limited environments (e.g. [8–10]) but see Fornara [11] and Lekberg [12]. Whether plants rely on AMF as a secondary source of P or not is likely more complicated and dependent on multiple factors including: the soil's character (e.g. capacity to immobilize P, predominant P form) and predominant nutrient uptake traits of a plant community [4, 13–15]. Because of the many factors affecting P availability and cycling, more research is needed to understand when and where modes of P acquisition are favored and affect plant coexistence, composition, and soil structure and function.

Dryland ecosystems cover a third of global ice-free land and are primarily used to support livestock [16]. Drylands' soil is often distinct (e.g. alkaline, low organic matter) with large amounts of inorganic carbon in the subsoil [17, 18] which contribute to low levels of soluble P (e.g. [13, 19–21]). Reinhart [13] demonstrated that additions of inorganic carbon (i.e. CaCO_3 powder) increased subsoil pH and reduced soluble P by increasing calcium-bound P, and AMF were not more beneficial where P was more limiting. Soil CaCO_3 can negatively affect fungal development and limit the beneficial effects of mycorrhizal fungi on plants [22]. The distinct soil character of drylands acts as a lever favoring plants with autonomous P-acquisition strategies over mycorrhizal collaboration [13] (Fig. 1). In contrast, AMF are more ecologically important (e.g. affect plant dominance, soil structure) in more humid ecosystems, like tallgrass prairie of Kansas, USA [23, 24] and montane grasslands of Switzerland with weakly calcareous soil [25]. In tallgrass prairies, soils have comparatively more organic matter, development, depth, and contain little CaCO_3 [26]. They are also likely to have comparatively more P in the root zone cycled through organic than inorganic (Ca-P) pathways [26] which Reinhart [13] and Tarafdar and Marschner [7] posited favors collaboration with mycorrhizal fungi (Fig. 1).

To help fill knowledge gaps for drylands, we conducted a field experiment and tested the effect of AMF suppression on plant community composition of a semiarid calcareous grassland in the Northern Great Plains of North America. We predicted suppression of AMF would disrupt grassland community composition by decreasing C_4 grasses, other mycorrhizal dependent species (e.g. [24, 28]), and have nontarget effects on fungal symbionts of lichens [29]. Furthermore, we predicted AMF would benefit plants during periods of drought stress and that AMF suppression effects on plant community composition would be greater during and after than before a natural drought [30–32]. We also predicted that 5 years of AMF suppression in the field would degrade soil structure (i.e. macroaggregate stability) due to the important role of AMF for promoting aggregate stability [23, 25, 33] which could degrade soil function, especially soil water transport [34]. Insight from the field experiment informed design of complementary pot bioassays which addressed apparent competition between two dominant grasses. We hypothesized AMF promote coexistence of competing *Koeleria macrantha* and *Hesperostipa*

Soil phosphorus & climosequence

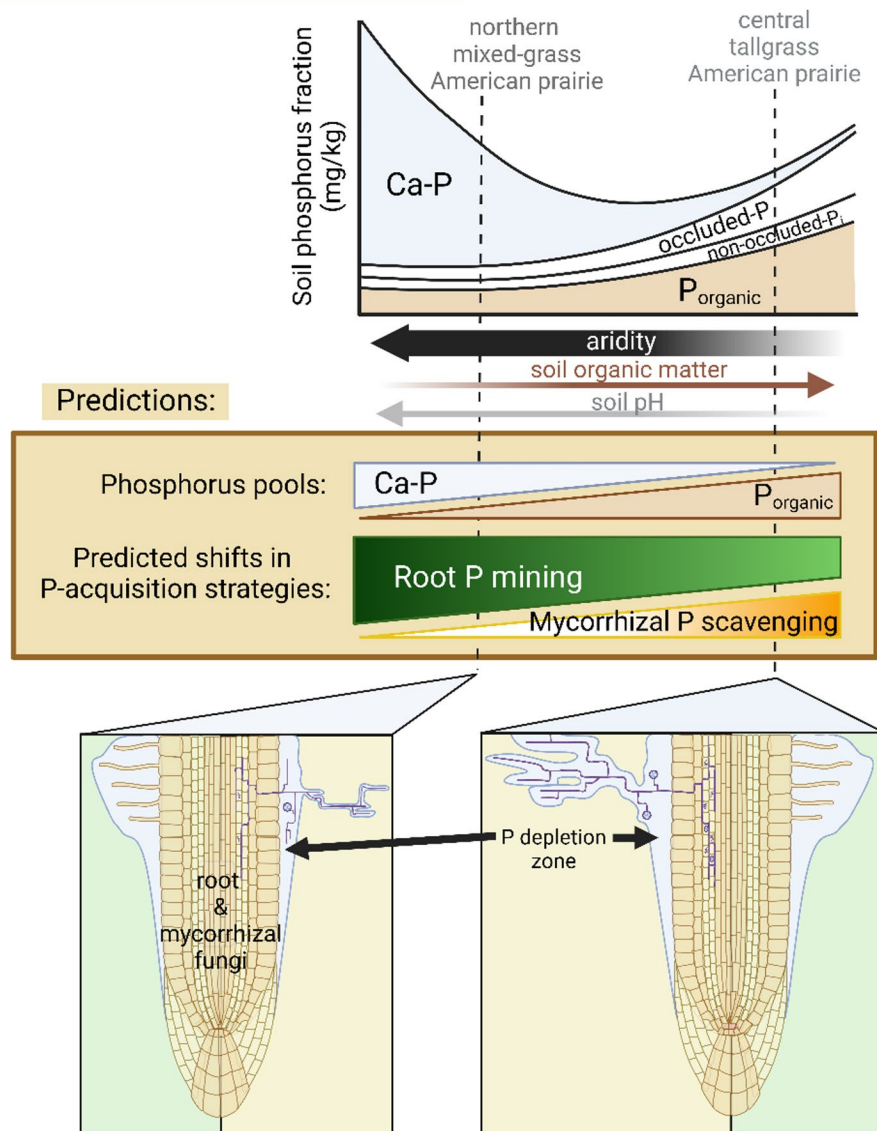


Fig. 1 Soil phosphorus (P) fractions, soil organic matter (SOM), and pH along climosequences may influence variation in grassland plant's dependency on arbuscular mycorrhizal fungi. The figures were originally illustrated by Reinhart, Vermeire [13] with climosequence panel portion based on Ippolito [26] and Feng [27]. This version has been slightly modified to incorporate soil properties (soil organic matter, soil pH) that covary with aridity. Ca-P = calcium phosphates in primary minerals, P_{organic} = organic P, occluded-P = P stabilized in secondary minerals, and non-occluded P = available [inorganic] phosphates. The graphic illustrates that the more Ca-P and less SOM favors root mining over mycorrhizal P scavenging based on Reinhart [13]. The opposite condition (less Ca-bound P and more SOM) favors collaboration with AMF and mycorrhizal P scavenging

comata while assuming *K. macrantha* was better at chelation-mediated root mining and *H. comata* was better at acquiring P by collaborating with AMF [13].

For both field and pot experiments, AMF suppression was also hypothesized to increase P limitation, as indicated by an increase in leaf nitrogen (N) to P (N: P) [35, 36] but see Craine [37]. Since AMF suppression is likely to degrade the capacity of AMF to function as a secondary source of P, our study also measured shoot [Mn]. Shoot [Mn] can indicate the release of carboxylate by roots to mobilize Ca-bound P [38, 39] but see

Wang [40]. We hypothesized treatments that suppress (1) AMF (field and pot experiments) and/or (2) soil biota (e.g. P-solubilizing bacteria [pot experiment]) will induce plants to increase root mining of P compared to control plants.

2 Methods

2.1 Study site and system

The experiment was conducted at the USDA-ARS Fort Keogh Livestock and Range Research Laboratory in eastern Montana, USA (46° 22' N, 105° 53' S). The field station is centrally located in the Northern Great Plains steppe ecoregion. This ecoregion contains expansive areas of intact semiarid grasslands (>22 million hectares) dominated by mixed-grass prairie and used as grazing lands (Martin et al. 1998). The average minimum temperature in January is −14.6 °C and the average July maximum temperature is 31.8 °C. The average frost-free growing season is 150 days but the true growing season is heavily influenced by available soil moisture. Annual precipitation averages 333 mm of precipitation with nearly half occurring April through June. Therefore, plant production is strongly influenced by April and May precipitation [41].

The field experiment was conducted on an upland plain (elevation = 850 m) with a calcareous grassland dominated by three C_3 graminoid species (*H. comata* > *Carex filifolia* > *K. macrantha*). Arbuscular mycorrhizal fungi are known to associate with [42] and affect many of the prairie's plants [28]. The experimental site had a gentle slope and fine loamy soil (57% sand: 33% silt: 10% clay; pH = 7.4; Epa loam soil series, frigid Aridic Argiustolls or Mollisols). In adjacent grassland with Epa loam, Reinhart and Vermeire [43] reported alkaline and calcareous subsoils which were shown to reduce soluble P and limit plant growth [13]. The experimental site was fenced (1999) to exclude livestock grazing.

2.2 Field experiment

We tested the effect of AMF suppression (fungicide, control) on plant composition, foliar nutrients, soil structure (macroaggregate stability), and soil functioning (soil water transport) with a 5-year field experiment. We also tested for nontarget fungicide effects on lichens and mosses. The experiment was a randomized complete block design with eight blocks (16 total plots). Each block had two plots, and blocks were distributed over a 0.6 ha grazing enclosure. Plots (3 × 3 m) were separated by ≥ 3-m gap to reduce potential effects of treatments on each other.

The systemic fungicide Topsin M (70% active ingredient [thiophanate-methyl]; Cerex-agri Inc., Philadelphia, PA) was applied as a soil drench (1.25 g × m^{−2} of active ingredient in 9 L per plot at 3-week intervals) to reduce the abundance of fungal communities, especially AMF (2010–2014) [44, 45]. Control plots received a similar amount of water. Treatments were applied from April 15 to July 8 and once in October (6 total applications per year). Previous field studies reported thiophanate-methyl reduced AMF abundance in the soil and/or AMF colonization of roots [35, 44–46] and increased a putative indicator of phosphorus limitation by plants (shoot N: P) [35]. Thiophanate-methyl and benomyl are a class of fungicides (Benzimidazole) thought to be consistently detrimental to mycorrhizal fungi [47].

We tested the effects of treatments on plant and soil properties. Plant properties included: plant community composition (measured 2010–2014), lichen and moss cover

(post-treatment, 2015), green shoot chemistry (2010–2013), and annual net primary productivity (post-treatment, 2014–2015). Plant community composition was characterized using the point-intercept method of 40 random points along each of three permanent transects per plot [48]. Transect data were collected from late May to early July and converted into % cover data. Plant production was determined July 2, 2014 and July 15–16, 2015, at peak annual production for each plot. In both years, we clipped three quadrats (ea. 0.1 m²). In 2014, quadrats were placed >0.5 m from the edge and distributed along the centerline of each plot. The following year, quadrats were spaced farther from the centerline and avoided areas that had been previously clipped. Samples from a clipped quadrat were sorted by succulents and year of growth. Current-year's growth was bagged, dried (60° C, 48 h), and weighed (g × 0.3 m⁻²). Because of the difficulty in determining the dry weights of succulents (*Opuntia* spp.), their dry weight was estimated with a correction calculation (0.2 × fresh weight). Analyses of plant biomass excluded *Opuntia*. We also measured the lichen and moss percent cover after measuring soil water transport (details below) on June 26 and July 2, 2015.

Shoot chemical composition was determined for two dominant perennial C₃ grasses (*H. comata* and *K. macrantha*) and used to interpret nutrient limitation from N: P (e.g [49]) but see [37] and nutrient acquisition strategy from shoot manganese (Mn) concentrations ([Mn], e.g [38]) but see [40]. Approximately, 5.5 g of whole green leaves per species per plot were collected August 13, 2010; June 28, 2011; July 3, 2012; and July 5, 2013. Leaf samples were dried, ground with a Wiley mill (1 mm mesh screen), and 2 g samples were submitted to a service center (Midwest Laboratories, Inc., Omaha, NE) for chemical analyses.

We tested the effects of AMF suppression treatments on soil structure (2014) and function (2015) following standard procedures described by Reinhart [50]. To measure soil structure, we collected two soil cores (0–10 cm depth) per plot on August 27, 2014. Cores were combined by plot and brought back to the laboratory. The soil was dried (55 °C, 48 h), gently crushed to pass a 4.75-mm screen, and dry-sieved [50]. The percentage of water-stable aggregates was determined for two macroaggregate size classes (0.25–1 and 1–2 mm) by wet-sieving. For soil functioning, we measured soil water transport of plots with dual-head infiltrometers (DHI) (Meter, Pullman, WA) June 26 and July 2, 2015. The DHIs measure field-saturated hydraulic conductivity (K_{sat}). The DHI applies water to a small circular area (15 cm diameter). Prior to preparing the DHI's insertion ring and performing an assay, the vegetation was clipped. Further details on the DHI can be found in the user's manual or Reinhart and Vermeire [51]. Following DHI measurements, mosses and lichens were hydrated and visually apparent in the infiltration ring areas. We measured their % cover by ocular estimation.

2.3 Pot bioassays

Based on insight from the field experiment, we hypothesized AMF promote coexistence of competing *K. macrantha* and *H. comata* while assuming (1) *K. macrantha* was better at root P mining, and (2) *H. comata* was better at acquiring P by collaborating with AMF. Each pot was planted with a 50/50 mix and four total plants. Seed was purchased (*H. comata*, Western Native Seed, Coaldale, CO; *K. macrantha*, Wind River Seed, Manderson, WY), surface sterilized (10% bleach, 10 min), rinsed with reverse-osmosis (RO)

water, and sown into moist autoclaved vermiculate. Grass germinants were then transplanted into pots.

The first bioassay had three soil inoculant treatments including: field soil inoculant (FSI, control), FSI and thiophanate-methyl fungicide (i.e. AMF suppression), and autoclaved FSI (3 treatments $\times$ 12 replications = 36 pots). The treatments enabled testing for (1) AMF effects (FSI [control] versus FSI with applications of fungicide to suppress AMF) and (2) soil biota effects (FSI versus autoclaved FSI). The treatments aimed to uncover the importance of different soil biota fractions and followed routine manipulative approaches used to study soil ecology (e.g [52]). For the FSI, we collected 12 samples ($\sim$ 0.5 L ea.) of surface soil (0–10 cm depth) from the field site. We separately air-dried and sieved (1-cm openings) each FSI sample and kept samples separate (*sensu stricto* [53]). In addition, we autoclaved a $\sim$ 120 mL portion of each FSI (12 autoclaved samples).

The majority (93%, v/v) of every pot (1.8 L, 14 cm dia.) was filled with sieved (6.5 mm) and autoclaved field soil (Eapa loam soil series) (Appendix S1: Table S1). Each pot included a FSI (120 mL, 7% of pot volume) that was topped with a small amount of autoclaved field soil. The inoculant amount has been shown sufficient to establish a similar soil microbial community [54]. For each sample, the “living” (not autoclaved) FSI portion was used to inoculate two pots (control, fungicide treated), and the autoclaved portion was used to inoculate a third pot. After transplanting germinants, pots were kept on benches with grow lights in a climate controlled room with growth conditions like those used to test for AMF effects on 68 regional species [28]. The experiment lasted 130 days and plants were watered with RO water as needed (March through July, 2012). The first thiophanate-methyl application was 1 month after transplanting germinants and was followed by three additional applications at 3-week intervals (4 total applications). Pots received amounts of thiophanate-methyl (or RO water) that were proportional (on a surface area basis) to the *Field experiment*. After 130 days, grass shoots were harvested, dried (60 °C, 48 h), and weighed by species ($\text{g} \times \text{pot}^{-1}$). To have sufficient material for foliar nutrient assays, we combined samples ($\sim$ 4 pots) into three composite samples per treatment $\times$ species combination, and samples were submitted to a service laboratory.

A second 130-day bioassay was conducted, and it was nearly identical to the first. The main differences were (1) the experimental treatments (which tested for AMF effects) and (2) use of a single inoculant mixture (per treatment) to inoculate pots. This experiment tested for AMF effects by comparing performance of competing plants grown with either a mixed AMF inoculant or an autoclaved AMF inoculant (2 treatments $\times$ 12 pot technical replications = 24 pots). We produced AMF inoculant following routine isolation and propagation techniques previously described by Reinhart [28]. The AMF inoculant consisted of a mixture of nine Sudan grass cultures started from single AMF spores of unknown identity isolated from soil samples of Custer County, MT. Reinhart and Anacker [42] attempted to identify the cultures using molecular methods and found the cultures consisted of four AMF OTUs including one unidentified *Glomus* sp. and three unknown AMF OTUs.

2.4 Analyses

We predicted plant community composition of plots with AMF suppression would diverge from control plots (per block) gradually over time. To test for compositional shifts, we first determined community dissimilarity of plot pairs (treated versus control)

per block per year with the `vegdist` function (`method = jaccard`, `binary = FALSE`) in the package `vegan` [55]. The relationship between year and community dissimilarity appeared hump-shaped and was better fit with nonlinear (inverse 2nd order polynomial, $R^2 = 0.14$) than linear models ($R^2 = 0.01$). We present only the nonlinear model result. We also modeled data for the three dominant plant species (i.e. *H. comata* > *C. filifolia* > *K. macrantha*), fixed effects included AMF suppression, species, year, and all possible interactions. To account for spatial and temporal non-independence, we included random intercepts for block and plot nested within block, and we utilized a compound symmetry covariance structure. If a significant three-way interaction was detected, then we ran separate models by species. We ran models in R 4.4.1 with the `nlme` package and `lme` function [56]. We transformed data when necessary to meet model assumptions and considered results significant at $\alpha = 0.05$. Model assumptions were checked with `shapiro.test` and `levene.test` functions. Results are presented as means $\pm$ standard error except where noted. To help visualize AMF suppression effects over time on % cover of the eight dominant vascular plant species, we determined log response ratios ($LRR = \ln[\text{treated/control}]$) of plot pairs when both plots contained the focal species.

For models of field data collected in two or more years (i.e. plant biomass, species richness, diversity, and shoot N: P and [Mn]), fixed effects included AMF suppression, year, and all possible interactions. We included random intercepts for block and plot nested within block. We utilized a compound symmetry covariance structure and type three sums of squares. Whenever any two-way interactions with year were significant, we ran separate models by year. Other data for the field experiment were collected in only a single year (i.e. lichen and moss cover, soil aggregate stability, and soil water transport) and were analyzed with a fixed effect for only AMF suppression. To account for spatial non-independence, we included a random intercept for block, and we utilized a compound symmetry covariance structure. We transformed data when necessary to meet model assumptions. The lichen and moss data, however, continued to violate the homogeneity of variance assumption because the data included many zeros. We then tested for AMF suppression effects with nonparametric Wilcoxon tests using the `wilcox.test` function.

For models of pot bioassays' data (i.e. plant biomass, leaf N: P and [Mn]) of 50/50 mixes of grasses (per pot), fixed effects included soil biota treatment, plant species, and all possible interactions. For the first bioassay, we tested for (1) an AMF effect by contrasting response variables of plants grown in pots with field soil inoculant (FSI, control) versus the FSI with AMF suppression treatments and (2) soil biota effect by contrasting response variables of plants grown in pots with FSI versus autoclaved FSI. To account for non-independence of soil samples and pots, we included random intercepts for pot nested within soil sample, and we utilized a compound symmetry covariance structure. The second bioassay compared the response variables of plants grown with AMF inoculant (control) versus autoclaved AMF inoculant. To account for non-independence of pots, we included random intercepts for pot and utilized a compound symmetry covariance structure.

3 Results

3.1 Field experiment

The AMF suppression treatments caused a marginally significant divergence in plant community composition to peak after the third year of application (2013) which

corresponded with a natural drought (Fig. 2A, B). Percent cover of dominant plants varied by AMF suppression, species, and year (Fig. 3). We detected overall species, species by fungicide, and species by year interactions on the percent cover of the three most dominant plants (Table 1). For *H. comata*, we observed a marginally significant negative effect of AMF suppression on percent cover and no fungicide by year interaction (Table 2). For cover of *K. macrantha*, we found no appreciable main effect of AMF suppression but a marginally significant fungicide by year interaction (Table 2) with positive AMF suppression effects peaking in 2013 then declining over time (Fig. 3). *C. filifolia* cover was not appreciably affected by AMF suppression treatment (Table 2; Fig. 3). Vascular plant species richness and Shannon's index of diversity were not affected by AMF suppression, and there was no fungicide by year interaction on diversity indices (Appendix S1: Table S2).

Following the 5 years of AMF suppression (2010–2014), treatments affected lichens, mosses and plant biomass. Specifically, lichen cover was 22% in control plots and 0% in fungicide treated plots in 2015 (Wilcoxon test, $W = 64$, $P < 0.001$; Fig. 4A). In contrast, moss cover was only 1% in control plots and 48% in fungicide treated plots ($W = 0$, $P < 0.001$; Fig. 4B). Plant biomass was 13% greater in fungicide treated than control plots (log transformed: $F_{\text{fungicide}} = 6.71$, $df = 1,7$, $P = 0.04$; $F_{\text{year}} = 43.0$, $df = 1,14$, $P < 0.0001$; $F_{\text{fungicide} \times \text{year}} = 0.92$, $df = 1,14$, $P = 0.35$; Fig. 4C). After 5 years of mycorrhizal suppression treatments, there was no effect of treatment on soil structure and soil water transport ($P \geq 0.76$). Specifically, there was no effect of AMF suppression on the percentage of water-stable small-sized (0.25–1 mm; $F_{1,7} = 0.08$, $P = 0.78$) and medium-sized macroaggregates (1–2 mm; $F_{1,7} = 0.06$, $P = 0.81$) (data not shown). Also, no effect of AMF suppression on soil water transport ($F_{1,7} = 0.10$, $P = 0.76$) (data not shown).

3.2 Pot bioassays

Pots were filled with autoclaved field soil with a neutral pH (7.0) and appeared to have sufficient phosphorus (i.e. $P = 18$ ppm; Appendix S1: Table S1) based on the Bray-1 P extractions which was designed for acidic and not neutral to alkaline soils [57]. For the bioassay with field soil inoculant, *K. macrantha* had greater shoot biomass than *H. comata* (autoclaved contrast comparison [fresh field soil versus autoclaved field soil]: $P_{\text{species}} = 0.003$, Appendix S1: Table S3; fungicide contrast [fresh field soil versus fungicide treated field soil]: $P_{\text{species}} < 0.0001$, Appendix S1: Table S4). Conversely, shoot mass did not vary by soil treatment (autoclaved contrast: $P_{\text{treatment}} = 0.87$; fungicide contrast: $P_{\text{treatment}} = 0.32$) (Appendix S1: Figure S1). Furthermore, there was no difference in shoot biomass proportions of immature *H. comata* and *K. macrantha* in fresh field soil versus autoclaved field soil ($P_{\text{treatment} \times \text{species}} = 0.81$, Appendix S1: Table S3) or versus fungicide treated ($P_{\text{treatment} \times \text{species}} = 0.19$, Appendix S1: Table S4) (Appendix S1: Figure S1). For the AMF inoculation experiment, *K. macrantha* again had greater shoot biomass than *H. comata* ($P_{\text{species}} < 0.0001$, Appendix S1: Table S5). Shoot biomass was not affected by AMF inoculant treatment ($P_{\text{treatment}} = 0.33$, Appendix S1: Table S5) (Appendix S1: Figure S1). We also observed no difference in biomass proportions of immature grasses competing in pots with AMF inoculant versus autoclaved AMF inoculant ($P_{\text{treatment} \times \text{species}} = 0.27$, Appendix S1: Table S5) (Appendix S1: Figure S1).

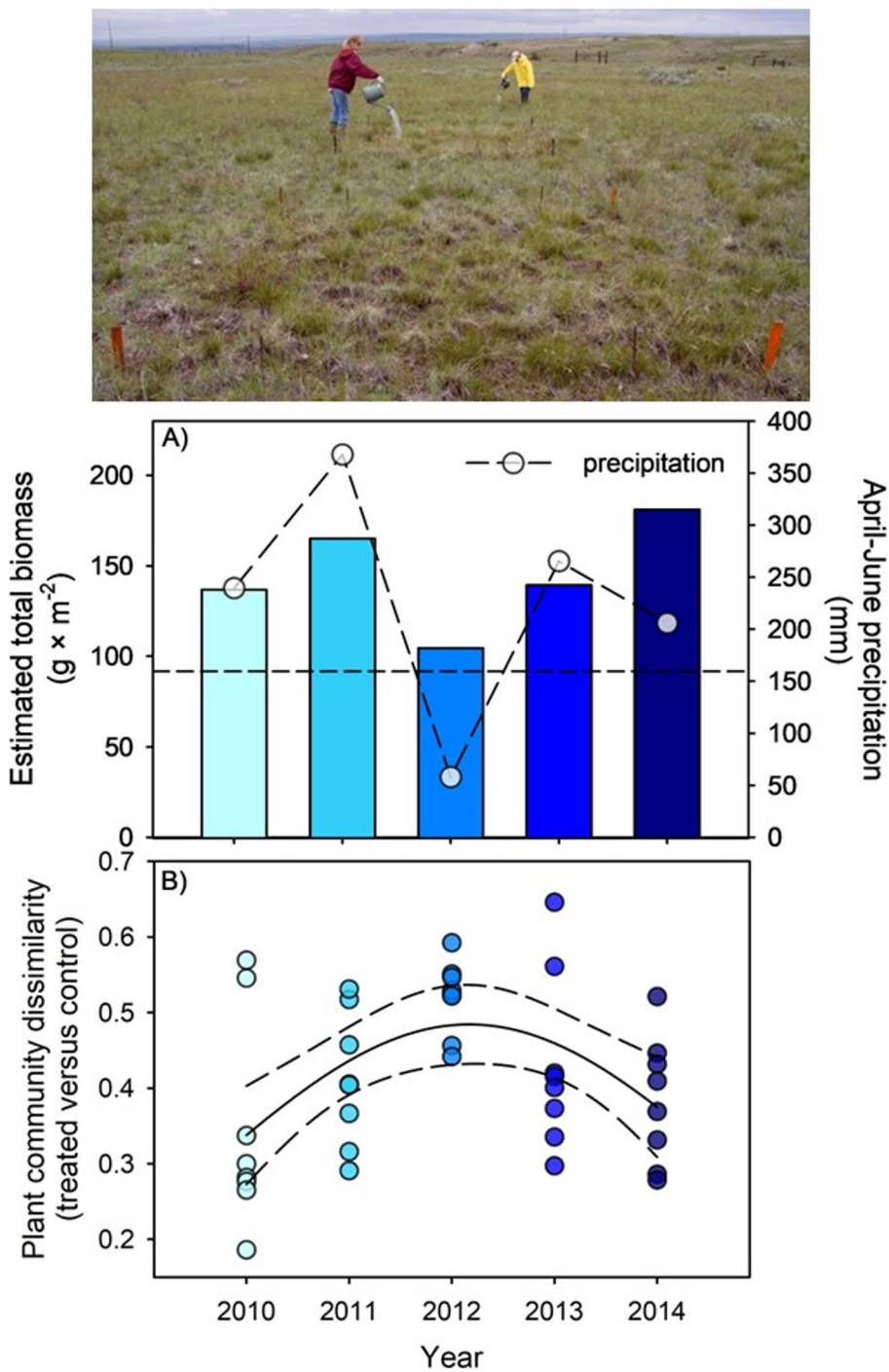


Fig. 2 Variation in precipitation and biomass (A) of a field experiment testing the effects of arbuscular mycorrhizal fungi (AMF) suppression (fungicide treated) on grassland community composition (B). We estimated biomass of the 5-year year experiment (A) from the relationship between total biomass and point-intercept data in 2014 ($y=0.7372x+45.17$, $r^2=0.11$). The 86-year median precipitation is depicted by a horizontal dashed line (A). Plant community dissimilarity of paired plots was measured with the Jaccard Index, and graph includes best-fit line (solid, second order polynomial: $R^2=0.14$, $P=0.061$) with 95% CI (dashed line) (B). Photo credit: K.O. Reinhart

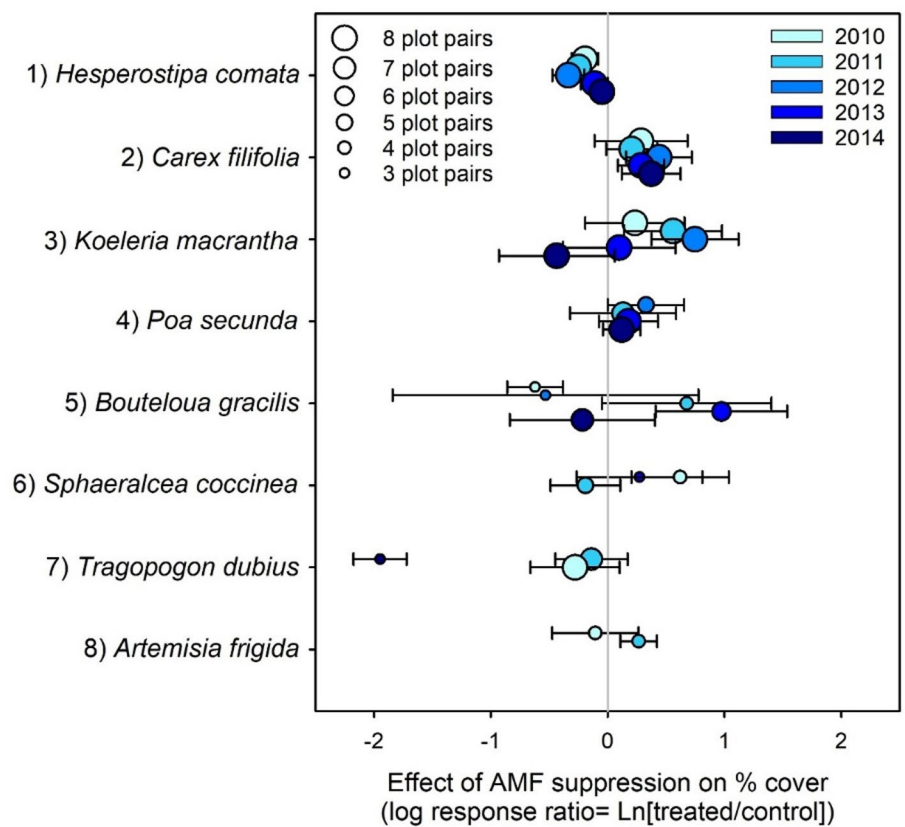


Fig. 3 Effect size (average $\pm$ 1 SE) of the % cover of the eight most abundant herbaceous species of the 5-year field experiment. Effect size was the log response ratio ($\ln[\text{treated}/\text{control}]$) of plot pairs treated with fungicide to suppress arbuscular mycorrhizal fungi (AMF) versus control. Species are ranked by abundance—graminoids dominated (species 1–5), forbs were subordinate (6–8), and one of eight species (#5) utilized C_4 photosynthetic pathway. To avoid inclusion of extreme values, we excluded effect sizes when there were less than three plot pairs (per year) and did not measure effect sizes when only one (or none) of the plot pairs contained cover of a species. Effects of treatment, year, and species on % cover were reported for the three most common species in Table 1

Table 1 Effects of arbuscular mycorrhizal fungi suppression (fungicide), year, and species on percent cover of three dominant Northern mixed-grass prairie graminoids (*Hesperostipa comata*, *Carex filifolia*, and *Koeleria macrantha*) of a 5-year field experiment

Experimental factor	d.f.	F	P-value
Fungicide	1,7	0.4	0.52
Species	2,196	99.2	< 0.0001
Year	4,196	0.7	0.63
Fungicide $\times$ species	2,196	8.6	0.0003
Species $\times$ year	8,196	4.9	< 0.0001
Fungicide $\times$ year	4,196	0.1	0.99
Fungicide $\times$ species $\times$ year	8,196	0.7	0.73

P-value $\leq$ 0.05 in bold. Tests for individual species are shown in Table 2

Repeated-measures ANOVA with a linear mixed-effects model included random effects for plot nested within block and type three sums of squares

3.3 Nutrient limitation and acquisition

Shoot nutrient stoichiometry (N: P) is a potential indicator of whether plants are N, P, or co-limited by N and P. Mature grasses in natural grasslands tended to have different N: P values by species (Table 3, Appendix S1: Figure S1) and marginally significant AMF suppression treatment interaction with year (Table 3). We also detected a significant

Table 2 Effect of arbuscular mycorrhizal fungi suppression (fungicide) and year on percent cover of three dominant grassland plants of a 5-year year field experiment

Plant species	Experimental factor	d.f.	F	P-value
<i>Hesperostipa comata</i>	Fungicide	1,7	5.3	0.06
	Year	4,56	5.1	0.0014
	Fungicide × year	4,56	0.9	0.49
<i>Carex filifolia</i>	Fungicide	1,7	2.8	0.14
	Year	4,56	27.1	<0.0001
	Fungicide × year	4,56	1.8	0.14
<i>Koeleria macrantha</i>	Fungicide	1,7	0.8	0.39
	Year	4,56	13.0	<0.0001
	Fungicide × year	4,56	2.5	0.06

P-value ≤ 0.05 in bold

Repeated-measures ANOVA with linear mixed-effects models included random effects for plot nested within block and type three sums of squares

interaction between AMF suppression treatment and species on leaf N: P (Table 3; Appendix S1: Figure S2). Separate analyses by species indicated that shoot N: P of *K. macrantha* was 11% greater (N: $P = 6.4$ vs. 5.8) in plots with AMF suppression treatments than controls ($P_{\text{treatment}} = 0.04$, Appendix S1: Figure S1), and we observed a treatment by year interaction ($P_{\text{treatment} \times \text{year}} = 0.04$). In contrast, *H. comata*'s foliar N: P (~6) was unaffected by fungicide treatment ($P_{\text{treatment}} = 0.35$) with no interaction between treatment and year ($P_{\text{treatment} \times \text{year}} = 0.46$, Appendix S1: Figure S1).

For the comparison of field soil inoculant (FSI, controls) versus either autoclaved FSI or fungicide treated FSI, shoot N: P was 2.5 and 2.4 times greater for immature *H. comata* than *K. macrantha* (autoclaved contrast: $P_{\text{species}} < 0.0001$, Appendix S1: Table S3; fungicide contrast: $P_{\text{species}} < 0.0001$, Appendix S1: Table S4, respectively) thereby suggesting *H. comata* (range of average N: $P = 15$ –17) was either co-limited by N and P (N: $P = 14$ to 16) or P limited (N: $P > 16$) while *K. macrantha* (average N: $P = 6$ –7) was most likely N limited (N: $P < 14$) (Appendix S1: Figure S1). Foliar N: P did not vary by soil autoclaving treatment ($P_{\text{treatment}} = 0.13$, Appendix S1: Table S3). For the FSI versus autoclaved FSI comparisons, we detected a marginally significant interaction between autoclaving treatment and grass species ($P_{\text{treatment} \times \text{species}} = 0.08$, Appendix S1: Table S3) with autoclaving tending to increase leaf N: P of *H. comata* more than *K. macrantha* (Appendix S1: Figure S1). For the contrast comparisons of immature grasses in pots with and without fungicide additions, we found no effect of fungicide additions and no interaction between treatment and species on foliar N: P (Appendix S1: Table S4). For the AMF inoculation experiment, leaf N: P for immature *H. comata* (~17–18) was 2.5 times that of *K. macrantha* (~7; Appendix S1: Figure S1 and Table S5). We detected a marginally significant negative effect of autoclaving AMF inoculant on leaf N: P ($P_{\text{treatment}} = 0.09$, Appendix S1: Table S5) and a marginally significant treatment and species interaction on leaf N: P ($P_{\text{treatment} \times \text{species}} = 0.10$, Appendix S1: Table S5).

Shoot [Mn] is a useful indicator of root exudates that mobilize calcium-bound P. For the field experiment, shoot [Mn] varied by mature grass species, and we detected an interaction between species and year (Table 3, Appendix S1: Figure S2). Shoot [Mn] was 4.1 times greater for mature *K. macrantha* than mature *H. comata* (Appendix S1: Figure S2). Shoot [Mn] of mature grasses did not vary by AMF suppression treatment, and we failed to detect an interaction between AMF suppression treatment and other factors on shoot [Mn] (Table 3).

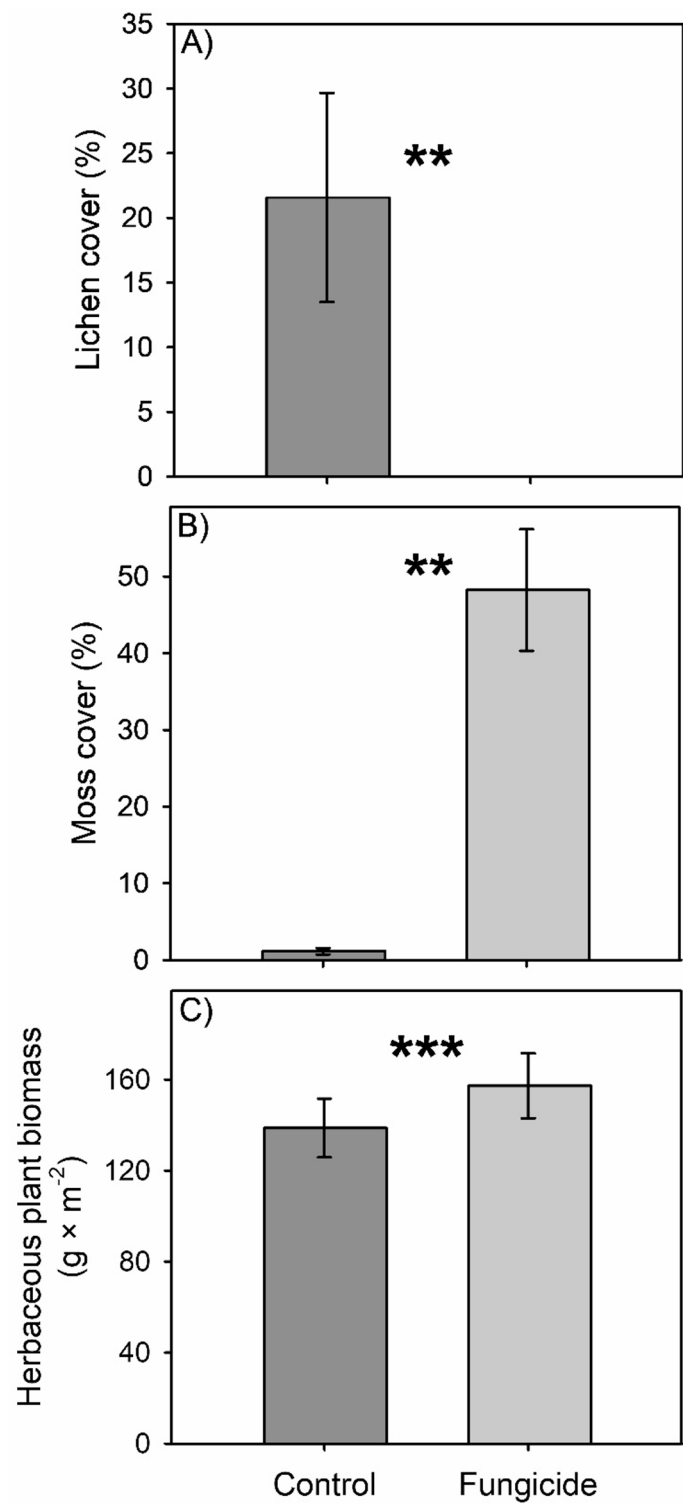


Fig. 4 Effect of fungicide treatment on average (± 1 SE) lichen cover (A), moss cover (B), and above-ground biomass of herbaceous plant biomass (C) of the field experiment ($n=8$ treatment replications [per year]). Fungicide was applied to suppress arbuscular mycorrhizal fungi of vascular plants. Moss and lichen data were collected the first year after ending fungicide applications, and plant biomass data were collected the first and second years after fungicide. ** $P<0.001$, *** $P<0.0001$

Table 3 Effects of arbuscular mycorrhizal fungi suppression (fungicide), year, and species on shoot N: P and [Mn] of mature plants of two dominant grasses (*Hesperostipa Comata* and *Koeleria macrantha*) in a Northern mixed-grass prairie

Plant parameter	Experimental factor	d.f.	F	P-value
Shoot N: P	Fungicide	1,7	6.4	0.04
	Species	2,96	3.0	0.09
	Year	3,96	66.8	<0.0001
	Fungicide × species	1,96	4.7	0.03
	Fungicide × year	3,96	2.3	0.08
	Species × year	3,96	8.6	<0.0001
	Fungicide × species × year	3,96	1.4	0.26
Shoot [Mn]†	Fungicide	1,7	0.8	0.40
	Species	2,96	1842.2	<0.0001
	Year	3,96	3.7	0.02
	Fungicide × species	1,96	0.2	0.65
	Fungicide × year	3,96	2.1	0.11
	Species × year	3,96	34.8	<0.0001
	Fungicide × species × year	3,96	0.3	0.84

P-value ≤ 0.05 in bold. Data are shown in Appendix S1: Figure S2

Repeated-measures ANOVA with linear mixed-effects model included random effects for plot nested within block and type three sums of squares. † Data were natural log transformed

For both contrast comparisons of the pot bioassay with field soil inoculant (FSI), shoot [Mn] of immature grasses was 2.4 and 2.2 times greater for *K. macrantha* than *H. comata* (autoclaved contrast: $P_{\text{species}} = 0.0004$, Appendix S1: Table S3; fungicide contrast: $P_{\text{species}} = 0.0006$, Appendix S1: Table S4), respectively. For the autoclaved versus fresh field soil comparison portion, shoot [Mn] was 2.7 times greater for immature grasses grown in autoclaved FSI than fresh FSI ($P_{\text{treatment}} = 0.0005$) with no interaction between soil treatment and grass species ($P_{\text{treatment} \times \text{species}} = 0.21$, Appendix S1: Table S3, Fig. 5). For the fresh FSI versus FSI with fungicide comparisons, shoot [Mn] of immature grasses was similar regardless of fungicide addition treatment and with no interaction between fungicide treatment and grass species ($P \geq 0.66$, Appendix S1: Table S4, Fig. 5). For the AMF inoculation experiment, shoot [Mn] was 3.6 times greater for immature *K. macrantha* than *H. comata* ($P_{\text{species}} < 0.0001$) (Appendix S1: Table S5, Fig. 5). Furthermore, shoot [Mn] was 1.3 times greater in assays with autoclaved AMF inoculant than fresh AMF inoculant ($P_{\text{treatment}} = 0.036$) with a significant interaction between AMF inoculant treatment and species on shoot [Mn] ($P_{\text{treatment} \times \text{species}} = 0.004$, Appendix S1: Table S5, Fig. 5). We observed a greater difference in shoot [Mn] of plants grown with fresh versus autoclaved AMF inoculant of *K. macrantha* than *H. comata*.

4 Discussion

In some humid grasslands, AMF affect plant co-existence, species composition, diversity, and soil structure. In the focal semiarid grassland, our results suggest 5 years of fungicide applications to suppress fungal mutualists had minimal effects on most ecosystem properties (e.g. plant diversity, soil structure, soil hydrologic function) and mainly affected interspecific (apparent) competition of two dominant C_3 perennial grasses and interactions between lichens and mosses. While AMF suppression had some effect on plant–plant interactions, plants were not strongly responsive to AMF and shifts in plant composition were often better explained by interspecific variation in traits for root exudate-mediated nutrient mining as inferred from elevated shoot [Mn].

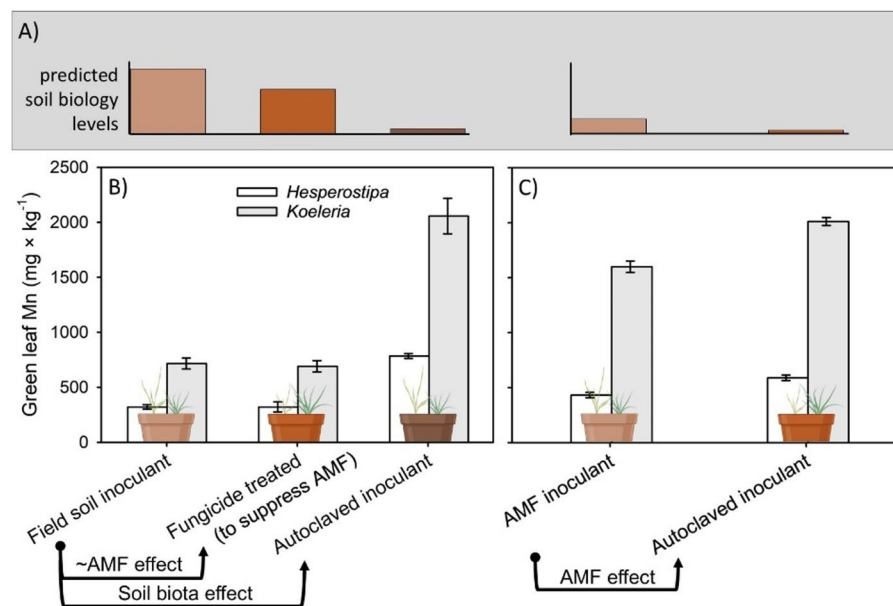


Fig. 5 Predicted effects of soil treatments on degree of intactness of soil biology (A), and effect of arbuscular mycorrhizal fungi (AMF) (and soil biota) on (average $\pm$ 1 SE) green shoot manganese concentration [Mn] (B, C) of C_3 grasses (*Hesperostipa comata*, *Koeleria macrantha*) planted in pots with a 50/50 mix ($n = 12$ replications). Shoot [Mn] is an indicator of phosphorus (P) mobilization (i.e. a proxy for rhizosphere carboxylate concentration) by plants. Results are reported in Appendix S1: Tables S3–S5

4.1 Effects of symbiotic fungi on Northern mixed-grass prairie

After 5 years of suppressing symbiotic fungi, we found evidence of (1) lichen suppressing mosses, (2) arbuscular mycorrhizal fungi (AMF) (or lichens) reducing plant biomass, and (3) AMF affecting interspecific competition of two dominant perennial C_3 grasses. Similar to experiments in a nearby northern mixed-grass prairie [58] and lowland heath in UK [29], we observed strong negative effects of suppressing symbiotic fungi on lichens. Subordinate mosses appeared to experience a form of competitive (or allelopathy) release as treatments eliminated lichens. Lawrey [59] proposed lichen release chemicals that suppress mosses and possibly vascular plants. In a field experiment, Slate, Callaway [60] manipulated lichen-moss biocrusts and interpreted biocrusts reduced the emergence and establishment of ca. 30% of assayed plant species, especially invasive plants in montane west-central Montana. A shift from lichens to mosses may also indicate shifts in unmeasured soil biogeochemistry [61] which may affect the plant community. While the observed shifts in biocrusts may be important, we have mixed evidence to interpret lichens (or mosses) directly affected vascular plants. These nontarget fungicide effects on lichens, however, clearly indicate the value of supplementing biocide field experiments with complementary pot experiments to better interpret plant–AMF interactions.

Despite strong effects on lichens, our main objective was to understand the effect of AMF on vascular plants of a dominant type of northern mixed-grass prairie [62]. Plant biomass was 13% greater in plots with AMF suppression than controls. Other field experiments of grasslands dominated by C_3 perennial grasses responded similarly [58, 63]. The precise mechanism for these effects is unclear. As suggested in the prior paragraph, suppression of lichens may have released plants from the negative effects of lichen leachates. Another interpretation is that AMF had parasitic effects and reduced

shoot production of a C_3 dominated grassland. Alternatively, the fungicide applications may have also suppressed pathogenic fungi. Maron [64] conducted a study on 16 plant species (two grasses and 14 forbs) at a fallow field site and reported *K. macrantha* benefitted from drenches of a biocide cocktail (fungicide & oomycetocide) intended to suppress soil-borne pathogens suggesting beneficial effects of release from pathogens. Our study included an additional set of plots (per block) treated with a biocide cocktail like Maron [64]. This additional treatment indicated mixed evidence of pathogen effects on biomass. Specifically, we observed no main effect of biocide but a marginally significant interaction between biocide and year on biomass suggesting a subtle negative effect of biocide treatment on plant biomass in 2014 and a positive effect in 2015 (Appendix S1: Figure S3). Thus, we failed to detect consistent effects of suppressing AMF versus pathogens on biomass. We suspect another nontarget effect contributes to the observed positive effect of AMF suppression on plant biomass—fungicide drenches may have suppressed fungal decomposition of aerial plant shoots and allowed more shoot biomass to persist annually. Other field studies in other grasslands reported no effect of AMF suppression treatment on plant biomass [24, 44]. It is also worth noting that a growth room pot experiment with nearly 70 regional plant species found only 19% had greater growth when colonized by indigenous AMF than not mycorrhized [28]. Conversely, 65% of plant species for tallgrass prairie in Kansas had greater growth when colonized by indigenous AMF than not mycorrhized [65]. Overall, our field results suggest weak effects of AMF and pathogens on mixed-grass prairie vegetation.

AMF suppression had no effect on plant diversity and species richness. Others have reported minimal effects of AMF suppression treatments on plant community composition of humid to dry grasslands already dominated by C_3 perennial grasses [66, 67] but additional studies have reported context-specific effects of AMF suppression on plant diversity metrics. In dry calcareous grassland in the Czech Republic and semiarid steppe in Inner Mongolia, AMF suppression decreased plant richness by increasing the prevalence of C_3 grasses which likely suppressed the abundance of other plant species [44, 68]. Similar results were reported for an early successional plant community [69]. Conversely in C_4 dominated tallgrass prairie, AMF suppression increased plant species richness, diversity, and evenness in Kansas [24]. In tallgrass prairie, subordinate C_3 grass and forb species experienced a form of competitive release when AMF dependent C_4 grasses were suppressed by AMF reduction treatments.

AMF suppression also had a small but significant effect on dominant perennial C_3 grasses resulting in less *H. comata* and more *K. macrantha*. A related mesocosm experiment with eight plant species detected something similar—AMF suppression increased the biomass of subordinate C_3 grasses including *K. macrantha* and decreased the abundance of dominant C_4 grasses [70]. Our field experiment suggests that *H. comata* is more responsive to AMF than *K. macrantha*; however, our pot experiments failed to corroborate this finding. Thus, the precise mechanism for these effects in the field is unclear. For example, AMF suppression can be incomplete (e.g. reduce colonization/biomass of some but not all AMF) [71] which may differentially affect grass species, AMF can affect interactions with soil-borne pathogens [72, 73] and grasses may vary in pathogen susceptibility, and/or AMF suppression treatments can have nontarget and species-specific effects (e.g. nematodes, soil-borne pathogens) [74]. AMF suppression may have affected individual C_3 grasses differently because the grasses associate with different AMF taxa

[42] which may vary in susceptibility to fungicide [71]. Alternatively, fungicide drenches may have had complex indirect effects such as impacts on the enemy (soil-borne pathogens) of my enemy (*H. comata*). For example, *H. comata* is known to experience negative plant–soil feedback effects while *K. macrantha* experiences neutral feedback [75]. Since colonization of roots by AMF can reduce soil-borne disease [72], AMF suppression may have intensified negative feedback for the dominant grass and released *K. macrantha* from interspecific competition. While plausible, it is unlikely [76]. As indicated above, our field experiment included a separate set of plots treated with a biocide cocktail (fungicide & oomycetocide) known to suppress soil-borne pathogen effects on plantings at a fallow site in western Montana, USA [64]. In our intact prairie site, the biocide cocktail had no effect on plant species composition (Appendix S1: Figure S3).

Pot bioassays complemented the field experiment and tested whether AMF suppression reduced *H. comata* and increased *K. macrantha*. We tested the effect of several soil inoculants and/or treatments on 50/50 mixes of *H. comata* and *K. macrantha*. Young *K. macrantha* plants outperformed *H. comata* regardless of soil treatment. Although not significant, there was a faint signal of immature *H. comata* closing the performance gap with AMF [i.e. field soil inoculant versus fungicide treated (Appendix S1: Figure S2D) and AMF inoculant versus autoclaved inoculant (Appendix S1: Figure S2E)]. More work is needed to resolve the apparent competition between these C₃ grasses [76].

If soil phosphorus (P) levels limit plant growth, then plants are predicted to collaborate with arbuscular mycorrhizal fungi [77]. Contrary to expectations for northern mixed-grass prairie [13], the soil in the field and pot experiments appeared to have sufficient P based on Bray-1 P extractions (Appendix S1: Table S1) (and see *Effects of AMF suppression on nutrient limitation and acquisition strategy*). However, Bray was designed for acidic soils [57] and will overestimate labile P in alkaline soils when there is appreciable Ca-bound P. The preferred P extraction for neutral to alkaline soils is Olsen instead of Bray-1 [57]. In adjacent grassland with the same soil series, Reinhart [13] reported topsoil had moderate P—based on Olsen P extractions and reference levels by Dinkins and Jones [57]. Reinhart [13] went on to conduct a biogeochemical experiment mimicking natural variation in subsoil calcium carbonate levels. Calcium carbonate additions increased subsoil pH (and Ca-bound P) and decreased water-soluble P, anion exchange membrane P, and plant growth but had no effect on Olsen P [13]. Thus, the calcareous soils in our experiments can limit P availability by reducing solution P intensity, while conventional soil test extractants such as Olsen and Bray are mostly sensitive to P quantity and therefore not indicative of solubility changes resulting from addition of Ca and pH increase. Changes in plant growth due to shifting P forms (i.e. increased Ca-bound P) are less observable with routine soil P extractants.

4.2 Effect of drought on plant–mycorrhizal interactions

A natural drought occurred midway through the 5-year field experiment which enabled examination of AMF effects in situ before, during, and after drought. The shifts in plant composition induced by AMF suppression varied from year-to-year and with drought. Drought is an interesting disturbance because it can simultaneously affect two levers (soil water and soil nutrient availability) thought to incentivize collaboration with AMF [10]. This is because (1) AMF can help individual plants (and plant communities) resist and recover from moderate droughts [30–32] and (2) dry soil conditions

can shift soil equilibrium dynamics and reduce available P in temperate grasslands [78] which depending on the soil's character (e.g. P solubility and lability) may favor either increased or decreased collaboration with AMF. AMF suppression induced incremental shifts in plant community composition from 2010 to 2012 and peaked in the drought year (2012) but effects did not strongly increase from 2011 to 2012 (Figs. 1 and 2). In the drought recovery period (2013–2014), effects of AMF suppression appeared to vanish. If the natural drought caused a pulse of available P across all plots because plants take up less P in dry soils and/or P is released as dry soils regain moisture due to destruction of organo-mineral complexes during drying [79], then the fertile conditions might have weakened the nutritional mutualism and plants' incentive to collaborate with AMF [9]. Droughted grassland soils, however, tend to have less available P and more available N [78] which should strongly increase the ratio of available N to P in the soil and result in less available P at least temporarily. Furthermore, nearby field experiments detected either similar [80] or lower levels of soil P (extracted from anion exchange membranes) and greater soil N in plots with simulated droughts than controls (Reinhart K.O., *unpublished data*). Whether plants are more (or less) P limited during and immediately after a drought is difficult to interpret because dry soil conditions will limit P uptake by roots because uptake depends on diffusion which requires a water film around soil particles [81]. During the recovery period, we anticipate that P will at least temporally be available (labile) but transition back to the pre-drought insoluble phase after soil Ca re-equilibrates with the solution i.e. formation of poorly soluble Ca phosphates under alkaline conditions [21]. As the proportion of insoluble P increases, *H. comata* and other plants may increase P acquisition by autonomous root mining in place of collaboration with AMF [6, 13].

4.3 Effects of AMF suppression on nutrient limitation and acquisition strategy

To help understand the importance of AMF in northern mixed-grass prairie, experiments combined AMF suppression (or inoculant) treatments with putative indicators of plant nutrient limitation (shoot N: P) and P acquisition strategy (shoot [Mn]). We found limited evidence that AMF suppression treatment affected shoot N: P of mature plants in situ. Specifically, AMF suppression increased shoot N: P of *K. macrantha* but not *H. comata* which conflicts with other results (Fig. 3) that suggest *H. comata* was more dependent on AMF than *K. macrantha*. We cautiously interpret that shoot N: P of mature specimens of the focal species may be an unreliable indicator of nutrient limitation. For example, others have shown the two grasses in situ have relatively constant shoot N: P values regardless of available soil N: P [82]. Craine [37] and Yan [83] also interpreted that shoot N: P is not necessarily a useful indicator of nutrient limitation of plants. The stability of shoot N: P values [82] may be the product of the grasses' capacities for shoot-to-root transfer of P and their root systems to store P as phytic acid [84]. This may ultimately allow mature perennial grasses to maintain shoot N: P year-to-year and mask the degree in which P limits their growth. Shoot N: P values are likely a useful indicator of nutrient limitation of plants without efficient P storage and those with highly plastic shoot N: P which appears to include some plants that are highly responsive to mycorrhization [9].

Our nutrient limitation interpretations can be aided by the pot bioassay portion that harvested immature grasses which had no prior years of growth to accumulate P stores

in root biomass. The bioassays revealed effects of soil treatments and grass species on shoot N: P. Consistent with observations of apparent competition in situ, shoot N: P values of ex situ 50/50 mixes of young grasses (without nutrient stores) indicated divergent nutrient limitation—*H. comata* was either co-limited by N and P or P limited (P-limited = N: P > 16) while *K. macrantha* was likely N limited (N: P < 14) (sensu stricto [85]).

To further aid interpretations of nutrient limitation and nutrient acquisition strategy, we measured a putative indicator of root exudation (shoot [Mn]) to mobilize calcium-bound P [86]. Grasslands are known to produce high levels of root exudates [87] and contain microbes with an abundance of soil P-cycling genes [88]. Furthermore, co-occurring grassland plants are known to have varying P acquisition strategies in Asian steppe [89]. Consistent with results indicating interspecific competition among grasses, shoot [Mn] varied by C₃ grass in situ (field experiment) and ex situ (pot experiment) suggesting greater root P mining by *K. macrantha* than *H. comata*. While related plants may share some root trait and metabolome characteristics, the species may continue to have ecologically important differences in root traits and exudates [90]. In field plots, suppression of AMF favored the C₃ grass (↑ *K. macrantha* and ↓ *H. comata*) with fine root structural traits (↑ specific root length and ↓ root diameter) indicative of fast potential growth and resource acquisition—4.2 times greater specific root length (162 versus 39 m × g⁻¹, respectively) and 52% smaller root diameter (0.15 versus 0.29 mm, respectively) [91].

In pot bioassays, grass shoot [Mn] increased as the intensity of inoculant sterilization increased [i.e. control = fungicide < autoclave (Fig. 5B) and AMF inoculant < autoclaved inoculant (Fig. 5C)] thereby suggesting some plasticity for inducible root mining which appeared to depend on the degree of intactness of the grassland's soil biota (Fig. 5A) and possibly the presence of phosphorus solubilizing bacteria. Autoclaving soil increases Mn that is extractable from soil [92] which may partly explain the effects of inoculant autoclaving on shoot [Mn]. Since the bulk of the soil in all pots was autoclaved (93%, v/v), the presence or absence of key P solubilizing soil biota (e.g. calcium phosphate-solubilizing bacteria) [93, 94] likely explains much of the dramatic difference in shoot [Mn] and root P mining of controls across bioassays (i.e. Fig. 5B field soil inoculant versus Fig. 5C AMF inoculant, respectively) relative to their autoclaved inoculant counterparts. Future studies should control for autoclaving-induced flushes of Mn by autoclaving (or irradiating) all soil and inoculating soils with varying microbial filtrate treatments (e.g [95]).

4.4 Effects of AMF suppression on soil structure and hydrologic function

AMF are a driver of soil aggregate stability, and aggregate stability is a putative indicator of ecosystem functions including nutrient cycles and hydrologic function [96, 97]. Our field experiment failed to uncover that AMF suppression affected soil structure (i.e. macroaggregate stability) and soil hydrologic function. Similarly, Reinhart [50] conducted a nearby field experiment and failed to detect (negative) effects of AMF suppression on the stability of water-stable macroaggregates and soil water transport. Furthermore, their meta-analysis of aggregate-infiltration relationships for northern mixed-grass prairie indicated no overall relationship between measures of aggregate stability and soil water transport. In calcareous soils, AMF suppression may have little effect on aggregate stability because the soil solution contains high levels of Ca which can increase aggregate stability [98]. In drylands, soil water transport is possibly more strongly related to

plant biomass [99] and/or plant species composition (e.g. biennial tap rooted forbs) [50] than soil aggregate stability. Conversely in Kansas tallgrass prairie dominated by AMF dependent C_4 grasses, AMF suppression decreased the proportion of macroaggregates and increased the proportion of microaggregates [23] which may have cascading negative effects on soil water transport.

4.5 Predicting modes of P acquisition

Complementary in situ and ex situ approaches strengthened our interpretations and understanding of the boundaries of where and when plants will depend on AMF, especially C_3 grasses in dryland systems. We cautiously interpret that one determinant of the predominant mode of P acquisition by plants is the ratio of soil organic to inorganic carbon (e.g. $CaCO_3$) (Fig. 1). Depending on which carbon form dominates (organic versus inorganic) will determine what P form is most dominant, and the dominant P form will impact P cycling and collaboration with AMF [13, 15] (Fig. 1). Generally, the proportion of soil's organic carbon content is inversely related to the inorganic carbon [100]. Plants in humid montane grasslands in soils of light calcareous nature favored collaboration with AMF [25] and also appear to follow our framework's predictions (Fig. 1). For example, the soils in van der Heijden's [25] experiment were described as, "rich in organic matter, contained considerable amounts of calcium carbonate and had a relatively high pH", in contrast to the low-organic carbon and semiarid soils of the current study and described by Reinhart [13] which contained two to 32 times more $CaCO_3$ and were not responsive to AMF. In other words, a relatively large proportion of organic to inorganic carbon should correspond with more P in the form of organic matter than calcium bound which should favor collaboration with AMF [15]. We interpret that main modes of P acquisition (root mining versus mycorrhizal scavenging) are likely driven by $CaCO_3$ and soil organic matter which determine the dominant P form (Fig. 1).

5 Conclusion

Suppression of arbuscular mycorrhizal fungi (AMF) had weak effects on plant community composition and no effects on soil structure and soil water transport. Suppression of fungal symbionts mainly eliminated lichens, shifted apparent competition of co-dominant C_3 grasses, and increased plant biomass. Both in situ and ex situ experiments indicate the majority of regional plants have weak responses to AMF [13, 28]. Our findings support the interpretation that dryland soils have distinct properties (e.g. alkaline, calcareous, infertile) that cause co-precipitation of calcium and phosphorus (P), which reduces P solubility and availability [13]. The resulting insoluble P character of many dryland soils is likely to favor autonomous P-acquisition strategies over collaboration with AMF [13]. Conversely, we cautiously interpret that collaboration with mycorrhizal fungi is likely favored where (e.g. Kansas tallgrass prairie) the soil's character (e.g. near neutral pH, non-calcareous, fertile) enables more labile P.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1007/s44378-025-00124-6>.

Supplementary Material 1.

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Author contributions

K.R., L.V., C.P., and Y.L. conceived the ideas. K.R. designed methodology, collected the data, and analyzed the data. K.R. and Y.L. led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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Data availability

Open Research: Data and code are provided via the following link: (<https://figshare.com/s/16f9d1934ef7c58ace4c>).

Declarations

Ethics approval and consent to participate

As an employee of the Fort Keogh Livestock and Range Research Laboratory, KR is permitted to collect soil and plant material (e.g. grass shoots) for research purposes from the research station.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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