Prairie plant communities and their associated phyllosphere fungal communities change across the steep precipitation gradient in Kansas USA, though individual plant species' phyllosphere communities may not.

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by

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Abstract

Tallgrass prairies in North America have endured substantial losses in diversity along with compositional shifts due to anthropogenic environmental change. One largely overlooked aspect of diversity in prairie systems is phyllosphere fungi. Many environmental drivers impact the composition and diversity of phyllosphere fungal communities including plant hosts and surrounding plant communities, precipitation, and land use history. Studies have mainly focused on the changes in foliar fungal communities associated with specific plant species. Few, however, have addressed the association between whole plant communities and their phyllosphere fungi. Additionally, though it is well documented that plant communities shift along precipitation gradients, the impact on phyllosphere fungi in prairies has yet to be explored. Further, little is known about how prairie plant phyllosphere fungi in post-agricultural fields differ from those in native prairie remnants or if they respond differently to environmental change. To determine how plant communities and their phyllosphere fungal communities respond to changes in precipitation and between land use histories, we sampled plant communities and associated phyllosphere fungal communities in native prairie remnants and post-agricultural prairie sites across the steep precipitation gradient (456 mm yr^{-1} – 1040 mm yr^{-1}) in the central plains in Kansas, USA. In addition, we sampled leaves of big bluestem (*Andropogon gerardii*, Poaceae) and leadplant (*Amorpha canescens*, Fabaceae) in five native prairie remnants across a shorter span of the same gradient (615 mm yr^{-1} – 1038 mm yr^{-1}) to dissect the response of phyllosphere fungi in individual prairie plant species across this precipitation gradient. Plant community cover data and MiSeq ITS2 metabarcode data of the phyllosphere fungal communities indicated that both plant and fungal community composition respond strongly to mean annual precipitation (MAP), but less so to land use (native prairie remnants vs. post-agricultural sites). Plant and fungal diversity were greater in the native remnant prairies than in post-agricultural sites and both plant diversity and the diversity of fungi in their phyllospheres increased with MAP. Additionally, communities in the arid and mesic parts of the precipitation gradient were distinct. We also found that *A. canescens* and *A. gerardii*, harbored comparable phyllosphere fungal communities which did not significantly change along the precipitation gradient. Similarly, the diversity of the phyllosphere fungi neither differed

between these two plant hosts nor changed with increasing precipitation. Although decoupling the drivers of fungal communities and their composition – whether abiotic or host-dependent – remains a challenge, our research highlights the distinct community responses to precipitation and the tight tracking of the plant communities by their associated fungal symbionts – though individual prairie plants may not show significant distinctions.

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Dedication

I would like to dedicate this work to my loving parents and grandparents, Amy Marie Dea, Darren Ray Dea, Dinah Krasselt, and Terry Doughty. In loving memory of Beverly Rae Dea and Donald Joseph Dea “and until we meet again, may God hold you in the hollow of his hand”.

Chapter 1 - Introduction

Microbial communities are ubiquitous and inhabit a wide variety of habitats. One such habitat is the phyllosphere – the aerial plant photosynthetic tissues. The phyllosphere is an extensive yet harsh habitat for microbial life which is influenced by many environmental factors. Despite the oligotrophic conditions and rapid fluctuation in environmental conditions such as temperature and moisture, the phyllosphere harbors a diverse ecosystem (Lindow and Leveau, 2002), of archaea, bacteria, fungi, protists, and viruses (Jumpponen and Jones, 2009; Martiny et al., 2011; Vorholt, 2012; Laforest-Lapointe and Whitaker, 2019). Phyllosphere fungi are of special note as they may impact the plant productivity and physiology (Saikkonen et al., 1998; Rodriguez et al., 2009; Meyer and Leveau, 2012; Zahn and Amend, 2019).

Phyllosphere fungi within leaf tissues (endophytic) and on the leaf surfaces (epiphytic) provide ecosystem services, making them of interest for ecosystem conservation. One important ecosystem service that phyllosphere fungi provide is increased plant productivity. Phyllosphere fungi can aid plant productivity through providing resistance of the host plant to pathogenic infection by spatial exclusion (Blakeman and Fokkema, 1982), reduction of harmful UV radiation affecting the leaf surfaces (Barrera et al., 2020; Solhaug et al., 2003), aid in host plant water retention (Rho and Kim 2017), and modulation of plant stress tolerance (Vorholt, 2012). Though phyllosphere communities include pathogenic fungi, colonization of endophytic fungal communities from asymptomatic leaves have been shown to reduce damage to plant tissues caused by pathogens (Arnold et al., 2003). The foliar fungal communities may be more sensitive to environmental factors than those of bacteria (Bernard et al., 2021) or ectomycorrhizal fungi (Bowman and Arnold, 2021). Because of this, it is necessary to determine the response of

phyllosphere fungal communities to wide variation in the environmental factors such as mean annual precipitation and land use.

In Chapter 2, we aim to answer three main questions: 1) how do plant communities and their associated phyllosphere fungal communities respond to mean annual precipitation (MAP) in their community estimates (richness, diversity, and evenness) and in their composition?; 2) does their response differ between different land use histories – namely remnant native prairies and post-agricultural prairies?; and, 3) are the plant and foliar fungal communities linked? In addition to sensitivity to climatic changes (Bowman and Arnold, 2021; Oita et al., 2021), phyllosphere fungal community composition may be sensitive to legacies of agricultural land use. This could be because of dispersal limitation from remnant native prairies (Turley et al., 2017) or possibly altered soil communities, differences in plant communities, and other changes to the sites that persist (Bellemare et al., 2002; Dupouey et al., 2002; Flinn and Marks, 2007). The interaction between these factors – mean annual precipitation and land use history – has yet to be elucidated. Further, studies on the linkage between plant and phyllosphere fungi remain rare.

In Chapter 3, we examined a shorter portion of the precipitation gradient and chose two focal prairie plant species: a common prairie legume *Amorpha canescens* Pursh (leadplant) and a dominant grass *Andropogon garardii* Vitman (Big Bluestem). We aimed to answer two primary research questions 1) how do foliar fungal communities respond to the mean annual precipitation within the range in which the two species co-occur? and 2) do the two co-occurring focal plant species recruit distinct foliar fungal communities? Legumes such as *A. canescens* associate with N-fixing bacteria in their roots leading to greater N-content in other plant organs such as the leaves (Adams et al., 2016). This relatively higher substrate quality because of the lower carbon

to nitrogen ratio compared to non-legumes may affect fungal communities as suggested in previous studies (Jumpponen and Jones 2010). Further, the difference between these two species may give key insights into conservation of prairie biodiversity by helping us to assess if target plants, such as *A. canescens*, are hot spots fungal diversity that should be prioritized in conservation or restoration efforts.

Chapter 2 - Precipitation, Not Land Use, Primarily Determines the Composition of Both Plant and Phyllosphere Fungal Communities[1]

Abstract

Plant communities and fungi inhabiting their phyllospheres change along precipitation gradients and often respond to changes in land use. Many studies have focused on the changes in foliar fungal communities on specific plant species, however, few have addressed the association between whole plant communities and their phyllosphere fungi. We sampled plant communities and associated phyllosphere fungal communities in native prairie remnants and post-agricultural sites across the steep precipitation gradient in the central plains in Kansas, USA. Plant community cover data and MiSeq ITS2 metabarcode data of the phyllosphere fungal communities indicated that both plant and fungal community composition respond strongly to mean annual precipitation (MAP), but less so to land use (native prairie remnants vs. post agricultural sites). However, plant and fungal diversity were greater in the native remnant prairies than in post-agricultural sites. Overall, both plant and fungal diversity increased with MAP and the communities in the arid and mesic parts of the gradient were distinct. Analyses of the linkages between plant and fungal communities (Mantel and Procrustes tests) identified strong correlations between the composition of the two. However, despite the strong correlations, regression models with plant richness, diversity, or composition (ordination axis scores) and land use as explanatory variables for fungal diversity and evenness did not improve the models

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[1] As printed in Dea, H. I., Urban, A., Kazarina, A., Houseman, G. R., Thomas, S. G., Loecke, T., Greer, M. J., Platt, T. G., Lee, S. & Jumpponen, A. (2022) Precipitation, Not Land Use, Primarily Determines the Composition of Both Plant and Phyllosphere Fungal Communities. *Frontiers in Fungal Biology*, 3:805225. doi: 10.3389/ffunb.2022.805225

compared to those with precipitation and land use ($\Delta AIC < 2$), even though the explanatory power of some plant variables was greater than that of MAP as measured by R^2. Indicator taxon analyses suggest that grass species are the primary taxa that differ in the plant communities. Similar analyses of the phyllosphere fungi indicated that many plant pathogens are disproportionately abundant either in the arid or mesic environments. Although decoupling the drivers of fungal communities and their composition – whether abiotic or host-dependent – remains a challenge, our study highlights the distinct community responses to precipitation and the tight tracking of the plant communities by their associated fungal symbionts.

Introduction

Aerial plant photosynthetic tissues – the phyllosphere – are among the most extensive microbial habitats on Earth (Morris et al., 2002). This habitat can be oligotrophic and exposed to rapid fluctuations in environmental conditions including shifts in temperature, humidity, and radiation (Lindow and Brandl, 2003). Yet, the phyllosphere represents a diverse ecosystem (Lindow and Leveau, 2002), colonized by hyperdiverse communities of bacteria, archaea, virus, protists, and fungi all living on (epiphytes) and within (endophytes) the leaves (Jumpponen and Jones, 2009; Martiny et al., 2011; Vorholt, 2012; Laforest-Lapointe & Whitaker, 2019). These diverse communities drive ecosystem function (Song et al., 2017; Laforest-Lapointe and Whitaker, 2019) and can contribute to nitrogen cycling by fixing nitrogen *in situ* (Furnkranz et al., 2008). Phyllosphere communities can also affect plant fitness and productivity (Davison, 1988; Schauer and Kutchera, 2011) through their modulation of stress tolerance (Vorholt, 2012) or pathogen resistance (Innerebner et al., 2011). Further, the phyllosphere communities may drive plant community dynamics (Aschehough et al., 2014; Whitaker et al., 2017; Laforest-Lapointe and Whitaker, 2019) thereby linking the phyllosphere communities to plant communities and their productivity (Laforest-Lapointe et al., 2017).

Phyllospheres are clearly important for ecosystem function and as a hotspot for microbial diversity (Arnold and Lutzoni, 2007; Laforest-Lapointe et al., 2017). Foliar fungi are among the most diverse members that can impact plant productivity and physiology within the phyllosphere (Saikkonen et al., 1998; Rodriguez et al., 2009; Meyer and Leveau, 2012; Zahn and Amend,

2019). These fungi presumably occupy photosynthetic tissues of all species and in all divisions of land plants (Bacon and White, 2000). While present in the foliage, these communities include taxa that are directly and functionally associated with the plant tissues (e.g., pathogens, foliar parasites or endophytes) as well as those that may be observable on these tissues but neither penetrate the cuticle nor directly functionally interact with the host plant (i.e., epiphytes that may utilize nutrients available on the foliar surfaces but never cross the cuticular barrier) (see Gomez et al., 2018). The foliar fungal communities may be more sensitive to environmental factors than those of bacteria (Bernard et al., 2021) or ectomycorrhizal fungi (Bowman and Arnold, 2021). A recent study of Hibiscus tiliaceus trees in Hawaii (Bernard et al., 2021) reported that while bacterial community composition was better explained by the plant organ macrohabitat, location within a steep environmental gradient better predicted variation in fungal community composition (see also Zimmerman and Vitousek, 2012). Similarly, Bowman and Arnold (2021) concluded that while the distribution of ectomycorrhizal fungi was mainly constrained by dispersal, foliar fungi were more constrained by climate factors such as mean annual precipitation and mean annual temperature. These studies exemplify the value of studying steep environmental gradients as a means to better understand how environmental variation influences the composition and assembly of fungal communities (Fraser et al., 2015; Rudgers et al., 2021).

In addition to the environment, communities can be impacted by a variety of human factors. The anthropogenic conversion of natural ecosystems presents a substantial threat to biodiversity (Foley et al., 2005; Newbold et al., 2015; Perreault and Laforest-Lapointe, 2021). Human land-use, including agriculture and silviculture, can have long-lasting legacies wherein the altered ecosystem attributes persist after cessation of human land-use (Dupouey et al., 2002; Foster et al., 2003; Flinn et al., 2005; McLauchlan, 2006; Cramer et al., 2008). These systems struggle with the establishment of native plant communities after the human land-use abandonment (Kuussaari et al., 2009; Moreno-Mateos et al., 2017). For example, compared to systems that have no history of human use, former agricultural lands may possess altered soils, nonnative plant communities, and other distinct ecosystem properties for decades and even millennia following farm abandonment (Bellemere et al., 2002; Dupouey et al., 2002; Flinn and Marks, 2007). Similarly, agricultural land-use history can reduce soil-inhabiting fungal diversity and result in communities distinct from those in native remnants that have never been used for production agriculture (Oehl et al., 2003; Wagg et al., 2018; Turley et al., 2020). Phyllosphere

communities may be less responsive to edaphic factors as they do not directly interact with the soil matrix, whose biogeochemical attributes may strongly influence soil-inhabiting communities. Consistent with this, community composition of the foliar fungi often reflects climatic factors (Bowman and Arnold, 2021) such as mean annual temperature and precipitation (Oita et al., 2021), rather than variation in soil properties. This is particularly true if phyllosphere communities are assessed broadly and include casual epiphytes that may only utilize readily available resources on the leaf surfaces. Even within the phyllosphere, controls of communities in foliar compartments may differ, as the communities of leaf epiphytes and endophytes may be shaped by distinct environmental controls (Gomes et al., 2018).

Although plant and fungal communities and their responses to environmental gradients have been targets of many studies, analyses to better establish linkages among them are still rare. Large-scale studies have reported correlations between plant and fungal richness (Arnold and Lutzoni, 2007; Tedersoo et al., 2020) that may often stem from collinearities and/or correlations between plants and associated fungal communities. In this contribution, we attempt to concurrently dissect plant communities as well as those fungal communities that occupy their photosynthetic tissues. Many studies thus far have focused on diversity at the local scales (Allan et al., 2014; Newbold et al., 2015) but neglected changes at larger spatial scales. We utilized the steep precipitation gradient in the state of Kansas (USA) located in the Great Plains to assess how plant communities and their foliar fungal communities may respond to this precipitation gradient, how the plant and fungal communities may differ across two distinct historic land uses (post-agricultural sites and native remnant prairies), whether the communities within these two historic land uses respond differently to precipitation, and how the plant communities and their foliar communities may be linked to each other. Agricultural systems that have a history of intensive human land-use are a common focus of restoration efforts but how post-agricultural fields compare to native prairie remnants remains unclear particularly for fungal communities that occupy photosynthetic tissues. We hypothesized that 1) plant and their phyllosphere fungal communities increase in richness, evenness, and diversity with increasing the mean annual precipitation; 2) post-agricultural sites – as a result of their previous intensive agricultural use – have a lower richness and diversity as well as distinct communities when compared to native prairie sites; 3) native prairie remnants and post-agricultural sites differ in their responses to the precipitation gradient such that richness, diversity, and evenness in the remnant prairie sites

respond more strongly to precipitation than post-agricultural prairies; and 4) fungal communities correlate with plant communities in diversity and composition. We emphasize that the approaches linking aboveground plant diversity with fungal richness and diversity are rare (Cho et al., 2017) and that studies across land-use systems and plant diversity are required to enable sound recommendations for sustainable land-use (Monkai et al., 2017).

Materials and Methods

Study Sites and Sampling

During the summer of 2019, we located eight post-agricultural and eight native remnant prairie sites along the steep precipitation gradient in Kansas for a total of sixteen sites with mean annual precipitation (MAP) ranging from 455.74 mm yr^{-1} to 1040.46 mm yr^{-1} and mean annual temperature (MAT) ranging from 11.31°C to 13.30°C (Figure 1A; Table 1). We specifically targeted sites that would represent the precipitation gradient while stratifying our sampling to similar soil types along the Kansas River watershed, which runs across the state east-to-west at approximately the 39th parallel (longitude ranging from 095° 16’ 21.42”W to 101° 47’ 06.31”W). The chosen sites had similar edaphic characteristics and occurred in a similar landscape position (e.g., toe slope or flood slope terrace). Additionally, we sampled sites alternating between the arid and mesic ends of the gradient to minimize the potential for temporally confounding factors in a sampling that required a little over three months (June 12th–September 18th, 2019).

At each site, we established a 25 m x 23 m plot. Within each plot, we established a 1m x 1m subplot in each of the four corners for a total of four subplots (Figure 1B). The GPS location was recorded using an Eos Arrow 100 Submeter GNSS Receiver (Eos® Positioning Systems, Inc., Terrebonne Canada), and the subplot corners marked to permit sampling of the plant and fungal communities even if the teams sampling plants and fungi could not sample simultaneously. Within each subplot, we identified all plant individuals to species. When this was not possible, we reverted to genus (e.g., species within *Cyperus* and *Carex* were pooled). We quantified plant abundance by comparing the canopy cover of each species or morphospecies within the subplot to a cover card that was marked with cover percentages of various sizes. For species that occupied large areas, we counted the number of cover card areas required to match

the cover of a particular species. These values were then summed to get the subplot cover for that species. We recorded values to the nearest percentage or fraction of a percentage for values below 1%. We then measured the modal height of each species in 10-cm height classes in the subplot. Plant height varied strongly across the sites because of the differences in the plant communities (short to tallgrass prairie) and the timing of sampling (plant phenology). Because we used a visual comparison of the cover card to plant cover, there was a potential for error if the cover card was held at different distances within and among observers due to the foreshortening effect (distance between eye, cover card, and plant height could appear to have different values). To minimize this problem, all observers held the cover card in the same way each time. We then adjusted for differences in the eye hand relationships of each observer and differences in plant height. To do this, each observer measured swatches of a known size at various heights in the lab. These data were used to calculate an observer-specific plant height correction that was applied to final cover values (Watson et al., 2021).

In order for fungal community samples to reflect the cooccurring plant communities, we sampled fungal communities using a systematic gridline intersection sampling. In each of the four subplots, we placed a 1 m x 1 m quadrate gridded 20 cm apart for a total of 25 intersects (Figure 1C). At each intersect, we lowered a wooden dowel rod and excised the first, topmost leaf the dowel rod touched and placed the leaves individually in sterile plastic bags skipping the middle intersect for a total of 24 leaf samples within each subplot and 96 for each full plot (single land use within a precipitation band). Samples were placed on ice in a cooler and processed in the laboratory within 24 hours of collection. Our sampling allowed us to capture representative plant taxa and minimize plant height bias due to the spatial (four representative 1 m x 1 m subplots) and temporal (samples taken throughout the growing season) heterogeneity of our sampling efforts.

DNA Extraction, PCR Amplification, and Sequencing of Fungal Communities

To extract total environmental DNA from the sampled leaves, we excised two 3 mm disks from each of the 24 leaves from each subplot using sterile Ted Pella Biopsy Punches (Ted Pella Inc., Redding, CA). We followed the ThermoFisher Phire Plant Direct kit manufacturer's protocol (ThermoScientific, Pittsburg USA) to isolate total DNA from the leaf tissues. In brief, we suspended the two leaf disks in 40 µl of dilution buffer and crushed the leaf disks with round

tip forceps. We then pooled 20 µl of each of the twenty-four extractions for each subplot into one representative sample (4 subsamples for each of the 16 plots).

To choose the optimal dilution for PCR-amplification, we diluted the extracts (10^0 - 10^{-3}) in sterile molecular grade RNA and DNA-free water. In pilot reactions, the 10^{-2} dilution consistently and reliably produced PCR-amplicons and was chosen for library preparation. To analyze the fungal communities, we PCR-amplified the Internal Transcribed Spacer (ITS2) of the ribosomal RNA gene (Schoch et al., 2012) with the forward fITS7 (Ihrmark et al., 2012) and reverse ITS4 (White et al., 1989) primers in 50 µl duplicate PCR reactions. Both the forward and reverse primers included a sample specific 12 bp Molecular Identifier DNA (MID) (Caporaso et al., 2012). The volumes and final concentrations of reagents were as follows: 2.5 µl forward and reverse primer (0.5 µM), 5 µL 10-2 diluted template DNA, 25 µL of 2X Phire Plant Direct PCR Master Mix, and 17.5 µL molecular grade water. The PCR reactions included an initial denaturing step for 30 s (98°C) and were followed by 30-35 cycles of 10 s of denaturing (98°C); 30 s of annealing (54°C); 1 min of extension (72°C); and concluding with a 9 min final extension (72°C). When 30 cycles did not amplify, we repeated the reactions with 35 cycles. The PCR reactions included sterile molecular grade RNA- and DNA-free water as a negative control and a fungal mock community as a positive control to calculate internal sequencing error as described in Mothur Standard Operation Protocol (SOP) (Kozich et al., 2013). We constructed the fungal mock community from nine fungal pure cultures that broadly represent fungal taxa (Ascomycota: *Aspergillus niger*, *Chaetomium globosum*, *Penicilium notatum* (synonym *Penicillium chrysogenum*), *Saccharomyces cerevisiae*, *Sordaria fimicola*; Basidiomycota: *Coprinopsis cinerea*; Chytridiomycota: *Phlyctochytrium acuminatum* (synonym *Spizellomyces acuminatus*); Mucoromycota: *Phycomyces blakesleeanus*, *Rhizopus stolonifer*). We extracted DNA from two-week old cultures with the DNeasy PowerSoil DNA Isolation Kit (Qiagen, Germantown, Maryland) as per the manufacturer's protocol and equal volumes of 2 ng/µL of each extraction were pooled. We combined a total of 45 µL of each duplicate PCR amplicon for each sample including positive and negative controls. We purified the pooled 90 µl volumes using the MagBind RXNPure Clean-up system (Omega Bio-Tek Inc., NorCross, Georgia) following a modified manufacturer's protocol with a 1:1 ratio of PCR product to AMPure solution and two rinse steps with 80% ethanol. A total of 250 ng of purified DNA per sample was pooled into one.

As the negative control did not yield quantifiable amplicons, the whole 90 µl volume was included in the pool.

Illumina adapters and indices were added using four PCR cycles, KAPA Hyper Prep Kit (Roche, Pleasenton, CA USA), and 0.5 µg starting DNA. The library was sequenced (2 x 300 cycles) using the Illumina MiSeq Personal Sequencing System at the Integrated Genomics Facility (Kansas State University, Manhattan KS USA). The sequence data are available through the Sequence Read Archive under BioProject PRJNA795108; BioSamples SAMN24688311-SAMN24688331.

Sequence Data Processing

The sequence data were processed using the mothur pipeline (v. 1.44.3; Schloss et al., 2009) following mainly the MiSeq standard operating protocol to generate ASV (Amplified Sequence Variant) and OTU (Operational Taxonomic Unit) data. In brief, the sequence data for each experimental unit were identified by Molecular Identifier DNAs (MIDs; Caporaso et al., 2012), extracted from the paired-end.fastq files and assembled into contigs. Sequences with more than 1 bp difference with the primers, without an exact match to the MIDs, or with long homopolymers (maxhomop = 8) were omitted. Since the four sub-plot samples were not independent but rather represented one site, we pooled the libraries to one per plot (for a total of 16 experimental units). We considered this necessary to avoid pseudo-replication, as the adjacent subplots would not represent true replicates of the main effects (mean annual precipitation and land-use) in our models. Sequences were truncated to the length equal to the shortest high-quality read (237 bp excluding primers and MIDs), pre-clustered (Huse et al., 2010), and potential chimeras identified (UCHIME; Edgar et al., 2011) and culled. The remaining sequences were assigned to taxon affinities using the Naïve Bayesian Classifier (Wang et al., 2007) and the UNITE taxonomy reference (Abarenkov et al., 2021). Non-target reads (those with no match in the UNITE-curated INSD or assigned to Protista and Plantae) were removed from further analyses. The quality-screened sequences were assigned to ASVs and subsequently clustered to OTUs at 97% similarity using vsearch (Rognes et al., 2016). Rare ASVs and OTUs represented by fewer than ten reads were removed (Brown et al., 2015; Oliver et al., 2015).

Data Analyses

Fungal communities were analyzed as both ASVs and OTUs. Consistent with other analyses comparing ASVs and OTUs (Glassman and Martiny, 2018; Tipton et al., 2021;

Tawidian et al., 2021), our analyses also yielded comparable results. As a result, we present the OTU analyses here, whereas the ASV analyses are available as a supplement (see Appendix B)We iteratively (100 iterations) calculated observed (S_{Obs}) richness, Shannon's diversity (H'), and evenness based on Shannon's diversity (E_H) using the mothur pipeline (v. 1.44.3; Schloss et al., 2009). We subsampled the sequence data to 90000 sequences per sample, as recommended in (Gihring et al., 2012) to avoid biased comparisons of estimators in samples with unequal sequence yields.

Statistical analyses were performed using program R (R Core Team 2021-2022). We used multiple linear regression analyses to predict plant and fungal richness, diversity, evenness, sample scores of the first and second PCoA axes (i.e., sample coordinates in ordination space), and adjusted Floristic Quality Index (FQI_{adj}) responses to Mean Annual Precipitation (MAP) normalized around the mean (730.01 mm yr^{-1}), land use history (LU), and their interaction using the "lm()" function in program R. For plant communities, we relativized all cover values by total plant cover. Plant community diversity metrics were calculated using the "community structure()" function in the "codyn" package in program R (v. 2.0.5; Hallett et al., 2020). We used E_{var} as our metric for plant communities because it is least sensitive to differences in species richness (Smith and Wilson, 1996). We also estimated FQI_{adj} in each plot (Freyman et al., 2016). FQI_{adj} was initially developed by Wilhelm and Kane County (1977) and is a commonly used conservation indicator that provides a numerical value for the ecological value for restoration success of a site and uses ratios between native and total species richness (see Watson et al., 2021 for further details). We visually evaluated residuals to confirm that they did not present any blatant violations of assumptions of linear regression analyses and performed outlier analyses. Three samples (LVN_N ASV and OTU S_{Obs}; TRB_N plant H' and plant PCoA axis 2; and TRB_P plant FQI_{adj}) represented potential outliers (values greater than 2 standard deviations from the mean). We analyzed our data both with and without these data points to determine if they drove patterns in our results.

To determine if any other explanatory variables were superior to MAP in explaining variation in plant and fungal community estimators, we replaced MAP with geographic distance (change in longitude) for responses of plant (richness, diversity, evenness, FQI_{adj}, or first PCoA axis) and fungal (richness, diversity, evenness, or first PCoA axis) community estimators in models combining it with LU and their interaction as predictors. We also compared models with

MAP replaced by plant community estimators (richness, diversity, evenness, FQI_{adj}, or first PCoA axis) as explanatory variables in models combining LU and interaction terms for responses in fungal community estimators (richness, diversity, evenness, or first PCoA axis). We compared the change in AIC values to identify the superior models ($\Delta AIC > 2$) as described by Burnham and Anderson (2004).

To visualize and infer compositional differences within plant and fungal communities, we calculated pairwise Bray-Curtis distances and visualized these data with Principal Coordinates Analysis (PCoA) using function "ordinate()" (method = 'PCoA') in R package "phyloseq" (v. 1.38.0; McMurdie and Holmes, 2013). To control for library size, i.e. sequencing depth, we rarefied our community abundance data to 95000 sequences for ASVs and 99000 sequences for OTUs using function "rarefy_even_depth()" in "phyloseq" (v. 1.38.0; McMurdie and Holmes, 2013). To test for the main and interactive effects of land use history and MAP (grouped into "arid" for 455.7 – 634.9 mm yr^{-1} and "mesic" for 760.9 – 1040.5 mm yr^{-1}), we used a nonparametric permutational analysis of variance (PERMANOVA) on the Bray-Curtis distance matrix using function "adonis()" in "vegan" (v. 2.5-7; Oksanen et al., 2020). We further analyzed community composition using a constrained ordination, distance-based redundancy analyses, which allowed for use of MAP as a continuous explanatory variable, using main effects of MAP, MAT, longitude, and LU for plant communities with the addition of plant first PCoA axis to explain variation in fungal communities using function "ordinate()" (method = 'CAP') in R package "phyloseq" (v. 1.38.0; McMurdie and Holmes, 2013) and inferred differences using function "anova()". We tested the null hypothesis that experimental units have similar multivariate dispersion using the "betadisper()" function in "vegan" (v. 2.57; Oksanen et al., 2020). To determine if any plants, ASVs, or OTUs were disproportionately abundant in the arid or mesic precipitation habitats, we used indicator species analyses with the "multipatt()" function in R package "indicspecies" (v. 1.7.12; De Caceres and Legendre, 2009) on the 50 most abundant plants, 100 most abundant OTUs, and 200 most abundant ASVs and corrected P-values for multiple testing using function "p.adjust ()" with false discovery rate (FDR) method in program R. To test the association between plant and fungal communities, we used Mantel tests to compare Bray-Curtis distance matrices using function "mantel()" in "vegan" (v. 2.5-7; Oksanen et al., 2020). Similarly, to test the association of geographic distance with plant and fungal communities, we calculated the pairwise Haversine distance between sample coordinates

using “distm()” function in R package “geosphere” (v. 1.5-14; Hijmans, 2021) and used Mantel tests to compare with Bray-Curtis distances. Additionally, to compare the plant and fungal PCoA ordinations, we used Procrustes analyses of the plant and fungal PCoA ordinations using function “procrustes()” in “vegan” (v. 2.5-7; Oksanen et al., 2020).

Results

Community Description

In the 16 total samples from eight native remnant prairies and eight post-agricultural sites, we observed a total of 160 plant species representing a total of 36 families. The Family Poaceae was dominant (34 species and 62.7% of total cover), followed by Family Asteraceae (38 species and 19.0% total cover), Family Fabaceae (21 species and 6.4% total cover), Family Amaranthaceae (2 species and 3.3% total cover), Cyperaceae (3 species and 1.7% total cover), and Family Anacardiaceae (1 species and 1.5% total). Other families represented < 1% of the total cover (Figure 9A). The plant community cover data and taxonomic information are listed in Supplementary Files 1 and 2.

Following quality control and removal of rare sequences, we retained a total of 3,328,786 high quality sequences that clustered into 4,385 OTUs. The sequencing yields ranged from 99,025 to 366,866 per sample with a mean yield of 208,049 ± 92,819.02 (SD). The OTUs, their observed frequencies, and taxonomic assignments are listed in Supplementary Files 3 and 4.

Our data were dominated by the Phylum Ascomycota (63.6% sequences and 50.4% OTUs), the Phylum Basidiomycota (16.0% sequences and 18.1% OTUs), and a fairly large portion of unidentified taxa (18.2% sequences and 17.6% OTUs), followed by the Phylum Glomeromycota (1.3% sequences and 7.4% OTUs), Chytridiomycota (0.4% sequences and 4.3% OTUs), and several Phyla that made up <1% of sequences and OTUs (Mortierellomycota, Mucoromycota, Kickxellomycota, Rozellomycota, Olpidiomycota, Entorrhizomycota, Aphelidiomycota, Entomophthoromycota, Aphelidiomycota, Calcarisporiellomycota, and Blastocladiomycota) (following Tedersoo et al., 2018). Relative abundance of fungal orders can be found in Figure 9B. OTUs were assigned to a total of 774 genera. A large proportion of the OTUs (2,078 OTUs) were not assigned to a genus (47.3%). Among those with genus level assignments, the most abundant were *Alternaria* with 7 OTUs (4% sequences and > 0.2% of all OTUs), followed by *Cladosporium* with 2 OTUs (3.5%) and *Dissoconium* with 8 OTUs (2.8%

sequences). The ten most abundant genera were common phyllosphere inhabitants including *Alternaria*, *Dissoconium*, *Phaeosphaera*, *Puccinia*, *Fusarium*, *Blumeria*, and *Aureobasidium*.

Alpha Diversity and Regression Analyses

Our regression model — using MAP normalized around the mean precipitation (730.01 mm yr^{-1}), LU, and their interaction as predictors — predicted plant richness and explained a large proportion of its variation (Table 2). Plant richness increased with MAP, and prairie remnants had greater plant richness than the post-agricultural sites. We observed no evidence for an interaction between MAP and land-use suggesting that the plant richness increased similarly in both land-uses (Table 2; Figure 2A). AIC comparisons suggest that replacing MAP with geographic distance did not result in a superior model for predicting plant richness (Table 6). In contrast to plant richness, our regression models poorly predicted fungal richness (S_{Obs}) and explained only a small proportion of the variation. These analyses provided no evidence for fungal richness responses to MAP, LU, or their interaction (Table 3; Figure 3A). This result did not change whether or not the potential outlier (LVN_N) was excluded from the analysis (Table 7). AIC comparisons suggest that plant predictors or geographic distance were not superior to MAP (Table 8) except in the case of plant richness which was a better predictor for OTU richness ($F_{3,12} = 2.435$, $R^2_{adj} = 0.223$, $P = 0.115$). However, in general, none of these alternative models performed well in predicting fungal richness overall.

Our regression models — using MAP normalized around the mean precipitation (730.01 mm yr^{-1}), LU, and their interaction as predictors — predicted both plant and fungal diversity (H') and explained a large proportion of the variation in both communities (Tables 2, 3; Figures 2B, 3B). Plant diversity increased with MAP and native prairie remnants harbored greater plant diversity than post-agricultural sites. However, we found no evidence of interaction between MAP and land-use (Table 2; Figure 2B). When the potential low outlier (TRB_N) was removed, our model explained more of the variation in plant diversity, the land-use term had a greater explanatory power, whereas MAP decreased in explanatory power (Table 7). However, because the potential outlier represents the dry terminal end of the precipitation gradient, it likely represents an accurate value for the site. AIC comparisons suggested that replacing MAP with geographic distance did not result in a superior model in predicting plant diversity (Table 6). There was evidence for interaction between MAP and LU in models predicting fungal diversity: fungal diversity increased with MAP in the native prairie remnants but did not significantly

change with increasing MAP in post-agricultural sites. There was also evidence for a land-use main effect that indicated greater fungal diversity in native remnant prairies than post-agricultural sites (Table 3; Figure 3B). AIC comparisons suggest that FQI_{adj} and geographic distance were comparable to MAP in explaining fungal diversity (Table 8)

Our regression models – using MAP normalized around the mean precipitation (730.01 mm yr^{-1}), LU, and their interaction as predictors – neither predicted plant community evenness (E_{var}) nor explained much of its variation (Table 2; Figure 2C). Plant community evenness was not influenced by MAP, LU, or their interaction. AIC comparisons suggest that replacing MAP with geographic distance did not result in superior model for predicting plant evenness (Table 6). In contrast, our regression model predicted fungal community evenness (E_H) and explained a considerable proportion of its variation (Table 3; Figure 3C). There was some evidence for interaction between MAP and LU. Fungal evenness seemed to increase with MAP in native prairies, but did not change in post-agricultural sites. There was also evidence for a land-use main effect indicating greater fungal evenness in native remnant prairies than post-agricultural sites (Table 3; Figure 3C). AIC comparisons suggest that FQI_{adj} and geographic distance were comparable to MAP in explaining fungal evenness (Table 6).

In addition to plant richness and diversity, we estimated the adjusted Floristic Quality Index (FQI_{adj}) that aims to provide a numerical measure reflecting the quality of plant communities. Our model — using MAP normalized around the mean precipitation (730.01 mm yr^{-1}), LU, and their interaction as predictors — predicted plant FQI_{adj} and explained some of the variation in the model (Table 2; Figure 2F). In general, plant FQI_{adj} increased with MAP. There was also some marginal evidence suggesting that native prairie remnants had greater FQI_{adj} than post-agricultural sites. Similarly, there was some evidence for an interaction between MAP and land-use suggesting that, while the FQI_{adj} increased in native prairies, it did not in the post-agricultural sites. When the potential outlier (TRB_P) was removed, our model predicted FQI_{adj} and explained more of the variation. The marginal interactive effects became highly significant suggesting that the native prairie FQI_{adj} increased with MAP, whereas it decreased in the post-agricultural sites. However, since the two regression lines now intersect the intercept, the main effect of land use is no longer significant. Taken together, these analyses suggest that the two land uses have comparable FQI_{adj} at the average MAP mid-gradient, but may differ at the arid

and mesic extremes. Since these conclusions were primarily driven by the outliers in the post-agricultural site, the results are suspect to caution (Table 7).

Community Analyses

We used PCoA and PERMANOVA to visualize and test for any community responses to MAP and land-use (Figure 4). In these analyses where we divided the MAP gradient into arid and mesic categories, we observed no evidence for interaction between MAP and land-use in either plant or fungal community composition (PERMANOVA: Plant: $F_{1,15} = 0.99$, $R_2 = 0.096$, $P = 0.375$; OTU: $F_{1,15} = 0.97$, $R_2 = 0.058$, $P=0.506$). However, both plant and fungal communities differed compositionally between the arid and mesic habitats (PERMANOVA: Plant: $F_{1,15} = 5.77$, $R_2 = 0.290$, $P = 0.001$; OTU: $F_{1,15} = 2.78$, $R_2 = 0.166$, $P = 0.001$). In contrast to many richness and diversity analyses, there was no evidence for difference in community composition between native prairie remnants and post-agricultural sites (PERMANOVA: Plant: $F_{1,15} = 1.18$, $R_2 = 0.059$, $P = 0.243$; OTU: $F_{1,15} = 1.02$, $R_2 = 0.061$, $P = 0.395$). In addition to our PERMANOVA analyses, in which we simply divided the precipitation gradient to arid and mesic habitats, we analyzed the PCoA axis scores using multiple linear regressions similar to those we used for community richness and diversity estimators. These models successfully predicted changes in composition and explained a substantial proportion of the variation in the first but not the second PCoA axis of both the plant and fungal communities (Tables 2 and 3; Figures 2D, E and 3D, E). The first plant PCoA axis scores linearly increased, whereas the first fungal PCoA axis scores linearly decreased with MAP with no evidence for either land-use effects or interaction between the MAP and land-use (Tables 2, 3; Figures 2D and 3D). In contrast to the first PCoA axis, there was no evidence for MAP, land-use, or interaction effects for the second PCoA axis (Tables 2, 3; Figures 2E and 3E). This did not change when the potential low outlier (TRB_N) was removed (Table 7).

To further explore differences in community composition and its responses to environmental and anthropogenic factors, we used constrained ordinations, distance-based redundancy analyses, using main effects of MAP, MAT, longitude, and LU for plant communities. We used similar analyses for fungal communities with the addition of the first plant PCoA axis to explain variation in fungal communities. These analyses further confirmed that climate variables (MAP and MAT) had a greater influence on plant and fungal community compositions than land-use. However, the environmental variables may be correlated as

indicated by the similar direction of environmental vectors in ordination space (Figure 10). To also assess the heterogeneity in plant and fungal community composition, we tested for community dispersion. Neither plant nor fungal communities differed in their dispersion between the arid and mesic habitats (Plant: $F_{1,15} = 0.093$, $P = 0.783$; OTU: $F_{1,15} = 1.577$, $P = 0.199$) or between native prairie remnants and post-agricultural sites (Plant: $F_{1,15} = 1.18$, $P = 0.302$; OTU: $F_{1,15} = 3.264$, $P = 0.079$).

To identify plant taxa that may underlie the observed community differences, we used indicator taxon analyses (De Caceres and Legendre, 2009) including fifty most abundant plant species. Our correction for false detection (FDR) proved conservative and resulted in the loss of all or most significant indicators. Consequently, we present both corrected and uncorrected values (Supplementary File 5) for readers' information. We identified eight arid and six mesic plant indicators before the FDR correction which highlight the transition from mixed grass to tallgrass prairie with increasing precipitation (Supplementary File 5); one arid (*Pascopyrum smithii*) and two mesic (*Andropogon gerardii* and *Panicum virgatum*) indicators remained after the FDR correction. Before the FDR correction, arid indicators included six members of the family Poaceae including common mixed grass prairie taxa such as *Bromus japonicus*, *Bouteloua dactyloides*, and *Sporobolus cryptandrus*, as well as two members of the family Asteraceae (*Ambrosia psilostachya* and *Conyza canadensis*). Most indicators for mesic sites represented the family Poaceae and included the four dominant tallgrass prairie species: *Andropogon gerardii*, *Sorghastrum nutans*, *Panicum virgatum*, and *Schizachyrium scoparium* as well as *Sporobolus compositus*. One indicator represented the family Cyperaceae with various species of the genus *Carex* (Supplementary File 5).

Similar indicator taxon analyses of the 100 most abundant fungal OTUs identified 17 arid and 14 mesic indicator OTUs before FDR correction (Supplementary File 6). Indicators represented Phylum Ascomycota (15 arid and 11 mesic) and Basidiomycota (2 arid and 3 mesic). Eight arid and seven mesic indicators remained after FDR correction (arid: *Blumeria sp*., *Phaeoseptoriella zeae*, *Neostagonospora sp*., *Dinemasporium bambusicola*, *Gibberella tricincta*, *Alternaria sp*., *Cyphellophora sp*., and *Darksidea sp*.; mesic: *Phyllosticta sorghina*, Capnodiales sp., Herpotrichiellaceae sp., *Dissoconium sp*., Eurotiomycetes sp., *Neocosmospora falciformis*, and another Eurotiomycetes sp.). Many of the most abundant indicators were plant pathogens or other plant-associated fungi (Supplementary File 6). Among the most abundant fungal indicators

for arid sites was *Blumeria sp*., a member of the order Erysiphales (powdery mildews) which are obligate plant pathogens (Takamatsu, 2013); *Phaeoseptoriella zeae*, a foliar pathogen of *Zea mays* (Crous et al., 2019; Tennakoon et al., 2020); and *Neostagonospora sp*., a member of a genus of common pathogens of *Carex* (Quaedvlieg et al., 2013). Among the most abundant fungal indicators for the mesic sites was *Phyllosticta sorghina*, a common cereal crop pathogen (Oliveira et al., 2018); *Dissoconium sp*. anamorph (teleomorph Mycosphaerella; Crous et al., 2007), a representative of a genus with many foliar pathogens (Li et al., 2012); and, a member of the family Herpotrichiellaceae, with many documented decomposers of plants or fungi (Untereiner and Malloch, 1999). Among indicators that were significant prior to FDR correction were *Puccinia andropogonis*, a common rust pathogen of the dominant grasses in the Great Plains (Szabo, 2006) and *Phyllozyma linderae* (basionym *Sporobolomyces linderae* Nakase, M. Takash. & Hamam.), a basidiomycetous phyllosphere yeast in the Phylum Pucciniomycotina, whose ecology remains elusive (Wang et al., 2015).

Linkages Between the Plant and Fungal Communities

Our co-located sampling of plant and fungal communities was designed to permit testing whether the two communities correlate. Our Mantel tests indicated that the Bray-Curtis distance matrices characterizing the community dissimilarities among the plots were highly correlated between the plant and fungal communities ($R^2 = 0.673$, $P = 0.001$). Further, Mantel tests indicated that geographic distance did not correlate with plant communities ($R^2 = 0.078$, $P = 0.170$) but correlated with fungal communities ($R^2 = 0.228$, $P = 0.024$). Additionally, we utilized Procrustes analyses that compare two or more multidimensional shapes by translation, rotation and scaling the ordinations to maximize their superimposition (Figure 5). Corroborating the Mantel tests, these analyses highlighted the strong correlation between the plant and fungal two-dimensional PCoA ordinations ($R^2 = 0.573$, $P = 0.001$).

Discussion

We sampled the steep precipitation gradient in the central United States to better understand how plant and fungal communities vary with MAP, among native prairie remnants and post-agricultural sites, and how these communities may be linked. Our data indicate that both plant and fungal communities shift compositionally and increase in their diversity with MAP and had greater diversity in native remnant prairies than in post-agricultural sites. Further,

although plant community richness also increased with MAP, fungal community richness did not. This lack of fungal richness response to MAP is surprising, given that the plant and fungal communities were correlated in composition. Although it is impossible to decouple MAP and other potential correlates, our analyses suggest the importance of MAP gradient and land-use history in controlling plant and fungal communities.

Our data supported our hypotheses that plant communities change in composition and increase in richness and diversity with MAP. Temperate grasslands in central North America range from 200 to 1200 $mm \cdot yr^{-1}$ in MAP (Lauenroth et al., 1999) resulting in distinct ecosystems ranging from the shortgrass steppes with very low annual net primary productivity to the highly productive tallgrass prairies (Sala et al., 1988; Lauenroth et al., 1999). Our study covered a substantial proportion of this gradient (455.7–1040.5 mm yr^{-1}) and our results are consistent with the transition from shortgrass steppes and mixed grass prairies to tallgrass prairies along the west-east precipitation gradient. The broad variability in MAP not only affects ecosystem annual net primary productivity, but also plant community composition, cover, and diversity (Lauenroth et al., 1978; Watson et al., 2021). Our results are congruent with Watson et al. (2021) and suggest that MAP is an important plant diversity predictor for regionally distinct plant communities. Our indicator taxon analyses highlighted that it is indeed the dominant graminoids that define these grassland communities, particularly so in the mesic tallgrass prairies.

Interestingly, our data suggested that floristic quality response to MAP depended on the land-use such that FQI_{adj} increased with MAP in native remnants but not in the post-agricultural fields. When we excluded the potential outliers, these responses became even more obvious and indicated an actual decline in FQI_{adj} with MAP in the post-agricultural fields. The stochastic niche hypothesis (Tilman, 2004) predicts that plant communities with greater species richness would be less subject to establishment of new species – in our case also non-native species – than communities that have low species richness. This resistance to invasion is posited to stem more from resource exhaustion by the large number of potentially competing species with differing niches than from community diversity itself (McKane et al., 2002; Reich et al., 2012; Lannes et al., 2020). Plant species richness increased with MAP in both native prairie and post-agricultural sites in our analyses. As a result, our FQI_{adj} results in the native prairie remnants seem consistent with this hypothesis but not in the post-agricultural sites. In contrast, in the post-

agricultural sites, the decline in the FQI_{adj} in sites with greater species richness suggests that the agricultural land use legacy results in communities that are increasingly of lesser floristic quality and include a greater proportion of non-native species the greater the richness of comparable native sites is. It remains an open question whether the post-agricultural sites differ from the native prairies as a result of differences in available soil resources that reflect the past anthropogenic inputs during row crop production.

Plant communities and their shifts along gradients have been extensively studied (see Watson et al., 2021), whereas similar studies on fungal communities and/or their diversity are less common (but see e.g., Tedersoo et al., 2014; Glynou et al., 2016; Rudgers et al., 2021). Factors that may affect fungal communities include latitude (Arnold et al., 2000; Arnold and Lutzoni, 2007; Tedersoo et al., 2014), climate (McGuire et al., 2012; U′ren et al., 2012; Zimmerman and Vitousek, 2012; Eusemann et al., 2016; Oita et al., 2021; Rudgers et al., 2021), soil (Tedersoo et al., 2020; Bowman and Arnold, 2021; Rudgers et al., 2021), plant host (Hoffman and Arnold, 2008; U'ren et al., 2012; Lau et al., 2013; Kembel and Mueller, 2014; Tedersoo et al., 2020; Rudgers et al., 2021), and disturbance (Delgado-Baquirizo et al., 2021). Some studies highlight strong host species and/or climatic/edaphic effects (e.g., Hoffman and Arnold, 2008; Tedersoo et al., 2020; Rudgers et al., 2021), whereas others find no support for correlations between plant community diversity and fungal communities (e.g., McGuire et al., 2012; Tedersoo et al., 2014). While soil- and root-inhabiting fungal communities may be buffered against climatic drivers (Rudgers et al., 2021) or correlate with plant diversity (Shen et al., 2021), phyllosphere communities may be particularly sensitive to climatic drivers whilst buffered against edaphic factors (Bowman and Arnold, 2021; Oita et al., 2021). Consistent with our hypotheses and predictions, our data strongly suggest that phyllosphere fungal communities respond to MAP. These conclusions agree with others who have concluded that climatic factors strongly influence the phyllosphere fungal communities and their assembly (Carroll and Carroll, 1978; Zimmerman and Vitousek, 2012; U'Ren et al., 2012; Oita et al., 2021).

In addition to environmental factors, fungal communities respond to host species (Rudgers et al., 2021), although not necessarily to plant diversity or richness (McGuire et al., 2012; Tedersoo et al., 2014, but see Hooper et al., 2000; Shen et al., 2021). Our data clearly indicate that plant and fungal communities correlate, even though fungal richness neither strongly correlated with MAP nor was well predicted by climatic or plant community variables.

Differences in plant metabolites and plant physiology may control phyllosphere community diversity and composition (Bailey et al., 2005; Rajala et al., 2014; Eusemann et al., 2016), resulting in greater fungal diversity in systems with greater plant diversity. We hypothesize that our observed compositional correlations likely stem from the niche heterogeneity provided by diverse plant communities that then may host diverse and distinct phyllosphere fungi. Indeed, some of our most common fungal indicator taxa were directly linked to their hosts, exemplified by foliar plant pathogens (e.g., *Phyllosticta sorghina* and *Blumeria* sp.). In sum, as host species communities shift, so does the probability of distinct fungal associates in the phyllosphere.

Ranking factors for their importance in structuring fungal communities is not simple. Some studies have suggested that edaphic factors can override the influence of host plant identity (Glynou et al., 2016), whereas others have suggested that the importance of edaphic factors varies among host species (Rudgers et al., 2021). In our study, MAP and plant community composition or diversity are inherently collinear and evaluating their relative importance in phyllosphere community assembly is therefore challenging. The controls may also differ among fungal guilds. McGuire et al. (2012) targeted lowland tropical rain forests with high plant richness in Panama and concluded that the compositionally distinct communities in soil and leaf litter differed in their compositional controls. (2012) targeted lowland tropical rain forests with high plant richness in Panama and concluded that the compositionally distinct fungal communities in soil and leaf litter differed in their compositional controls. Although the former correlated with MAP but not with plant richness, the latter correlated with neither MAP nor plant diversity. Further experiments that manipulated litter richness suggested that plant diversity may be less important in determining fungal richness than MAP as the fungal richness did not track the plant richness. In contrast to those studies, Shen et al. (2021) manipulated herbaceous plant community richness in a greenhouse experiment and concluded that the soil fungal richness correlated with that of the plant communities. Clearly, experimental systems, targeted fungal guilds and included host taxa appear essential controls of fungal communities. To better understand the relative importance of environmental factors and plant community estimators in the current experiment, we compared models using the main and interactive effects of land use and either MAP or plant estimators (richness, diversity, evenness, FQI_{adj}, or PCoA axis 1). These simple model comparisons suggested that MAP is usually a superior predictor for fungal diversity and evenness. Although our studies emphasize the importance of climatic factors (see

also Oita et al., 2021), further and more detailed studies may be needed to better resolve these issues. Understanding how climatic or edaphic variables can influence host-associated fungal communities is becoming increasingly important as the ongoing environmental change has the potential to disrupt host microbe interactions (Ranelli et al., 2015; Glynou et al., 2016; Vetrovsky et al., 2019; Steidinger et al., 2020). Analysis of environmental gradients, such as MAP here, is a powerful approach to dissect such patterns (Rudgers et al., 2021).

Contrary to our hypotheses and predictions, we observed no strong evidence for differences in community composition and dispersion of plants or their phyllosphere fungi among the post-agricultural fields and native prairie remnants. However, our data indicate that native remnant prairies harbor greater plant richness and diversity as well as greater phyllosphere fungal diversity and evenness. Land-use and particularly its intensification have been posited as major drivers of biodiversity loss (Sala et al., 2000; Foley et al., 2005; Gossner et al., 2016; Brinkmann et al., 2019) and biotic and ecological homogenization (Gossner et al., 2016; Brinkmann et al., 2019; Delgado-Baquirizo et al., 2021). Some have suggested that the communities in post-agricultural sites remain distinct from those in native sites because of fungal dispersal limitations from native remnants (Turley et al., 2020), as has been reported for plants (Turley et al., 2017). The establishment of fungal communities in post-agricultural sites may also be a result of poor recovery of soil conditions after intensive agriculture (Bellemare et al., 2002; Dupouey et al., 2002; Flinn and Marks, 2007). Although the phyllosphere fungal communities correlate with phyllosphere chemistry and have been reported to differ among land-use types (e.g., Jumpponen and Jones, 2010), they may be less affected directly by the altered post-agricultural soil conditions than the soil- or root-inhabiting fungal communities are. Dispersal limitations for the phyllosphere communities may also be less restrictive than they are for soil-dwelling fungi (Bowman and Arnold, 2021). Our results are congruent with those of many others that emphasize agricultural legacy effects on bacterial and fungal communities decades after agricultural abandonment (Lauber et al., 2008; Upchurch et al., 2008; Jangild et al., 2011; Hui et al., 2018; Turley et al., 2020) as well as those that report strong biotic and ecological homogenization by anthropogenic land-use (e.g., McKinney and Lockwood, 1999; Groffmann et al., 2014; Gossner et al., 2016; Delgado-Baquirizo et al., 2021; Kotze et al., 2021). Our data indicate that land-use is an important driver of phyllosphere communities across broad environmental gradients such as the steep precipitation gradient sampled here. Taken together,

our study suggests that the phyllosphere communities in these systems closely track plant communities whose diversity has been impacted by the land-use legacies.

We simultaneously analyzed plant communities and their phyllosphere fungal communities to assess responses to MAP and land-use history across a precipitation gradient extending much of the known range of the temperate grasslands in the central Great Plains. Our data indicate strong climatic controls of both the plant and phyllosphere fungal communities and the lesser impact of the historic land-uses on community composition. Interestingly, these data highlight the resilience of the species-rich tallgrass prairies and comparatively lesser floristic quality of post-agricultural sites in the more mesic regions of this MAP gradient. The phyllosphere fungal communities also responded strongly to MAP, whereas the historic land-use appeared to have minimal to no effects on the composition of these communities. However, our data indicate greater plant richness and diversity as well as greater fungal diversity and evenness in native remnant prairies than in post-agricultural sites. Although our model comparisons highlighted that MAP was commonly a stronger predictor of phyllosphere fungal community metrics than plant richness or community composition, the fungal communities closely tracked plant community composition suggesting that plant communities likely serve as a key driver for foliar fungal communities.

Tables and Figures

Table 1 Site details including site identifiers, land use history (native prairie remnant or post-agricultural site), coordinates, mean annual temperature (MAT), mean annual precipitation (MAP) (acquired from PRISM Climate Group at Oregon State University; https://prism.oregonstate.edu/), soil type (as defined in USDA Natural Resources Conservation Service SSURGO database) and Sequence Read Archive accession under BioProject PRJNA795108.

Sample	Land Use	Coordinates (DMS)	MAT (ºC)	MAP (mm yr^{-1})	Soil Type	Accession
TRB_N	Native	38° 28' 10.17"N 101° 46' 56.05"W	11.31	455.74	Richfield (1761)	SAMN24688330
TRB_P	Post-ag	38° 28' 18.95"N 101° 47' 06.31"W	11.31	455.74	Richfield (1761)	SAMN24688331
SVR_N	Native	38° 52' 27.35"N 100° 59' 03.49"W	11.68	477	Ulysses (1857)	SAMN24688326
SVR_P	Post-ag	38° 51' 58.07"N 100° 59' 43.36"W	11.68	477	Ulysses (1857)	SAMN24688327
HAY_N	Native	38° 50' 07.50"N 099° 18' 12.15"W	12.28	604.68	Harney (2612)	SAMN24688313
HAY_P	Post-ag	38° 50' 40.11"N 099° 18' 58.83"W	12.23	602.91	Armo (2518)	SAMN24688314
RKS_N	Native	39° 10' 29.79"N 099° 09' 00.90"W	11.9	634.91	Heizer-Harney-Brownell-Bogue-Armo (s2536)	SAMN24688323
RKS_P	Post-ag	39° 09' 50.84"N 099° 09' 43.72"W	11.9	634.91	Heizer-Harney-Brownell-Bogue-Armo (s2536)	SAMN24688324
TLI_N	Native	38° 58' 11.13"N 097° 28' 08.50"W	13.3	760.86	Hord (3755)	SAMN24688318
TLI_P	Post-ag	38° 45' 58.94"N 097° 34' 26.57"W	13.21	781.65	McCook (2347)	SAMN24688319
KNZ_N	Native	39° 06' 20.06"N 096° 36' 36.65"W	12.74	850.63	Reading (7174)	SAMN24688315
KNZ_P	Post-ag	39° 06' 12.33"N 096° 36' 15.92"W	12.53	860.23	Reading (7170)	SAMN24688316
LVN_N	Native	39° 15' 31.46"N 094° 58' 42.46"W	12.55	1003.32	Shelby-Sharpsburg (s2389)	SAMN24688320
LVN_P	Post-ag	39° 15' 38.52"N 095° 00' 57.49"W	12.58	997.13	Pawnee-Grundy (s2386)	SAMN24688321
EKS_N	Native	38° 10' 52.44"N 095° 16' 21.42"W	13.23	1040.46	Kenoma-Olpe (8780)	SAMN24688311
EKS_P	Post-ag	38° 10' 52.32"N 095° 16' 27.12"W	13.23	1040.46	Kenoma (8875)	SAMN24688312

Table 2 Multiple linear regression model statistics for plant community diversity, richness, evenness, and compositional estimates predicted by land use history (LU) and mean annual precipitation (MAP) normalized around the mean precipitation (730.01 mm yr^{-1}) main effects and their interaction (LU x MAP) with native prairie remnants as reference (0) compared to post-agricultural sites (1). Statistically significant models and predictors (P<0.05) are bold-faced. Parameter estimate significances are denoted as 'ns' for not significant, '(*)' for 0.05≤P < 0.10, '*' for 0.01≤P < 0.05, '' for 0.001≤P < 0.01, and '***' for P < 0.001. Response variables with outliers were analyzed with and without the identified outliers. Models shown here include potential outliers, Table 7 provides model details with outliers removed.**

Response	Model	Predictor	Estimate ± SE	|t-value|
Plant FQI_{adj}[2]	**$F_{3,12}$ =4.53*, R^2_{adj}=0.414**	**Intercept**	**31.78 ± 3.42**	**9.29*****
	AIC=123.44	Land Use (LU)	–9.19 ± 4.84	–1.90$^{(*)}$
		MAP	**5.16x10^{-2}± 1.64x10^{-2}**	**3.16****
		LU x MAP	–4.63x10^{-2} ± 2.31x10^{-2}	–2.00$^{(*)}$
Plant Richness (S_{Obs})	**$F_{3,12}$ = 9.99*, R^2_{adj}=0.643**	**Intercept**	**27.44 ± 2.10**	**13.05*****
	AIC=107.85	**LU**	**–7.60 ± 2.97**	**–2.56***
		MAP	**4.15x10^{-2}± 1.00x10^{-2}**	**4.13****
		LU x MAP	–1.59x10^{-2} ± 1.42x10^{-2}	–1.12^{ns}
Plant Diversity (H')[3]	**$F_{3,12}$ = 6.24*, R^2_{adj}=0.512**	**Intercept**	**2.41 ± 1.51x10^{-1}**	**15.96*****
	AIC=23.61	**LU**	**–5.55x10^{-1} ± 2.14x10^{-1}**	**–2.60***
		MAP	**1.72x10^{-3}± 7.22x10^{-4}**	**2.39***
		LU x MAP	1.02x10^{-4}± 1.02x10^{-3}	0.10^{ns}
Plant Evenness (E_{var})	$F_{3,12}$ =0.55^{ns}, R^2_{adj}=–0.098	**Intercept**	**2.21x10^{-1}± 2.33x10^{-2}**	**9.50*****
	AIC= –36.28	LU	–1.55x10^{-2} ± 3.29x10^{-2}	–0.47^{ns}
		MAP	4.85x10^{-8}± 1.11x10^{-4}	0.00^{ns}
		LU x MAP	–1.32x10^{-4} ± 1.57x10^{-4}	–0.84^{ns}
Plant PCoA Axis 1	**$F_{3,12}$ =8.47**, R^2_{adj}=0.599**	Intercept	–2.90x10^{-2} ± 7.88x10^{-2}	–3.78 ^{ns}
	AIC=2.75	LU	5.83x10^{-2}± 1.11x10^{-1}	0.52^{ns}

[2] Contained a low potential outlier in TRB_P retained in this analysis.

[3] Contained a low potential outlier in TRB_N retained in this analysis.

		MAP	$1.44x10^{-3} \pm 3.76x10^{-4}$	3.82^{**}
		LU x MAP	$-2.17x10^{-4} \pm 5.32x10^{-4}$	-0.41^{ns}
Plant PCoA Axis 2[4]	$F_{3,12} = 0.31^{ns}$, R^2_{adj}=–0.160 AIC=4.10	Intercept	$1.60x10^{-2} \pm 8.22x10^{-2}$	0.19^{ns}
		LU	$-3.27x10^{-1} \pm 1.16x10^{-1}$	-0.28^{ns}
		MAP	$-3.29x10^{-4} \pm 3.92x10^{-4}$	-0.84^{ns}
		LU x MAP	$4.80x10^{-4} \pm 5.54x10^{-4}$	0.87^{ns}

Table 3 Multiple linear regression model statistics for fungal Operational Taxonomic Unit (OTU) community diversity, richness, evenness, and compositional estimates predicted by land use history (LU) and mean annual precipitation (MAP) normalized around the mean precipitation (730.01 mm yr^{-1}) main effects and their interaction (LU x MAP) with native prairie remnants as reference (0) compared to post-agricultural sites (1). Statistically significant models and predictors (P<0.05) are bold-faced. Parameter estimate significances are denoted as 'ns' for not significant, '(*)' for 0.05≤P<0.10, '*' for 0.01≤P<0.05, '' for 0.001≤P<0.01, and '***' for P<0.001. Response variables with outliers were analyzed with and without the identified outliers Models shown here include potential outliers, Table 7 provides model details with outliers removed.**

Response	Model	Predictor	Estimate ± SE	|t-value|
OTU Richness (S_{Obs})[5]	$F_{3,12} = 1.17^{ns}$, R^2_{adj}=0.033 AIC=202.93	**Intercept**	**780.37 ± 41.03**	**19.02^{***}**
		LU	–83.71 ± 58.02	-1.44^{ns}
		MAP	$1.29x10^{-1} \pm 1.96x10^{-1}$	0.66^{ns}
		LU x MAP	$6.90x10^{-2} \pm 2.77x10^{-1}$	0.25^{ns}
OTU Diversity (H')	**$F_{3,12} = 6.89^{*}$, R^2_{adj}=0.541 AIC=13.21**	**Intercept**	**$4.87 \pm 1.09x10^{-1}$**	**44.63^{***}**
		LU	**$5.65x10^{-1} \pm 1.54x10^{-1}$**	**-3.66^{**}**
		MAP	**$1.30x10^{-3} \pm 5.22x10^{-4}$**	**2.49^{*}**
		LU x MAP	**$-1.84x10^{-3} \pm 7.37x10^{-4}$**	**-2.50^{*}**
OTU Evenness (E_H)	**$F_{3,12} = 4.70^{*}$, R^2_{adj}=0.426 AIC= –44.69**	**Intercept**	**$7.32x10^{-1} \pm 1.79x10^{-2}$**	**40.95^{***}**
		LU	**$-7.17x10^{-2} \pm 2.53x10^{-2}$**	**-2.84^{*}**
		MAP	$1.80x10^{-4} \pm 8.54x10^{-5}$	$2.10^{(*)}$
		LU x MAP	**$-2.90x10^{-4} \pm 1.21x10^{-4}$**	**-2.40^{*}**

[4] Contained a low potential outlier in TRB_N retained in this analysis

[5] Contained a high potential outlier in LVN_N retained in this analysis.

OTU PCoA Axis 1	**$F_{3,12}$ = 42.88***, R^2_{adj}=0.893**	Intercept	$4.18 \times 10^{-3} \pm 2.76 \times 10^{-2}$	0.15^{ns}
	AIC= –30.80	LU	$-8.34 \times 10^{-3} \pm 3.90 \times 10^{-2}$	-0.21^{ns}
		MAP	**$-1.05 \times 10^{-3} \pm 1.32 \times 10^{-4}$**	**-7.95^{***}**
		LU x MAP	$-1.48 \times 10^{-5} \pm 1.86 \times 10^{-4}$	-0.08^{ns}
OTU PCoA Axis 2	$F_{3,12}$ = 0.03^{ns}, R^2_{adj}= –0.240	Intercept	$1.41 \times 10^{-2} \pm 7.34 \times 10^{-2}$	0.19^{ns}
	AIC=0.49	LU	$-2.81 \times 10^{-2} \pm 1.04 \times 10^{-1}$	-0.27^{ns}
		MAP	$5.64 \times 10^{-5} \pm 3.51 \times 10^{-4}$	0.16^{ns}
		LU x MAP	$-3.72 \times 10^{-5} \pm 4.95 \times 10^{-4}$	-0.08^{ns}

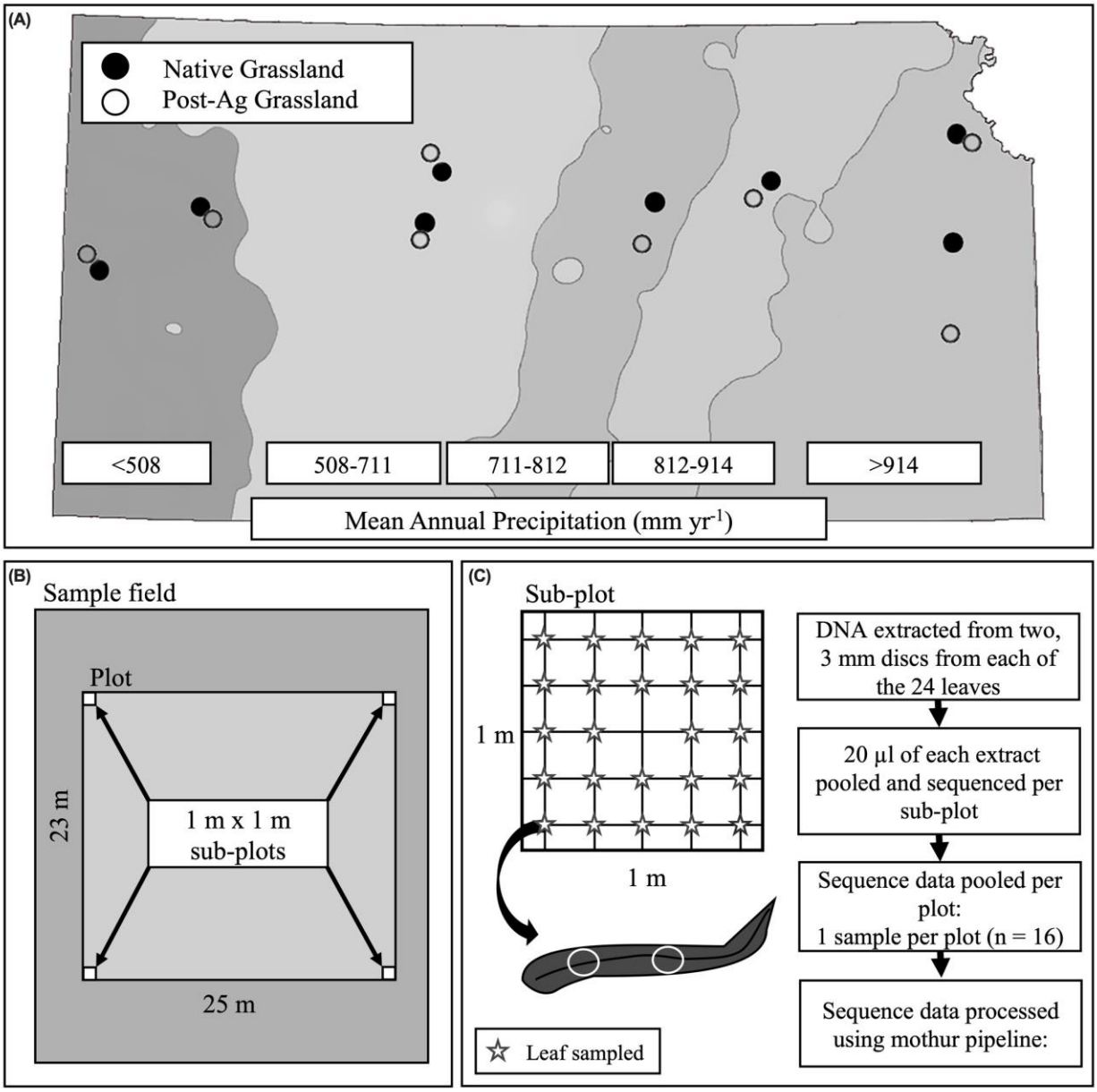

Figure 1 A schematic of the experimental design including site locations across the precipitation gradient of Kansas, USA with mean annual precipitation (MAP) bands indicated with shading. Points indicate sampled sites (open circles – post-agricultural sites; solid circles – native prairie remnants) (A), plot and sub-plot layout within each site (B), and leaf sampling within each sub-plot and subsequent sample and sequence data processing (C).

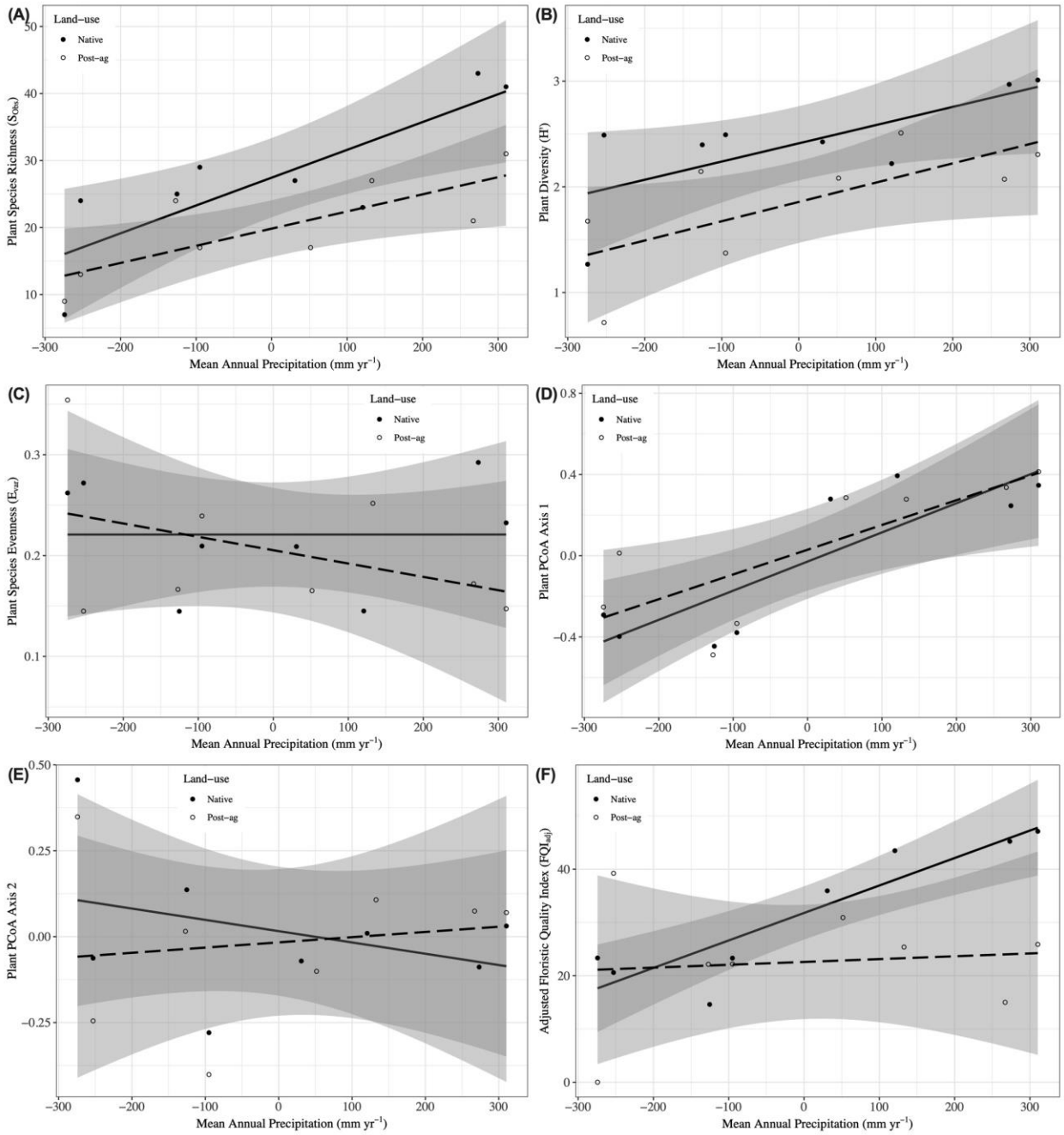

Figure 2 Plant community responses to mean annual precipitation (MAP) normalized around the mean precipitation (730.01 mm yr^{-1}) in native prairie remnants (solid line and filled symbols) and post-agricultural sites (dashed line and open symbols). Models predict observed species richness (S_{Obs}) (A), Shannon diversity (H') (B), evenness (E_H) (C), PCoA Axis 1 scores (D), PCoA Axis 2 scores (E), Adjusted Floristic Quality Index (FQI_{adj}). The shaded areas represent 95% confidence intervals around the model predictions.

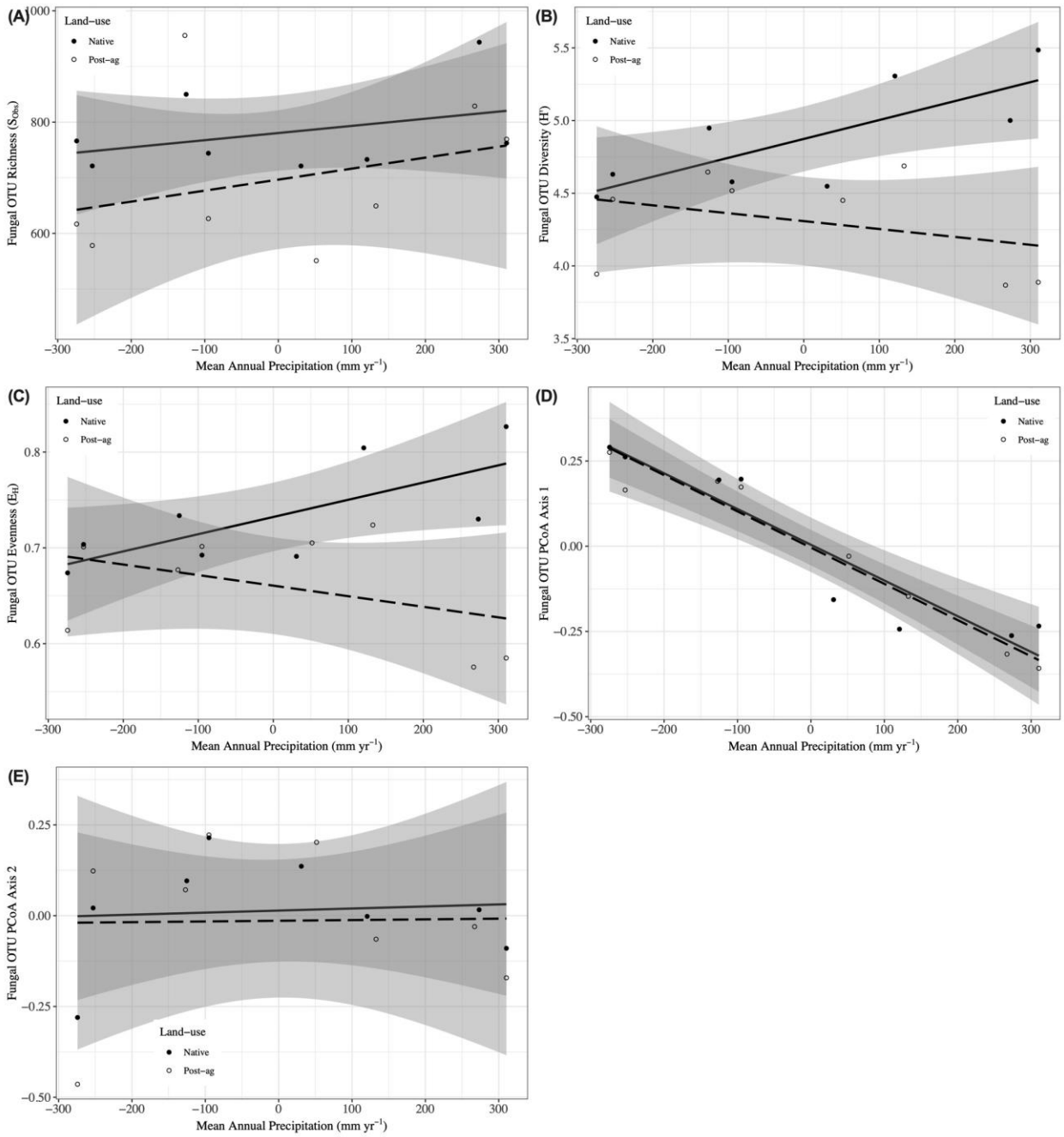

Figure 3 Fungal Operational Taxonomic Unit (OTU) responses to mean annual precipitation (MAP) normalized around the mean precipitation (730.01 mm yr^{-1}) in native prairie remnants (solid line and filled symbols) and post-agricultural sites (dashed line and open symbols). Models predict observed species richness (S_{Obs}) (A), Shannon diversity (H') (B), evenness (E_H) (C), PCoA Axis 1 scores (D), PCoA Axis 2 scores (E). The shaded areas represent 95% confidence intervals around the model predictions.

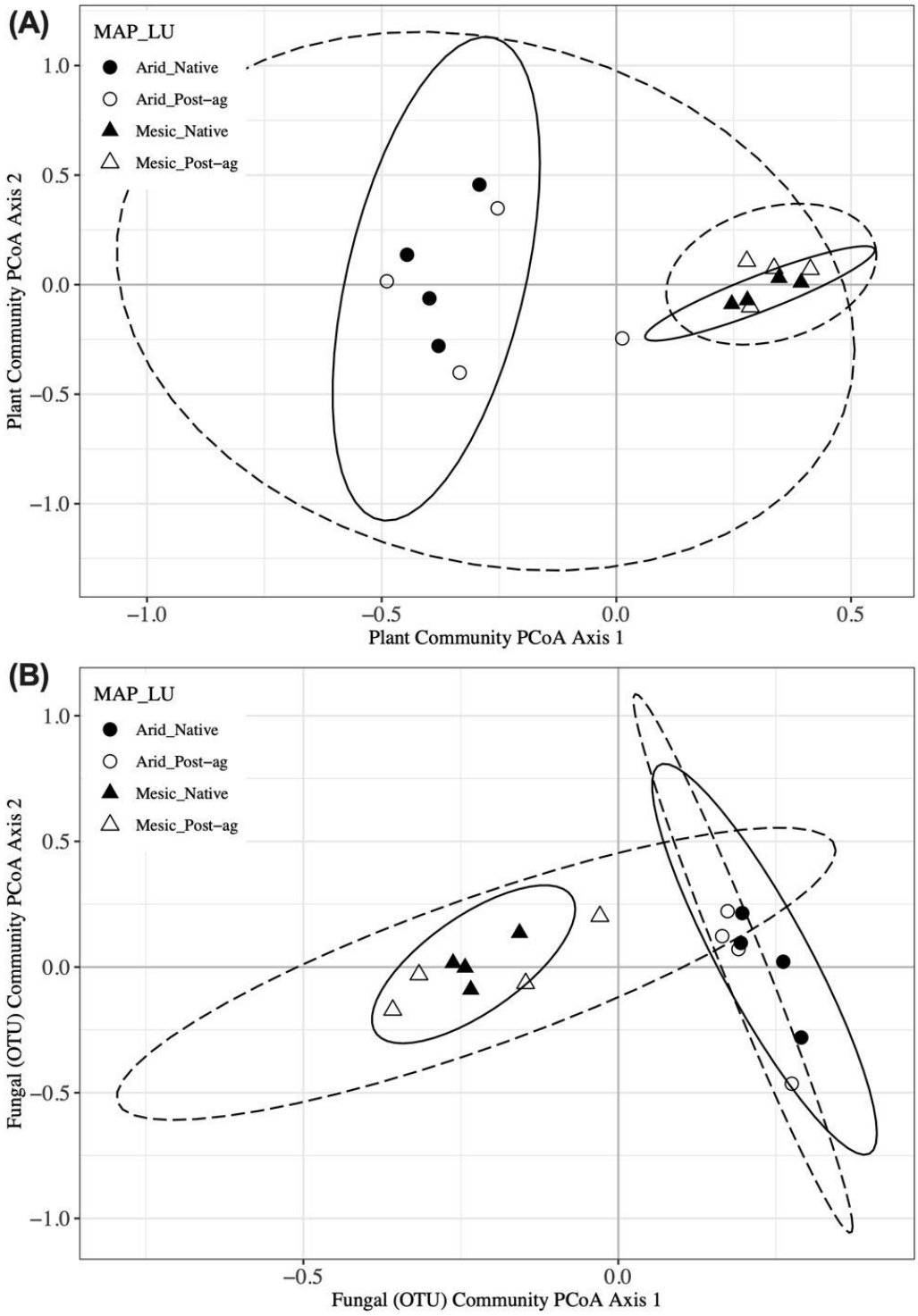

Figure 4 Principal Coordinates Analyses (PCoA) of Plant (A) and fungal community composition using Operational Taxonomic Units (OTUs) (B) in native prairie remnants (solid line and filled symbols) and post-agricultural sites (dashed line and open symbols). Circles indicate the arid end of the precipitation gradient (455.7-634.0 mm yr^{-1}), whereas

trianges indicate the mesic end (760.9-1040.5 mm yr^{-1}). Lines indicate the 95% confidence intervals around PCoA centroid for each group in the PCoA ordination.

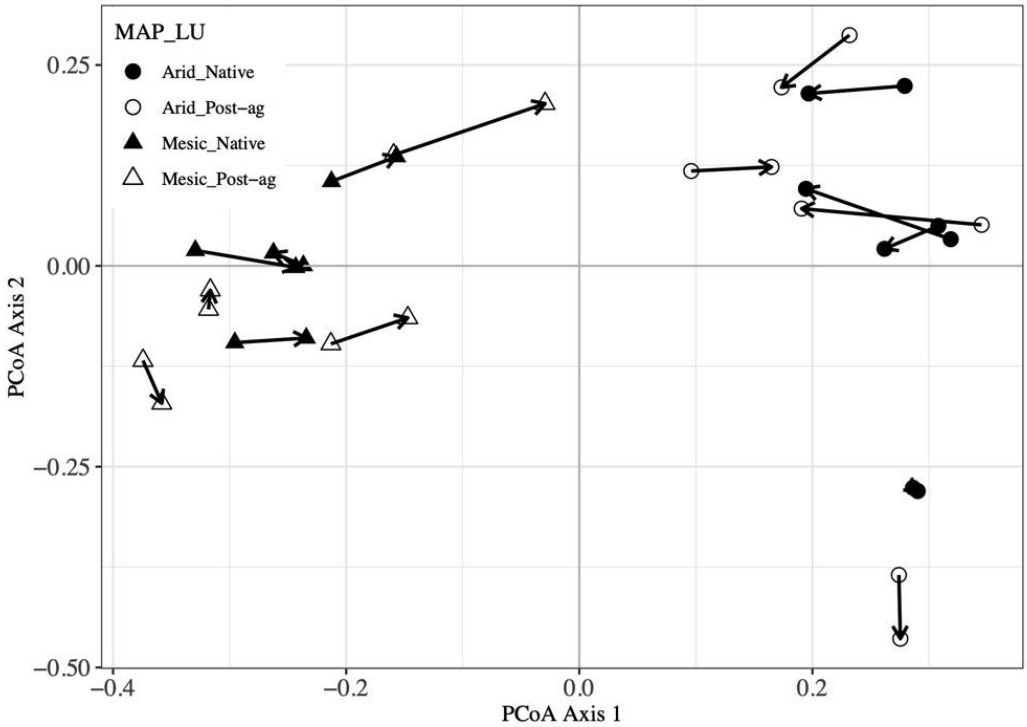

Figure 5 Procrustes analysis of plant community Principal Coordinates Analysis (PCoA) first and second ordination axes compared with Fungal Operational Taxonomic Unit (OUT) PCoA axes. Arrows point from plant community sample to the corresponding fungal community sample within a site.

Data Availability Statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: Sequence Read Archive (https://www.ncbi.nlm.nih.gov/sra/4) under BioProject PRJNA795108, BioSamples SAMN24688311- SAMN24688331.

Author Contributions

HD and AJ sampled the fungal communities, prepared samples for sequencing, conducted bioinformatic analyses, analyzed data, and prepared manuscript. AU collected plant data and aided in manuscript revision. AK aided in sample collection, preparation for fungal

sequencing, and manuscript revisions. GH prepared plant community data for analysis, estimated alpha diversity metrics, aided in plant community analysis and manuscript preparation and revisions. ST managed data in support of the site selection, and manuscript revisions. TL, TP, SL, and MG aided in site selection, sample collection and manuscript revision. All authors contributed to the article and approved the submitted version.

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Chapter 3 - *Amorpha canescens* and *Andropogon gerardii* recruit comparable foliar fungal communities across the steep precipitation gradient in Kansas[6]

Abstract

Tallgrass prairies in North America have endured substantial losses due to anthropogenic environmental change. Plant-associated phyllosphere fungi are a largely overlooked aspect of diversity in grassland systems. Phyllosphere fungi are important in plant health as pathogens, commensals, and potential mutualists. We aimed to determine how host plant species affected the community-level diversity of phyllosphere fungi across a steep precipitation gradient and two co-occurring prairie species – big bluestem (*Andropogon gerardii*, Poaceae) and leadplant (*Amorpha canescens*, Fabaceae). We sampled leaves of both plant species from five sites across a portion of the precipitation gradient in Kansas, USA, each site with a different mean annual precipitation (MAP; 615 mm – 1038 mm). Leaf disks were homogenized, and the extracted DNA was Illumina MiSeq sequenced to characterize the foliar fungal communities. We compared several richness and diversity estimates using Wilcoxon rank sum tests and multiple linear regression analyses and tested for compositional differences among the communities using permutational analysis of variance analogs (PERMANOVA). The data suggested minor differences in the fungal communities between the two hosts, whereas MAP had no effect. Similarly, linear regression analyses indicated no host or precipitation gradient effects on the

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[6] Reprinted with permission from "*Amorpha canescens* and *Andropogon gerardii* recruit comparable foliar fungal communities across the steep precipitation gradient in Kansas " by H.I. Dea, D.A. McKenzie, B. Clark, A. Jumpponen 2023. Transactions of the Kansas Academy of Science, Vol. 126, no. 1-2 P. 31-50. Copyright 2023 by Kansas Academy of Science.

fungal community composition. Both phyllosphere fungal communities and the factors affecting them in prairie systems remain poorly understood and results suggest that more research is needed to fully understand the compounding biodiversity which includes microbial communities within this system.

Introduction

Prairie ecosystem total area has declined across the United States' Great Plains due to anthropogenic environmental change, particularly due to conversion to monocrop agriculture (Lark et al., 2020). Tallgrass prairie systems have suffered the greatest losses compared to other grassland types and have been reduced to 4% of their original extent (Samson and Knopf, 1994). The largest contiguous tracts of tallgrass prairie today are in Kansas, whereas numerous other states have lost up to 99% of their tallgrass prairie cover (Samson and Knopf, 1994; Eilers and Roosa, 1994). These losses have resulted in declines in plant, animal, and microbial diversity, richness, and functional groups (Sala et al., 2000; Hirsh et al., 2013; Jain et al., 2014). Prairies are important because they provide crucial ecosystem services such as carbon storage, water filtration, and soil degradation mitigation (DeLuca and Zabinski, 2011). They also provide forage for livestock and other animals. Dominant plants in the tallgrass prairie are grasses such as *Andropogon gerardii* Vitman (big bluestem), *Sorghastrum nutans* (L.) Nash (Indiangrass), *Panicum virgatum* L. (switchgrass), and *Schizachyrium scoparium* (Michx.) Nash (little bluestem). Forbs contribute substantially to floristic diversity and can increase in abundance after fires or other disturbances (Fuhlendorf and Engle, 2004; Weir and Scasta, 2017). These diverse plant communities host hyperdiverse fungal communities reflective of the plant diversity (Peršoh, 2015) and lead to compounding diversity across trophic levels. Such diverse fungal

communities can even occur on the scale of plant leaves (e.g., Dea et al., 2022), as well as vary with nutrient quality within plants (Borruso et al., 2021; Lekberg et al., 2021).

One example of a unique fungal community is that of the phyllosphere fungi. These fungi can either occur on the leaf surface (epiphytic fungi) or within leaf tissues (endophytic fungi), all collectively called phyllosphere fungi. Phyllosphere fungi include plant pathogens but may also include taxa that can reduce pathogen infection in their host plants by spatial exclusion on the leaf surface otherwise open for pathogens (Blakeman and Fokkema, 1982). Epiphytic fungi can also reduce the harmful UV radiation affecting the leaf surfaces (Barrera et al., 2020; Solhaug et al., 2003) and can aid the host plant water retention (Rho and Kim, 2017). Even as a single plant organ (e.g., leaves) can support a diverse fungal community, individual host plant species can filter fungal communities in prairies, resulting in distinct fungal assemblages associating with different host plants (Yao et al., 2019; DeMers and May, 2021; Liu et al., 2021). Therefore, fungal diversity is an exaggerated reflection of botanical diversity, and fungal endophytes represent a diverse component of the prairie community, from within an individual plant to the ecosystem level (Saikkonen et al., 2015).

Climate factors, like precipitation, can affect fungal community assembly and alter fungal diversity and community composition on plants and in soils (House and Bever, 2018; Lagueux et al., 2020; Rudgers et al., 2021; Dea et al., 2022). For example, using Bray-Curtis dissimilarity matrices, DeMers and May (2021) documented a potential fungal community-level endophyte gradient along a precipitation gradient in Minnesota prairies. Data on the phyllosphere fungal diversity of tallgrass prairie plants are lacking and studies that focus on how these fungal communities differ among dissimilar host species are few (LeBlanc et al., 2014). For example, LeBlanc et al. (2014) found rhizosphere fungal diversity to increase with plant diversity and

fungal community structure to differ between species in the Fabaceae and Poaceae. *Andropogon gerardii* is a dominant, almost ubiquitous grass (Poaceae) in the tallgrass prairie ecosystem that depends on its symbiosis with mycorrhizal fungi (Anderson et al., 1994; Hartnett and Wilson, 1999). *Andropogon gerardii* can provide almost 100% of the canopy cover in some Kansas prairies (Hulbert, 1986) and can account for up to 24% of the aboveground biomass (Owensby et al., 1993). However, the phyllosphere fungi that associate with this important dominant plant remain to be explored. *Amorpha canescens* Pursh (leadplant) coexists with *A. gerardii* and is a common semi-perennial legume (Fabaceae) in tallgrass prairies with N-fixing symbionts in its root nodules. This symbiosis with N-fixing rhizobia results in higher N content in *A. canescens* tissues compared to non-leguminous plants in the tallgrass prairie ecosystem (Adams et al., 2016). Further, *A. canescens* is widely distributed within the tallgrass prairie region and can occur at a high density relative to co-occurring forbs (Towne and Knapp, 1996). We use these two important, but functionally distinct, prairie species as examples of hosts for fungal communities.

Worldwide, nitrogen content in leaves of N_2 fixing plants, such as those in the Fabaceae, is greater than leaf nitrogen content of non-N_2 fixing plants, such as grasses (Adams et al., 2016; Averill et al., 2019). Substrate nitrogen content may affect fungal growth rate, and some evidence suggests a correlation between foliar fungal communities and foliar nitrogen content in northeastern Kansas (Jumpponen and Jones, 2010). Similarly, fungal communities respond to available nitrogen; atmospheric nitrogen deposition can increase fungal community diversity and functional groups in prairie soil and on deciduous tree leaves (Borruso et al., 2021; Lekberg et al., 2021). Because *A. canescens* is a N-fixing species, and *A. gerardii* is a grass, they would

differ in N content and their C:N ratios in our areas of study and thus, in quality as a resource for fungal communities (Thomas and Asakawa, 1993).

We assessed the effects of two common tallgrass prairie host species (*A. gerardii* and *A. canescens)* that likely differ in foliar tissue quality as habitat for foliar fungal communities along with effects of MAP gradient on phyllosphere fungi. We sampled leaves and analyzed MiSeq sequence data to characterize the phyllosphere fungal communities. We hypothesized that our host species would present unique environments and would support different but overlapping fungal assemblages. Adding to host-based differences, we hypothesized that the steep precipitation gradient in Kansas would further modify habitat suitability to fungal communities. We expected that fungal communities on the mesic end of the precipitation gradient and those on *A. canescens* would be the most diverse.

Materials and Methods

Field Methods

Our study was conducted along a west to east precipitation gradient across the state of Kansas. Study sites were located at Hays Agricultural Research Center (HAR), a private prairie in Saline County (SAL), Konza Prairie Biological Station (KNZ), Rockefeller Prairie (JEF), and Welda Prairie (WEL). All sites were predominantly tallgrass prairie with management practices ranging from grazing and burning to haying, and along a precipitation that ranged from 615 to 1,038 mm annually (Table 4). Mean annual temperatures ranged from 12.3-13.4°C among sites. The dominant flora at the sites was characteristic of typical tallgrass prairie with grasses such as *A. gerardii*, *S. nutans*, *S. scoparium*, and various forb species such as *A. canescens*, *Solidago* L. spp. (goldenrods), and *Helianthus* L. spp. (sunflowers). We chose the two focal host plants for this study because of functional differences and because of their widespread distribution and

frequent occurrence in tallgrass prairies throughout the Great Plains (Great Plains Flora Association 1986).

We laid 100 m transects starting at a random point at each site. Transects followed elevation isolines and represented the trajectory with the least variation in elevation from the starting point. A sample collection point (n = 10 per transect) was located every 5 meters along the transect to avoid overlapping the 5m search radius of each point (Figure 6). Plants from which clippings were taken were the individuals of each species closest to the sampling point, and within the 5 m radius of each point along the transect. A total of ten plant individuals were sampled along each transect (five individuals for each of the two focal species). Only leaves without any clear pathogen infection were sampled to avoid skewing fungal community characteristics. For *A. canescens*, we sampled distal stems with new foliage, and for *A. gerardii*, we cut 2-4 mature blades at the base of each plant, excluding leaves on the flowering stalk. *A. canescens* was absent from the SAL site, potentially because of selective grazing by cattle. We included the five *A. gerardii* leaf samples from this site in our analyses. Leaf samples were sealed into plastic bags and transported on ice to the laboratory within 24 hours and stored in a -20 °C freezer for at least 24 hours before sample processing.

Laboratory Methods

A total of 16, 2 mm (diam.) discs were excised with a sterile biopsy punch from each individual plant (total surface area sampled = 0.5 cm^2 per plant). To remove unattached fungal spores and hyphae, discs were removed from the punch using a sterile dissecting needle and transferred to a 1.5 mL Eppendorf tube filled with 1,000 μL of 0.1% Triton-X. The discs were then shaken vigorously by hand for 30 s. After shaking, the solution was removed with a micropipette and the discs were rinsed by shaking them three times in sterile, deionized water for

30 s. The rinsed discs were transferred to a PowerSoil homogenization tube (2 mL) with PowerSoil beads (1.4 mm bead size, Qiagen, Hilden, Germany), two 2.4 mm zirconium beads, and 500 µL of dilution buffer (Plant Direct Phire Kit, Thermo Scientific) to homogenize the samples. The homogenization tubes with discs and beads were kept on ice and stored at -20°C until all samples had been processed. The leaf disks were homogenized in a Savant Fastprep FP120 (MP Biomedicals, Irvine, CA) at speed 6. Because Poaceae leaf tissues tend to be tougher than those of Fabaceae, we conducted a pilot study on extra host material to determine the optimal homogenization for each host. Based on that pilot study, the *A. canescens* discs were homogenized for 30 seconds, whereas the *A. gerardii* discs were homogenized for 60 seconds, with the addition of 200 µL of 0.15 mm garnet beads.

To choose the optimal dilution for PCR-amplification with the Phire Plant Direct Kits, we 10-fold diluted the leaf extracts (10^0 - 10^{-3}) in sterile molecular grade RNA- and DNA-free water and compared the Internal Transcribed Spacer (ITS2) metabarcode amplification success with the forward primer fITS7 (Ihrmark et al., 2012) and the reverse primer ITS4 (White et al., 1990). The 10^{-2} dilution produced PCR-amplicons consistently and was chosen for library preparation. The ITS2 amplicons were generated with fITS7-ITS4 primers in 30 cycles with primer pads that permitted sample-specific indexing and MiSeq adapter addition in five PCR cycles. The MiSeq libraries were prepared at the Integrated Genomics Facility at Kansas State University. The raw sequence data are available at the sequence read archive under BioProject number PRJNA934065.

The data set initially consisted of 3,848,465 raw fungal sequences, which were processed using the mothur pipeline (v. 1.44.1, Schloss et al., 2009) as per the MiSeq standard operation protocol (Kozich et al., 2013) where possible. Sequences were extracted from paired-end .fastq

files, contiged and any sequences with ambiguous bases, sequences with more than 1base pair (bp) mismatch with primer, or homopolymers longer than 9 bp were omitted. This resulted in a total of 3,007,181 sequences. The sequences were truncated to the length equal to the shortest high-quality read (237 bp excluding primers). The >99% similar sequences were pre-clustered (Huse et al., 2008), checked for potential chimeras using UCHIME algorithm (Edgar et al., 2011) and putative chimeras were removed. The remaining sequences were assigned to the Operational Taxonomic Units (OTUs) at 97% similarity and clustered using vsearch (Rognes et al., 2016). Rare OTUs (fewer than 10) and those that were detected in the negative controls were removed from further analyses. Remaining OTUs were assigned to taxa using the Naïve Bayesian Classifier (Wang et al., 2007) and International Nucleotide Sequence Database – reference database (UNITE) (Abarenkov et al., 2021). Non-target OTUs which did not match the UNITE dataset or were assigned to groups outside of the Kingdom Fungi were removed.

After removing poor quality, chimeric and rare sequences, the final dataset included 1,426,547 sequences representing 886 OTUs (Coverage: 0.999 ± 0.002). We iteratively (100 iterations) estimated fungal richness and diversity for each sample using mothur (v. 1.44.1, Schloss et al., 2009). To minimize biases resulting from differences in sequencing depths among the libraries, we rarefied the sequence data to 8,500 sequences per sample as recommended in Gihring, Green, and Schadt (2012). To estimate richness and diversity, we estimated observed OTU richness (S_{Obs}), Shannon Diversity (H'), and Shannon Evenness (E_H).

Statistical Analyses

We used R (R Core Team 2022) and RStudio (version 2022.7.1.554, RStudio Team 2022) for all statistical analyses and graphical data representations. Some experiment groups violated assumptions of normality (Shapiro Wilks tests) or homoskedasticity (Bartlett’s test), so

we chose to use a nonparametric test. To determine if one of the host species harbored greater fungal richness, diversity, or evenness, we used the Wilcoxon rank sum test to compare the fungal observed species richness (S_{Obs}), Shannon Diversity (H'), and Shannon Evenness (E_H) between plant host species within each site. To detect any relationships between fungal richness (S_{Obs}), diversity (H'), or evenness (E_H), and mean annual precipitation (MAP) or the two plant hosts, we used multiple linear regressions. Each model included the linear term "MAP" centered around the mean (861.11 mm yr^{-1}) and the categorical term "Host" as well as their interaction. We visually evaluated residuals to ensure they did not blatantly violate assumptions of linear regression analyses and performed outlier analyses. We identified outliers as those more than two standard deviations from the mean in each site-species combination and proceeded with linear regressions with and without outliers to evaluate the effects of potential outliers. These regressions yielded comparable results suggesting that the outliers minimally impacted our conclusions. As a result, we present models here with potential outliers retained. To further infer the ecology of the OTUs, we used the function "funguild_assign()" in R package "FunGuildR" (Nguyen et al., 2016). We used these data to test if the proportion of assigned plant pathogens differed between plant hosts and across the precipitation gradient. We calculated the percentage of sequences assigned to the guild "Plant Pathogen" and used Wilcoxon rank sum test and multiple linear regressions similar to those for richness and diversity (Host x MAP).

For community compositional analyses, we rarefied our community abundance data to 10,500 sequences per sample using the function "rarefy_even_depth()" in "phyloseq"(McMurdie and Holmes, 2013). This resulted in the removal of three low-yielding samples (one *A. gerardii* sample from site JEF and two *A. canescens* samples from HAR) which were excluded from compositional analyses. We calculated the Bray-Curtis distance matrix and visualized it with

Principal Coordinates Analysis (PCoA) using the "ordinate()" function in "phyloseq" (McMurdie and Holmes, 2013). To test for compositional differences in fungal communities associated with the two host plants (main effect term nested within site) and between the sites along the MAP gradient, we used a permutational analysis of variance (PERMANOVA) with 10,000 permutations with function "adonis()" in the R package "vegan" (Oksanen et al., 2020). To test for any linear relationships between MAP and ordination space, we used multiple linear regression models with the first three PCoA axes as the response. These models included the linear term "MAP" centered around the mean (861.11 mm yr^{-1}) and the categorical term "Host" as well as their interaction, similar to the analyses for fungal richness and diversity.

Finally, to identify OTUs disproportionally more abundant in either of the two host species or in the various sites, we used function "multiplatt()" in R package "indicspecies" (v. 1.7.12, De Caceres and Legendre, 2009) to run indicator taxon analysis (method = IndVal.g) and niche preference analysis (method=r.g). Both analyses compared either sites or plant hosts in three distinct data subsets: 1) the top 100 most abundant OTUs present in sites in which both hosts were sampled (SAL was excluded); 2) the top 100 most abundant OTUs in the two terminal sites (HAR and WEL); and 3) all OTUs assigned to the guild "Plant Pathogen" (64 OTUs total) present in sites in which both hosts were sampled (SAL was excluded). To correct for multiple testing, we used function "p.adjust()" and corrected acquired p-values for false discovery rate (FDR) in program R.

Results

After quality control and removal of rare sequences, a total of 1,426,547 high quality sequences and 886 OTUs remained. Sequence yields ranged from 3,817 to 65,371 per sample with a mean yield of 31,701 ± 15,375 (SD).

The fungal communities represented primarily Ascomycota (70.4% sequences and 68.3% OTUs) and Basidiomycota (28.2% sequences and 24.8% OTUs) with the remaining data (<1% sequences and ≤ 3% OTUs) representing Mortierellomycota, Chytridiomycota, Glomeromycota, Mucoromycota, Rozellomycota (following Tedersoo et al., 2018) and unclassified Fungi. Relative abundances of fungal orders can be found in Figure 11. A large majority of OTUs (78.0% sequences and 74.6% OTUs) were assigned to a genus. Among those with a genus-level assignment (373 genera total), the most abundant were *Darksidea* (3 OTUs, 4.57 % sequences), *Sporidiobolus* (2 OTUs, 4.46% sequences), and *Neoascochyta* (2 OTUs, 3.38% sequences). The additional ten most abundant genera were mainly fungi commonly observed in phyllosphere samples – including *Alternaria*, *Aureobasidium*, *Fusarium*, *Epicoccum*, *Magnaporthiopsis*, *Marasmiellus*, *Naganishia*, *Phaeosphaeria*, *Talaromyces*, and *Tilletiopsis*.

Our analyses provided no evidence for differences in fungal richness, diversity, or evenness between the two plant hosts within any of the sites (Wilcoxon: S_{Obs}: $W < 6$, $P > 0.222$; H': $W < 18$, $P > 0.310$; E_H: $W < 17$, $P > 0.111$). Multiple linear regression models explained a small proportion of the variation in fungal richness (Table 5). Fungal richness increased with MAP in *A. canescens* and there was some limited evidence that *A. canescens* harbored greater richness than *A. gerardii* as MAP increased (Table 5; Figure 7A). However, there was no evidence for a host plant main effect showing that at the mean precipitation, fungal richness did not differ between the two plant hosts.

In contrast to richness, models poorly explained variation in fungal diversity, evenness, and first or second PCoA axis scores (Table 5; Figure 7). Similarly, there was no evidence for an effect of plant host, MAP, or their interaction on fungal diversity, evenness, or first or second PCoA axes (Table 5; Figure 7B-E).

Although there was no evidence for the host or MAP effects on the first two PCoA axes, models predicted that the third PCoA axis explained some variation (Table 5). There was strong evidence that with increasing MAP, the third PCoA axis scores decreased in *A. canescens* plants (Figure 7F). Although third PCoA scores were not significantly different between the host plants at the mean precipitation, there was evidence for interaction between the hosts and MAP: *A. canescens* PCoA axis scores decreased whereas *A. gerardii* did not respond strongly.

Models explained a small proportion of the variation in percentage of fungi assigned to the guild plant pathogen in our samples (Table 5; Figure 7G). There was evidence for interaction between plant host and MAP – with *A. gerardii* harboring lower percentage of plant pathogens with increasing MAP whereas the percentage did not change in *A. canescens* (Table 5; Figure 7G). This was further shown in site-by-site comparisons of plant hosts: we found evidence of greater pathogen percent in *A. canescens* than *A. gerardii* in the terminal site WEL, while in sites with lower MAP, we found no evidence for differences between the hosts (Wilcoxon: HAR: W = 11.5, P > = 0.294; JEF: W = 7, P = 0.310; KNZ: W = 10.5, P = 0.753; WEL: W = 0, P = 0.019).

Our PERMANOVA provided no evidence for distinct fungal communities between the two host species ($F_{4,36}$ = 0.88, R^2 = 0.099, P = 0.764) or among the sites ($F_{3,36}$ = 0.98, R^2 = 0.083, P = 0.500) – a proxy for precipitation as each site differed in MAP (Figure 8). Similarly, analyses of dispersion aiming to evaluate the fungal community heterogeneity among samples provided no evidence for differences in dispersion between the two host species ($F_{1,36}$ = 0.04, P = 0.854) or between sites ($F_{3,36}$ = 0.737, P = 0.551).

Indicator taxon and niche preference analyses identified few or no indicator OTUs (P<0.05) between plant hosts or among the sites when we included the 100 most abundant OTUs

in sites where both hosts were sampled (SAL was excluded), the 100 most abundant OTUs in terminal sites (HAR and WEL), or OTUs assigned to the guild "Plant Pathogen" (see Figure 12 for guild proportions). No indicator OTUs in these analyses remained significant after false detection rate (FDR) correction for multiple testing (Tables 9 and 10). Among the indicators that were significant before the FDR correction were an unidentified member of the genus *Coniothyrium* as an indicator for WEL site; for *A. canescens*, an unidentified member of the order Pleosporales as an indicator for WEL site and *A. gerardii*; and a member of the genus *Curvularia* as an indicator for *A. canescens*. Our analyses identified far more indicator OTUs for *A. canescens* than for *A. gerardii* (~5 times more, Tables 9 and 10).

Discussion

We aimed to evaluate how foliar fungal communities associated with two host species representing two functional groups (a grass and a legume) would respond to MAP along the steep precipitation gradient in Kansas. Previous research has highlighted that edaphic (Glynou et al., 2016; Rudgers et al., 2021) and climatic (Dea et al., 2022; Oita et al., 2021) variables may control the assembly of host-associated fungal communities. Similarly, host-associated communities may correlate with host communities (Dea et al., 2022) or may differ among the host species (U'Ren et al., 2012; Kembel et al., 2014). Foliar fungal communities can be particularly sensitive to climatic drivers and buffered against edaphic factors (Bowman and Arnold, 2021; Oita et al., 2021) as the foliar communities do not directly interact with the soil matrix and are thus more susceptible to temporal and diurnal oscillations in the environment. Our data provided little support for responses to either host species or MAP. This is despite the two hosts representing distinct functional types and the relatively steep MAP gradient that ranged from 615mm to 1,038mm per year. Our conclusions disagree with others who have found that

climatic factors strongly influence the phyllosphere fungal communities and their assembly. These studies differed from ours in several ways, however. For example, Carroll and Carroll (1978) compared fungal communities on gymnosperms at both high (dry) and low (wet) elevation sites; Zimmerman and Vitousek (2012) compared communities across a strong hydrologic and elevational gradient in Hawaii on Mauna Loa Volcano; U'Ren et al. (2012) compared fungal communities along a precipitation gradient, but at continental scale; Oita et al. (2021) compared communities at a landscape scale, but this was in the tropics and also spanned an elevation gradient; and finally, Dea et al. (2022) studied leaf fungal communities of prairies in Kansas, but their work spanned the entire precipitation gradient in Kansas sampling whole plant communities rather than a single target plant.

A lack of distinction between the foliar communities of the two hosts was surprising, particularly because differences have even been reported between co-occurring *Dalea* L. (prairie clover) species in Minnesota prairies (DeMers and May, 2021). Equally surprising was the lack of any relationship between fungal diversity and MAP, because others (e.g., DeMers and May, 2021; Dea et al., 2022) have documented strong differences based on sampling locations and their position along the MAP gradient in the Midwest. Despite the lack of differences in fungal communities between our plant hosts or among sampling sites, fungi represent a significant source of biodiversity, and affect ecosystem function (Perreault and Laforest-Lapointe, 2022). It is of note that our sampling of 720 small leaf disks from 45 plant individuals (20 from *A. canescens* and 25 from *A. gerardii*) included 886 molecular OTUs thus emphasizing the hyperdiverse communities that phyllospheres host (see Arnold et al., 2000; Arnold et al., 2007; Jumpponen and Jones, 2010).

Our data, overall, agree with earlier high throughput sequencing analyses that highlight foliar fungal communities dominated by ascomycetes (see Jumpponen and Jones, 2009; Zimmerman and Vitousek, 2012; Oita et al., 2021; Dea et al., 2022). Approximately 70% of our sequence data were assigned to ascomycetes, whereas the next dominant taxon - Phylum Basidiomycota – represented less than 30% of the acquired data. Among the OTUs represented by the greatest sequence counts were common foliar inhabitants, e.g., Pleosporalean genera *Alternaria* and *Epicoccum*, Dothidealean genus *Aureobasidium*, Eurotialean genus *Talaromyces*, and the basidiomycetous yeast *Tilletiopsis*, representing potential plant pathogens and saprobes. The common OTUs also included some surprising taxa. Among these was the genus *Darksidea* to which three OTUs were assigned. Fungi in genus *Darksidea* are Pleosporalean root-colonizing endophytes that have been frequently reported in European (Knapp et al., 2012; Knapp et al., 2015) and North American grasslands (Romero-Jimenez et al., 2022). We lack a sound explanation for this observation, although it is possible that the fungi occupying belowground tissues may colonize their hosts systematically or adhere to the foliar tissues even after our surface washing.

Even though our community-wide PERMANOVAs provided no support for shifts in the foliar fungal communities, our indicator taxon analyses revealed several OTUs that were either more abundant in one host or occurred more frequently at one site than at the others before correction for multiple testing. OTUs associated with *A. gerardii* remained unclassified below the level of order and represented fungal groups that include many taxa commonly associated with plant tissues (OTUs assigned only to Sordariomycetes and Pleosporales). In contrast, the indicator OTUs for *A. canescens* included OTUs assigned to genus *Coniothyrium*, *Dictyosporella*, and *Mycena*. In general, these *A. canescens* indicators represent common leaf-

associated fungi. For example, genus *Coniothyrium* includes plant-associated Pleosporalean mycoparasites (e.g., Whipps et al., 2008) and plant endophytes, antagonists, and pathogens (e.g., Peters et al., 1998; Berg, 2009). Further, species of *Mycena* have been generally considered saprotrophic (Læssøe et al., 1996; Emmett et al., 2008), or when colonizing plant tissues, considered latent saprotrophs (Osono, 2010; Kohout et al., 2018). However, recent research has documented some as endophytes that associate with roots of many plant hosts (see, e.g., Roy et al., 2021; Thoen et al., 2020). The foliar *Mycena* spp. may indeed represent either latent foliar saprotrophs or indicate presence of leaf spot disease caused by some species of *Mycena* (e.g., Avelino et al., 2007). Why these taxa might be overrepresented in *A. canescens* remains unclear, but may reflect host preference for a landscape position, differences in host susceptibility, and/or quality of plant tissue available for fungal colonization. We also observed indicator OTUs (assigned to ascomycete genera *Coniothyrium*, *Phaeosphaeria*, and *Sclerostagonospora*, and the basidiomycete genus *Marasmiellus*) that were more abundant at sites in the mesic end of our gradient (KNZ and WEL). These OTUs are not unexpected as they represent common foliar tissue associates, plant endophytes, pathogens and saprobes (e.g., Peters et al., 1998; Phookamsak et al., 2014; Oliveira et al., 2019). It may be helpful to consider these OTUs infrequent or near absent in arid sites, suggesting that the more arid end of our gradient may lie at the edge of their environmental tolerances.

Our study is a snapshot of the phyllosphere fungal communities during one summer. Our sampling alternated between arid and mesic sites to avoid temporally confounding sampling as it was stretched between the months of June and July. The lack of support for our MAP hypothesis may be attributable to seasonal dynamics of fungal communities. Jumpponen and Jones (2010) dissected foliar fungal communities and sampled *Quercus macrocarpa* leaves in northeastern

Kansas six times during one growing season. They identified clear seasonal shifts in these phyllosphere communities, suggesting that 1) we might have detected greater variation among sampling areas and/or between hosts had we collected specimens over a longer period and 2) the seasonal heterogeneity in the fungal communities may have masked the hypothesized community differences. In addition to the seasonal variability, plant-associated fungal communities may have interannual dynamics and vary among years. For example, Farner, Spear, and Mordecai (2020) sampled the perennial bunchgrass, *Stipa pulchra* Hitchc., in California and documented interannual dynamics in culturable fungal pathogen communities.

Although our sampling covered nearly a two-fold range in precipitation, sampling over a greater span of precipitation could have been beneficial. Our observed (though often nonsignificant) trends suggest that sampling further into the drier west and further into the wetter southeast parts of the precipitation gradient might have provided a clearer separation of the communities as a function of MAP. However, locating co-occurring populations of the two target species became increasingly more challenging the further into the arid sites we traveled. Although our data did not support our hypotheses, our study contributes towards a better understanding of the hyperdiverse foliar fungal communities in prairie ecosystems. When considering the microbial communities in prairie restoration, this information is useful for understanding where inoculant soils could possibly be obtained for transfer to restored locations (Koziol and Bever, 2016).

Tables and Figures

Table 4. Characteristics of climate (MAP = mean annual precipitation, MAT = mean annual temperature), management, soil, and location of each site. Sites include Konza

Prairie Biological Station (KNZ), Hays Agricultural Research Center (HAR), Rockefeller Prairie (JEF), Welda Prairie (WEL), and a private prairie in Saline County (SAL).

Table 5 Multiple linear regression model statistics for fungal Operational Taxonomic Unit (OTU) community diversity, richness evenness, compositional estimates, and percentage of plant pathogen guild predicted by plant host species (Host) and mean annual precipitation

Site	Location	MAP (mm)	MAT (°C)	Management	Soil Type	County
WEL	38° 51', -99°23’	1038	13.4	patch-burn grazing, haying	Kenoma-Olpe (8780)	Anderson
JEF	39° 06', -99° 37'	982	12.6	haying,	Pawnee (7501)	Jefferson
KNZ	38° 54', -97° 59'	864	12.8	prescribed burns	Reading (7174)	Riley
SAL	39° 03', -95° 12'	742	12.7	grazing,	Wells-Edalgo (3495)	Saline
HAR	38° 11', -95° 16'	615	12.3	grazing	Harney (2612)	Ellis

centered around the mean (MAP) main effects and their interaction (Host x MAP) with *Amorpha canescens* as reference (0) compared to *Andropogon gerardii* (1). Statistically significant models and predictors (P<0.05) are bold-faced. Parameter estimate significances are denoted as 'ns' for not significant, ‘(*) ’for 0.05≤P<0.10, ‘* ’for 0.01≤P<0.05, ‘ ’for 0.001≤P<0.01, and ‘*** ’for P<0.001.**

Response	Model	Predictor	Estimate ± SE	t-value
Richness (S_{Obs})	**$F_{3,38}$ = 4.13*, R^2_{adj} = 0.186**	**Intercept**	**105.00±15.90**	**6.60*****
	AIC = 475.30	Host	–33.02±20.74	–1.59^{ns}
		MAP	**2.8x10^{-1}±1.1x10^{-1}**	**2.67***
		Host x MAP	–2.4x10^{-1}±1.4x10^{-1}	–1.79$^{(*)}$
Diversity (H')	$F_{3,38}$ = 0.45^{ns}, R^2_{adj} = –0.042	**Intercept**	**3.19±2.1x10^{-1}**	**15.27*****
	AIC=111.49	Host	1.3x10^{-2}±2.7x10^{-1}	0.05^{ns}
		MAP	1.5x10^{-3}±1.3x10^{-3}	1.14^{ns}
		Host x MAP	–1.5x10^{-3}±1.8x10^{-3}	–0.86^{ns}
Evenness (E_H)	$F_{3,38}$ = 1.70^{ns}, R^2_{adj} = 0.049	**Intercept**	**7.6x10^{-1}±3.2x10^{-2}**	**24.01*****

	AIC = –46.66	Host	$5.5x10^{-2}±4.2x10^{-2}$	1.34^{ns}
		MAP	$–2.3x10^{-4}±2.1x10^{-4}$	$–1.08^{ns}$
		Host x MAP	$4.8x10^{-5}±2.7x10^{-4}$	0.18^{ns}
PCoA Axis 1	$F_{3,38} = 0.59^{ns}$, $R^2_{adj} = –0.031$	**Intercept**	**$–6.6x10^{-2}±7.0x10^{-2}$**	**$–0.94^{***}$**
	AIC = 19.04	Host	$1.2x10^{-1}±9.1x10^{-2}$	1.29^{ns}
		MAP	$–8.5x10^{-6}±4.6x10^{-4}$	$–0.02^{ns}$
		Host x MAP	$9.6x10^{-5}±6.0x10^{-4}$	0.16^{ns}
PCoA Axis 2	$F_{3,38} = 0.09^{ns}$, $R^2_{adj} = –0.071$	Intercept	$–7.8x10^{-3}±5.1x10^{-2}$	$–0.15^{ns}$
	AIC = –6.44	Host	$8.2x10^{-3}±6.7x10^{-2}$	0.12^{ns}
		MAP	$9.9x10^{-5}±3.4x10^{-4}$	0.29^{ns}
		Host x MAP	$–2.2x10^{-4}±4.4x10^{-4}$	$–0.49^{ns}$
PCoA Axis 3	**$F_{3,38} = 3.33^{*}$, $R^2_{adj} = 0.146$**	Intercept	$3.4x10^{-2}±3.7x10^{-2}$	0.92^{ns}
	AIC = –33.66	Host	$–3.3x10^{-2}±4.9x10^{-2}$	$–0.68^{ns}$
		MAP	**$–7.3x10^{-4}±2.5x10^{-4}$**	**$–2.97^{**}$**
		Host x MAP	**$9.5x10^{-4}±3.2x10^{-4}$**	**2.97^{**}**
% Pathogens	$F_{3,38} = 2.78^{(*)}$, $R^2_{adj} = 0.115$	**Intercept**	**$3.31±6.7x10^{-1}$**	**4.93^{***}**
	AIC = 209.53	Host	$–7.6x10^{-1}±8.8x10^{-1}$	$–0.86^{ns}$
		MAP	$6.7x10^{-3}±4.5x10^{-3}$	1.50^{ns}
		Host x MAP	**$–1.5x10^{-2}±5.8x10^{-3}$**	**$–2.58^{*}$**

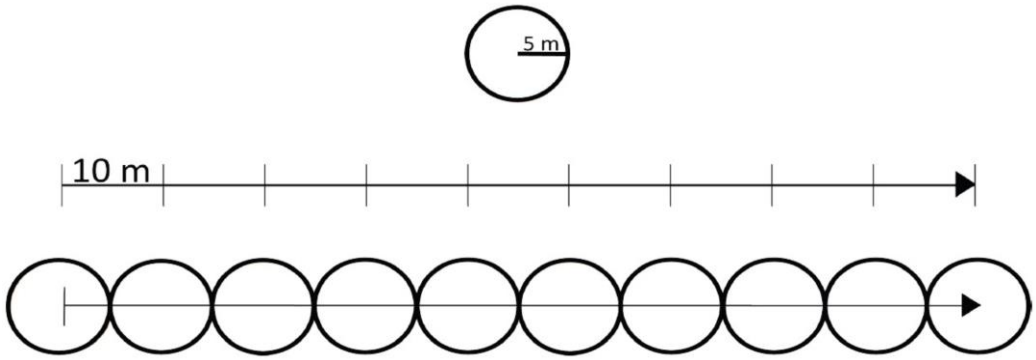

Figure 6. A schematic example of a transect used to gather host plant clippings. The center of each circle is a sampling start point 10 m from the next, each with a 5 m search radius for either *Andropogon gerardii* or *Amorpha canescens*, whichever was found first. Five specimens of each species were collected along each transect.

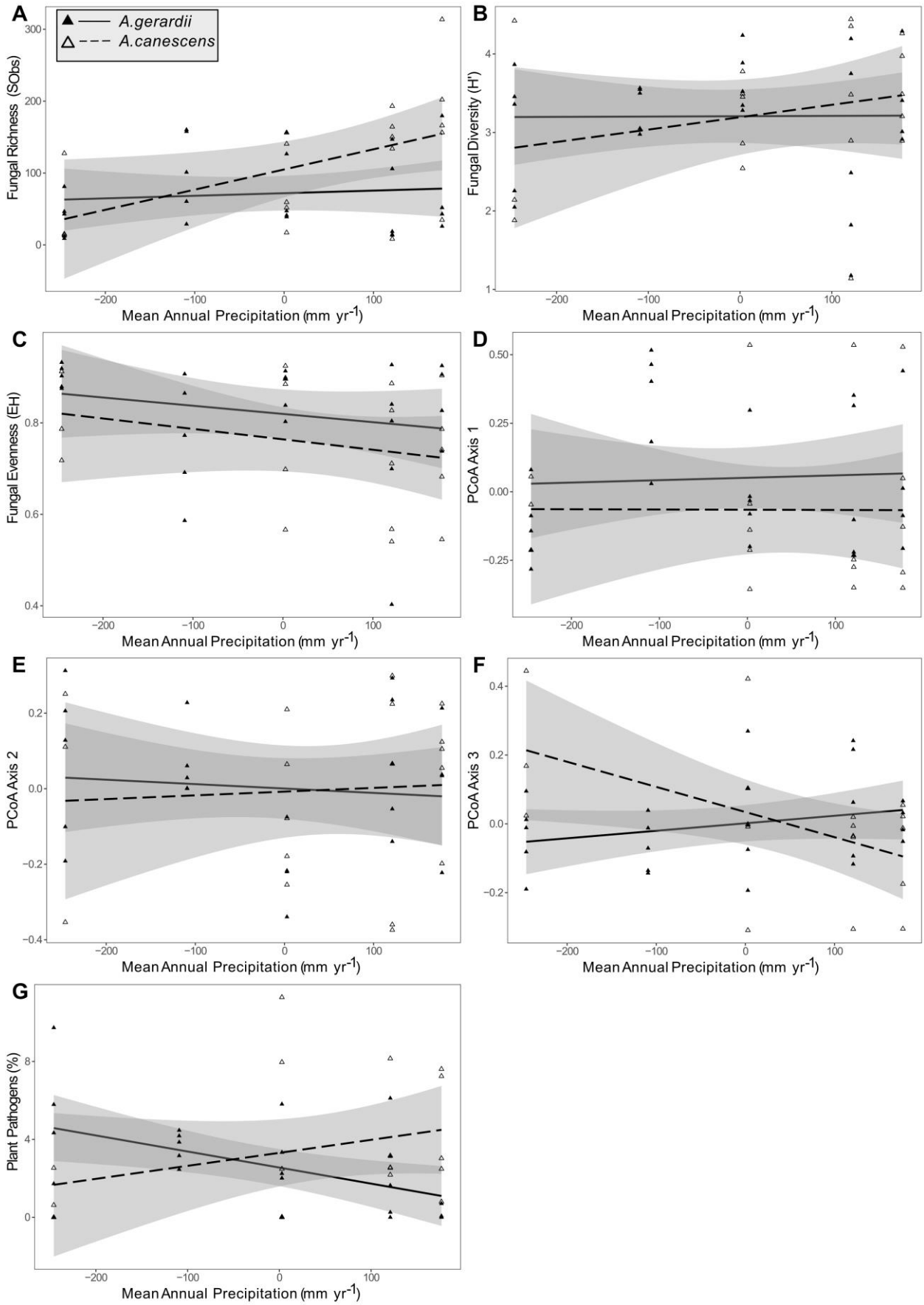
A
A.gerardii
A.canescens
Fungal Richness (SObs)
Mean Annual Precipitation (mm yr-1)
B
Fungal Diversity (H')
Mean Annual Precipitation (mm yr-1)
C
Fungal Evenness (EH)
Mean Annual Precipitation (mm yr-1)
D
PCoA Axis 1
Mean Annual Precipitation (mm yr-1)
E
PCoA Axis 2
Mean Annual Precipitation (mm yr-1)
F
PCoA Axis 3
Mean Annual Precipitation (mm yr-1)
G
Plant Pathogens (%)
Mean Annual Precipitation (mm yr-1)

Figure 7 Fungal Operational Taxonomic Unit (OTU) responses to mean annual precipitation (MAP) centered around the mean in *Amorpha canescens* (dashed line and open symbols) and *Andropogon gerardii* (solid line and filled symbols). Models predicting observed species richness (S_{Obs}) (A), Shannon diversity (H') (B), evenness (E_H) (C), PCoA Axis 1 scores (D), PCoA Axis 2 scores (E), PCoA Axis 3 (F), and percent of plant pathogen guild (G). The shaded areas represent 95% confidence intervals around the model predictions.

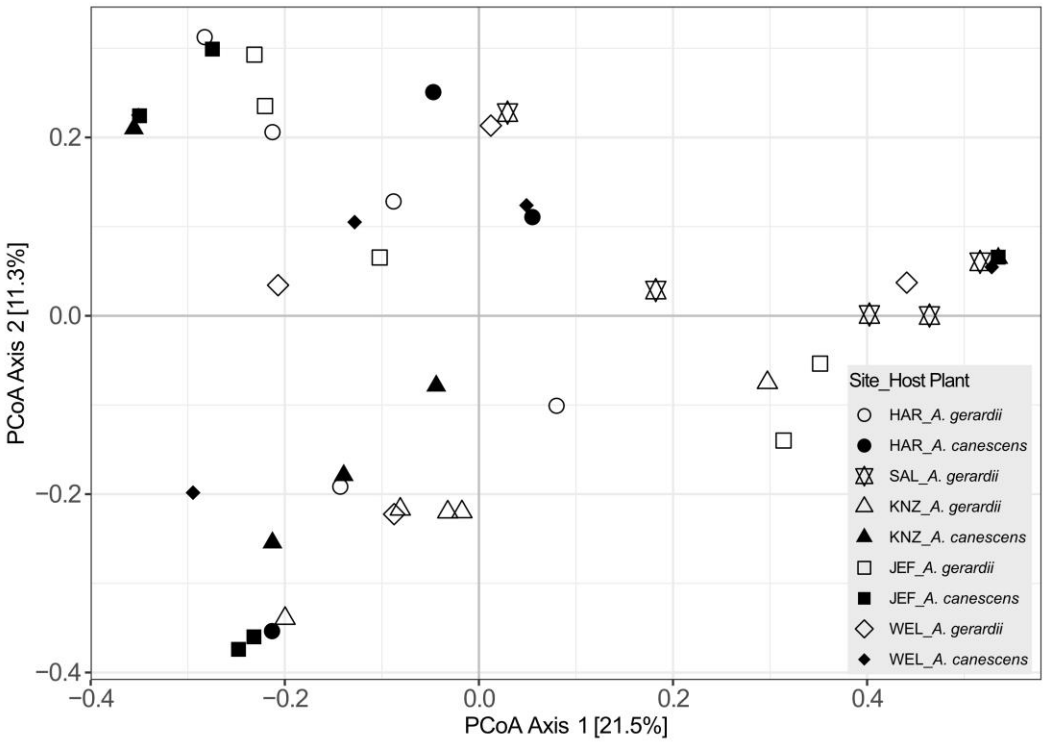

Figure 8 Principal Coordinates Analyses (PCoA) of fungal Operational Taxonomic Units (OTUs) in *Amorpha canescens* (open symbols) and *Andropogon gerardii* (filled symbols). Shapes indicate sites listed from least to most mean annual precipitation (circles = HAR, double triangle = SAL, triangle = KNZ, square = JEF, and diamond = WEL).

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Chapter 4 - Conclusion

Phyllosphere fungal communities are essential drivers of ecosystem functions and thus of importance for ecosystem conservation. The composition and diversity of these communities are driven by multiple, complex factors including climatic and anthropogenic influence. The results of this research show that, though the foliar endophytes of individual plant taxa may not respond to climatic factors such as mean annual precipitation, plant community shifts resulting from environmental gradients tightly couple with changes in the associated foliar fungi.

In Chapter 2, we address the question of whether plant communities and their associated phyllosphere fungal communities respond to changes in precipitation and land use history and whether the two communities are linked. Our results indicate that both plant and phyllosphere fungal communities change with mean annual precipitation – with the arid and mesic halves of the gradient harboring distinct communities. Additionally, plant richness, diversity, and FQI_{adj} increased with increasing mean annual precipitation with native prairies harboring greater richness and diversity than post-agricultural sites. Surprisingly, foliar fungal community richness did not respond to changes in precipitation while fungal diversity and evenness increased with precipitation in the remnant native sites but did not respond in the post-agricultural sites. This highlights the strong influence of mean annual precipitation on foliar fungal communities, congruent with studies on multiple climatic factors (Carroll and Carroll, 1978; Zimmerman and Vitousek, 2012; U'Ren et al., 2012; Oita et al., 2021), as well as the possibly severe loss of foliar diversity in fields with agricultural legacies, congruent with previous studies (Lauber et al., 2008; Upchurch et al., 2008; Jangild et al., 2011; Hui et al., 2018; Turley et al., 2020). This study builds upon previous research by showing that in mesic areas, this loss in foliar fungal communities becomes even more prominent and concerning. Further, though plant and fungal

diversity responded differently to increasing precipitation, their community compositions were tightly linked.

In Chapter 3, we addressed the question of whether two functionally distinct plant species, the legume *Amorpha canescens* and the grass *Andropogon gerardii*, recruited different fungal communities where they co-occur and whether these communities responded differently to changes in precipitation. Surprisingly and somewhat in contrast to results of studies described in Chapter 2, we found no significant difference between foliar fungal communities recruited by the two focal species. Further, foliar fungi increased in richness with increasing mean annual precipitation but did not strongly respond in their diversity and evenness.

We conclude that plant communities and phyllosphere fungal communities associated with a wide range of hosts respond strongly to changes in precipitation in their community composition, however, this response was not detected when focusing on two individual plant hosts' fungal endophyte communities. Some possible explanations for this may be that 1. There may be plant taxa other than our two focal species that harbor distinct communities, resulting in distinct fungal communities across the host plant communities, but not visible in these two species and 2. Epiphytic fungal communities may respond differently to environmental factors than endophytic communities (Gomes et al., 2018), and thus, the strong response reported in Chapter 2 study may be mainly driven by the epiphytic members of the phyllosphere communities that were not considered in Chapter 3. Overall, these studies highlight the tight linkage between plant communities and their associated fungal communities as well as the importance of climatic factors (mean annual precipitation) and land use in determining fungal community composition and diversity. Additionally, since plant communities shift along the precipitation gradient sampled, it is difficult to decouple the impact of mean annual precipitation

and plant communities on phyllosphere fungal communities – this decoupling of climate and plant community impacts warrants further research. Further studies that focus on mesic areas may be necessary to aid in selecting optimal areas to preserve the diversity of these important prairie plant symbionts.

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Appendix A - Supplementary Data

Supplementary Tables

Table 6 Multiple linear regression model adjusted R-squared (R^2_{adj}) and Akaike's Information Criteria (AIC) for plant community estimators predicted by land use history (LU) and listed main effect predictors and their interaction (LU x predictor) with native prairie as reference (0) compared to post-agricultural site (1).

Response variable	Longitude (DD)	MAP (mm yr^{-1})
Plant Richness (S_{obs})	R^2_{adj} = 0.67; AIC= 107.10	R^2_{adj} = 0.64; AIC= 107.85
Plant Diversity (H')	R^2_{adj} = 0.52; AIC= 23.16	R^2_{adj} = 0.51; AIC= 23.61
Plant Evenness (E_H)	R^2_{adj} =–0.06; AIC=–36.74	R^2_{adj} =–0.10; AIC=–36.28
Plant FQI_{adj}	R^2_{adj} = 0.39; AIC= 124.03	R^2_{adj} = 0.41; AIC= 123.44

Table 7 Multiple linear regression model statistics for plant and fungal community estimators predicted by land use history (LU) and mean annual precipitation (MAP) normalized around the mean precipitation (730.01 mm yr^{-1}) main effects and their interaction (LU x MAP) with native prairie as reference (0) compared to post-agricultural site (1). Statistically significant models and predictors (P<0.05) are bold-faced. Parameter estimate significances are denoted as 'ns' for not significant, '(*)' for 0.05≤P<0.10, '*' for 0.01≤P<0.05, '' for 0.001≤P<0.01, and '***' for P<0.001. Response variables with outliers were analyzed with and without the identified outliers. Models shown here do not include potential outliers.**

Response	Model	Predictor	Estimate±SE	|t –value|
Plant FQI_{adj}	**$F_{3,11}$ =9.57*, R^2_{adj} =0.647**	**Intercept**	**31.78±2.25**	**14.11*****
	AIC=103.47	Land Use	–5.15±3.34	–1.54^{ns}
		MAP	**5.16x10^{-2}±1.08x10^{-2}**	**4.79*****
		LU x MAP	**–7.13x10^{-2}±1.64x10^{-2}**	**–4.35****
Plant Diversity (H')	**$F_{3,11}$ =8.00*, R^2_{adj}=0.600**	**Intercept**	**2.54±1.43x10^{-1}**	**17.78*****
	AIC=18.15	**LU**	**–6.80x10^{-1} ±1.94x10^{-1}**	**–3.51****
		MAP	9.34x10^{-4}±7.20x10^{-4}	1.30^{ns}
		LU x MAP	8.91x10^{-4}±9.53x10^{-4}	0.94^{ns}
Plant PCoA Axis 2	$F_{3,12}$ =0.11^{ns}, R^2_{adj}=–0.237	Intercept	–4.94x10^{-2}±7.89x10^{-2}	–0.63^{ns}

	AIC= 0.39	LU	$3.27x10^{-2}$±$1.07x10^{-1}$	0.31^{ns}
		MAP	$8.24x10^{-5}$±$3.98x10^{-4}$	0.21^{ns}
		LU x MAP	$6.93x10^{-5}$±$5.27x10^{-4}$	0.13^{ns}
ASV Richness (S_{Obs})	$F_{3,11}$=0.19^{ns}, R^2_{adj}= –0.21	**Intercept**	**1151.76±81.06**	**14.21^{***}**
	AIC=208.31	LU	–$5.07x10^{-1}$±109.90	$–0.01^{ns}$
		MAP	–$6.02x10^{-2}$±$4.08x10^{-1}$	$–0.15^{ns}$
		LU x MAP	$3.21x10^{-1}$±$5.40x10^{-1}$	0.59^{ns}
OTU Richness (S_{Obs})	$F_{3,11}$=0.73^{ns}, R^2_{adj}= –0.061	**Intercept**	**755.85±43.05**	**17.56^{***}**
	AIC=189.33	LU	–59.19±58.37	$–1.01^{ns}$
		MAP	–$2.38x10^{-2}$±$2.17x10^{-1}$	$–0.11^{ns}$
		LU x MAP	$2.21x10^{-1}$±$2.87x10^{-1}$	0.77^{ns}

Table 8 Multiple linear regression model adjusted R-squared (R^2_{adj}) and Akaike's Information Criteria (AIC) for fungal community estimators predicted by land use history (LU) and listed predictor main effects and their interaction (LU x predictor) with native prairie as reference (0) compared to post-agricultural site (1). Superior models (ΔAIC > 2; Burnham and Anderson, 2004) are bold faced. If no model was superior, we used the model LU x MAP.

Predictor	OTU Richness (S_{obs})	OTU Diversity (H')	OTU Evenness (E_H)
Plant Richness (S_{obs})	**R^2_{adj} = 0.22; AIC=199.43**	R^2_{adj} =0.39; AIC=17.74	R^2_{adj} =0.24; AIC=–40.27
Plant Diversity (H')	R^2_{adj} = 0.11; AIC=201.64	R^2_{adj} =0.38; AIC=18.07	R^2_{adj} =0.25; AIC=–40.48
Plant Evenness (E_H)	R^2_{adj} = 0.01; AIC=203.29	R^2_{adj} =0.30; AIC=19.93	R^2_{adj} =0.18; AIC=–39.07
Plant PCoA Axis 1	R^2_{adj} =–0.05; AIC=204.30	R^2_{adj} =0.46; AIC=15.78	R^2_{adj} =0.33; AIC=–42.14
Plant FQI_{adj}	R^2_{adj} =–0.04; AIC=204.14	R^2_{adj} =0.53; AIC=13.69	R^2_{adj} =0.45; AIC=–45.25
Longitude (DD)	R^2_{adj} =0.05 ; AIC=202.59	R^2_{adj} =0.50; AIC=14.41	R^2_{adj} =0.38; AIC=–43.55
MAP (mm yr^{-1})	R^2_{adj} = 0.03; AIC=202.93	R^2_{adj} =0.54; AIC=13.21	R^2_{adj} =0.43; AIC=–44.69

Table 9 Indicator Taxon Analysis results from analyses run on 3 different subsets of the data: the top 100 most abundant OTUs in all sites where both species were found (SAL was excluded) listed as "All Sites", the top 100 most abundant OTUs in the terminal sites (HAR and WEL) listed as "Terminal", and all OTUs assigned exclusively to the guild "Plant Pathogen" by FunGuild (64 OTUs total) in all sites where both species were found. Analyses were run comparing the plant hosts or sites. Included also are OTU number, Group indicated, statistic, P-value, significance of P-value, P-value after correction for multiple testing (p.FDR), taxonomic resolution (Level), and Taxon name.

Data subset	Compare	OTU	Group	Stat	p.value	sig	p.FDR	Level	Taxon
All Sites	Hosts	Otu0077	*A. gerardii*	0.56	0.026	*	0.65	Order	Pleosporales
All Sites	Hosts	Otu0100	*A. canescens*	0.53	0.02	*	0.65	Genus	*Coniothyrium*
All Sites	Hosts	Otu0057	*A. canescens*	0.71	0.021	*	0.65	Genus	*Fusarium*
All Sites	Hosts	Otu0087	*A. canescens*	0.6	0.023	*	0.65	Species	*Mycena olida*
All Sites	Hosts	Otu0204	*A. canescens*	0.47	0.045	*	0.9	Genus	*Dictyosporella*
All Sites	Site	Otu0083	KNZ	0.63	0.008	**	0.2	Family	Mycosphaerellaceae
All Sites	Site	Otu0058	KNZ	0.71	0.017	*	0.34	Genus	*Sclerostagonospora*
All Sites	Site	Otu0043	KNZ	0.66	0.025	*	0.417	Species	*Marasmiellus tricolor*
All Sites	Site	Otu0022	WEL	0.76	0.003	**	0.2	Genus	*Phaeopoacea*
All Sites	Site	Otu0077	WEL	0.7	0.004	**	0.2	Order	Pleosporales
All Sites	Site	Otu0100	WEL	0.67	0.007	**	0.2	Genus	*Coniothyrium*
All Sites	Site	Otu0025	WEL	0.64	0.031	*	0.443	Species	*Phaeosphaeria microscopica*
Terminal	Hosts	Otu0077	*A. gerardii*	0.75	0.042	*	0.85	Order	Pleosporales

Pathogens	Hosts	Otu0092	*A. canescens*	0.6	0.016	*	0.398	Genus	*Curvularia*
Pathogens	Hosts	Otu0349	*A. canescens*	0.47	0.044	*	0.398	Genus	*Drechslera*
Pathogens	Hosts	Otu0433	*A. canescens*	0.47	0.045	*	0.398	Genus	*Monosporascus*
Pathogens	Hosts	Otu0488	*A. canescens*	0.47	0.045	*	0.398	Genus	*Monosporascus*
Terminal	Hosts	Otu0100	*A. canescens*	0.79	0.015	*	0.85	Genus	*Coniothyrium*
Terminal	Hosts	Otu0160	*A. canescens*	0.71	0.031	*	0.85	Order	Pleosporales
Terminal	Hosts	Otu0028	*A. canescens*	0.76	0.034	*	0.85	Genus	*Psathyrella*
Terminal	Hosts	Otu0092	*A. canescens*	0.71	0.05	*	0.85	Genus	*Curvularia*

Table 10 Niche Preference Analysis results from analyses run on 3 different subsets of the data: the top 100 most abundant OTUs in all sites where both species were found (SAL was excluded) listed as "All Sites", the top 100 most abundant OTUs in the terminal sites (HAR and WEL) listed as "Terminal", and all OTUs assigned exclusively to the guild "Plant Pathogen" by FunGuild (64 OTUs total) in all sites where both species were found. Analyses were run comparing the plant hosts or sites. Included also are OTU number, Group indicated, statistic, P-value, significance of P-value, P-value after correction for multiple testing (p.FDR), taxonomic resolution (Level), and Taxon name.

Data Subset	Compare	OTU	Group	Stat	p.value	sig	p.FDR	Level	Taxon
All Sites	Hosts	Otu0077	*A. gerardii*	0.26	0.02	*	0.7	Order	Pleosporales
All Sites	Hosts	Otu0047	*A. gerardii*	0.28	0.029	*	0.7	Class	Sordariomycetes
All Sites	Hosts	Otu0087	*A. canescens*	0.35	0.022	*	0.7	Species	*Mycena olida*
All Sites	Hosts	Otu0100	*A. canescens*	0.25	0.024	*	0.7	Genus	*Coniothyrium*
All Sites	Hosts	Otu0204	*A. canescens*	0.17	0.046	*	0.7	Genus	*Dictyosporella*
All Sites	Site	Otu0043	KNZ	0.51	0.01	**	0.73	Species	*Marasmiellus tricolor*

All Sites	Site	Otu0063	KNZ	0.37	0.031	*	0.73	Class	Sordariomycetes
All Sites	Site	Otu0022	WEL	0.47	0.022	*	0.73	Genus	*Phaeopoacea*
All Sites	Site	Otu0077	WEL	0.39	0.023	*	0.73	Order	Pleosporales
All Sites	Site	Otu0100	WEL	0.42	0.05	*	0.73	Genus	*Coniothyrium*
Pathogens	Hosts	Otu0092	*A. canescens*	0.26	0.037	*	0.438	Genus	*Curvularia*
Pathogens	Hosts	Otu0358	*A. canescens*	0.25	0.042	*	0.438	Species	*Curvularia inaequalis*
Terminal	Hosts	Otu0100	*A. canescens*	0.38	0.005	**	0.5	Genus	*Coniothyrium*
Terminal	Hosts	Otu0160	*A. canescens*	0.28	0.022	*	0.833	Order	Pleosporales
Terminal	Hosts	Otu0092	*A. canescens*	0.35	0.025	*	0.833	Genus	*Curvularia*
Terminal	Hosts	Otu0159	*A. canescens*	0.38	0.049	*	0.904	Genus	*Phaeosphaeria*
Terminal	Site	Otu0029	WEL	0.53	0.02	*	0.88	Order	Pleosporales
Terminal	Site	Otu0108	WEL	0.43	0.034	*	0.88	Species	*Rachicladosporium*
Terminal	Site	Otu0023	WEL	0.33	0.045	*	0.88	Family	Gomphillaceae

Supplementary Figures

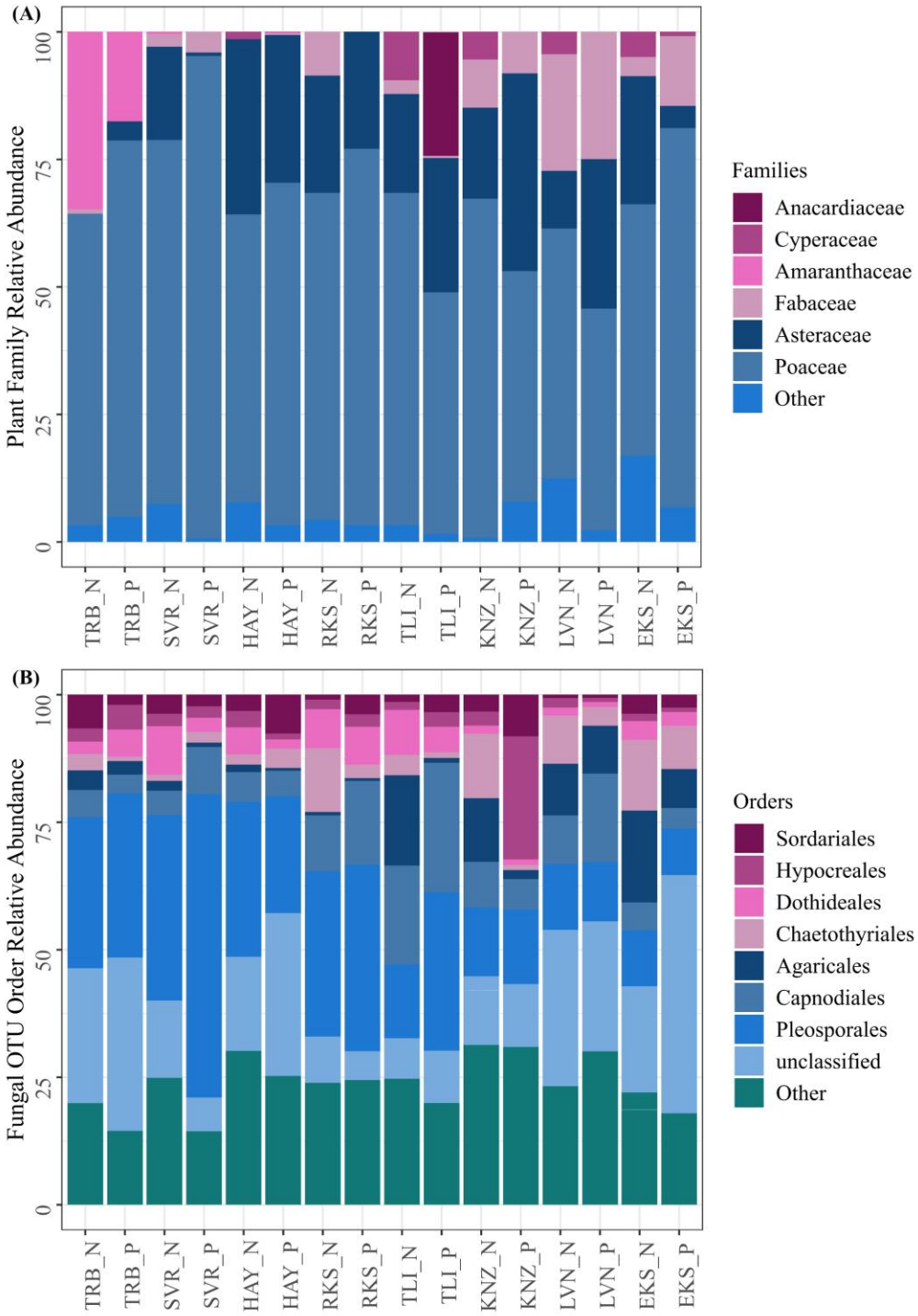

Figure 9 Relative abundance of plant families (A) and Operational Taxonomic Units (OTUs) order assignment (B). Plant families representing less than 1% and fungal orders representing less than 2% of the entire dataset are listed as "Other".

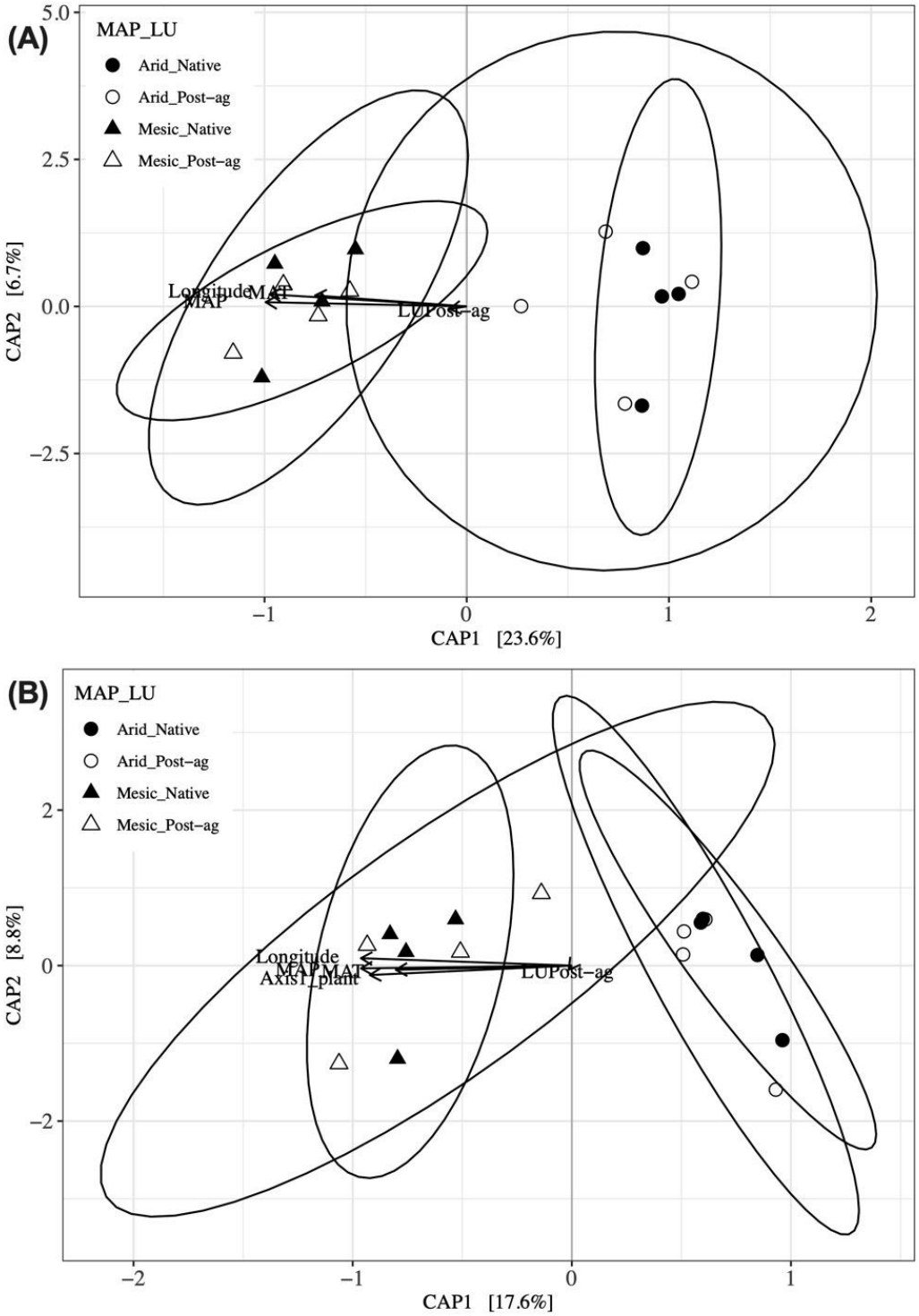

Figure 10 Distance Based Redundancy Analysis of the Bray-Curtis Distance of plant community ($F_{4,11}$ = 1.80, P = 0.006) (A) and phyllosphere fungal Operational Taxonomic Units (OTUs) ($F_{5,10}$ = 1.60, P = 0.001) (B). Main effects including Longitude (Plant: $F_{1,11}$ = 1.21, P = 0.223; OTU: $F_{1,10}$ = 1.58, P = 0.018), Mean Annual Precipitation (MAP) normalized around the mean precipitation (730.01 mm yr^{-1}) (Plant: $F_{1,11}$ = 2.24 , P = 0.026; OTU: $F_{1,10}$ =

1.58, P = 0.018), Mean Annual Temperature (MAT) (Plant: $F_{1,11}$ =2.76, P = 0.011; OTU: $F_{1,10}$ = 2.43, P = 0.001) and land-use (LU) (Plant: $F_{1,11}$ = 0.97, P = 0.389; OTU: $F_{1,10}$ = 1.06, P = 0.339) were used in models for both plant and fungal communities. The first Plant PCoA axis (OTU: $F_{1,10}$ = 1.35, P = 0.064) <u>was additionally included</u> for fungal communities. Arrows indicate the correlation of environmental variables with community composition.

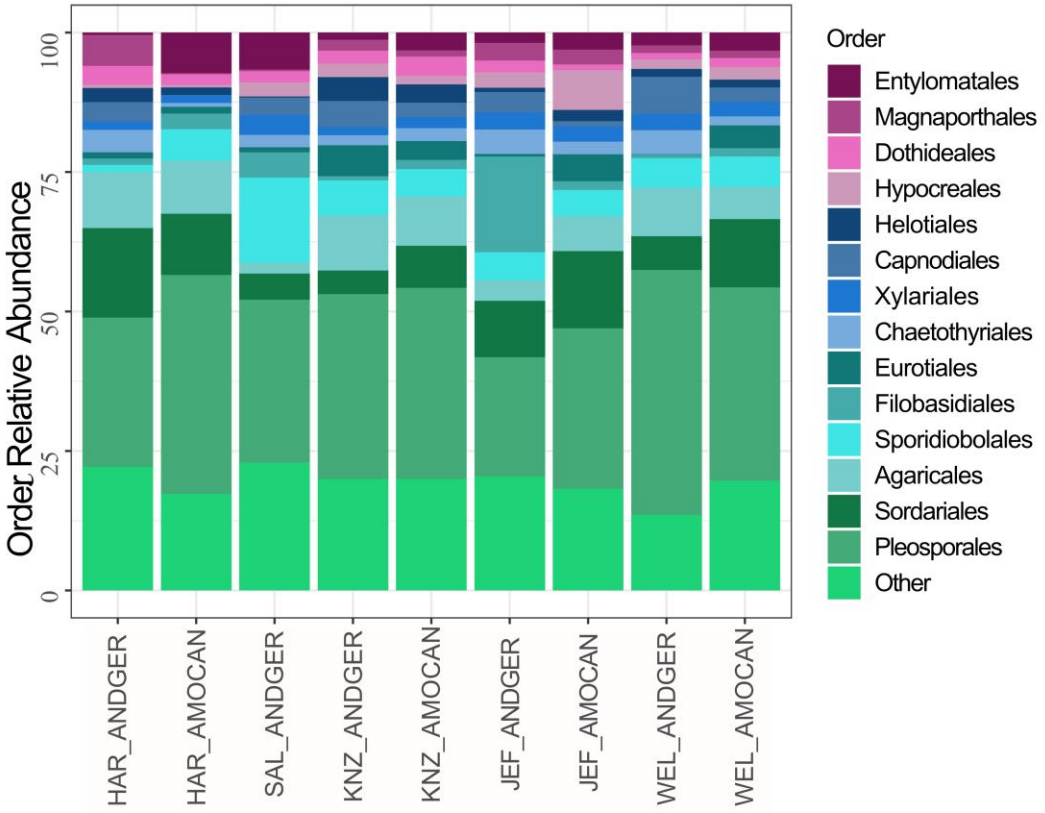

Figure 11 Relative abundance of fungal orders within each site-host combination with two letter identifiers for plant host (ANDGER = *Andropogon gerardii* and AMOCAN= *Amorpha canescens*). Orders that made up <2% total sequences were grouped into the "Other" category.

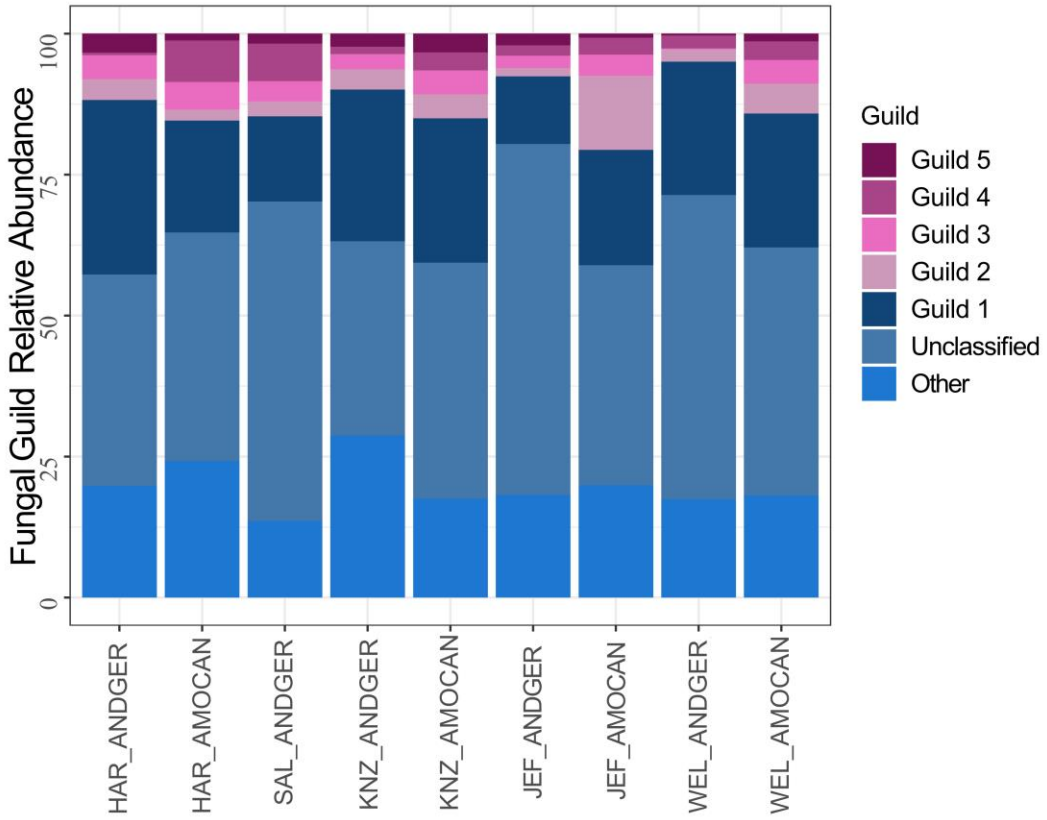

Figure 12 Abundance of fungal orders within each site-host combination with two letter identifiers for plant host (ANDGER= *Andropogon gerardii* and AMOCAN = *Amorpha canescens*). Guild 1 = Undefined Saprotroph; Guild 2 = Endophyte, Guild 3 = Plant Pathogen; Guild 4 = Endophyte-Litter Saprotroph-Undefined Saprotroph; Guild 5= Animal Pathogen-Endophyte-Epiphyte-Plant Pathogen-Undefined Saprotroph; Guilds that made up <2% total sequences were grouped into the "Other" category.

Appendix B - Results of ASV Analyses

Community descriptions

Following quality control and removal of rare sequences, we retained a total of 3,246,656 high quality sequences that clustered into 9,760 ASVs. The number of retained sequences differed between ASV and OTU pipelines because of the larger number of rare ASVs that were removed. The sequencing yields ranged from 95,925 to 359,387 per sample with a mean yield of 202,916 ± 90,823.6 (SD). The ASVs, their observed frequencies, and taxonomic assignments are listed in Supplemental Files 7 and 8.

Our ASV data were dominated by the Phylum Ascomycota (63.7% sequences and 59.0% ASVs), the Phylum Basidiomycota (16.0% sequences and 15.6 % ASVs), and a fairly large portion of unidentified taxa (18.1% sequences and 14.8 % ASVs), followed by the Phylum Glomeromycota (1.2% sequences and 6.9% ASVs), Chytridiomycota (0.4% sequences and 2.2% ASVs), and several Phyla that made up <1% of sequences and ASVs (Mortierellomycota, Mucoromycota, Kickxellomycota, Rozellomycota, Olpidiomycota, Entorrhizomycota, Aphelidiomycota, Entomophthoromycota, Aphelidiomycota, Entomophthoromycota, Calcarisporiellomycota, and Blastocladiomycota). Relative abundance of fungal Orders can be found in Figure 13. ASV were assigned to a total of 795 genera. a large proportion of ASVs were not assigned to the level of a genus – 3,783 ASVs (38.7%). Among those with genus level assignments, the most abundant were *Alternaria* with 37 ASVs (4.0% of all sequences and > 0.4% of all ASVs) followed by *Cladosporium* with 86 ASVs (3.5% sequences), and *Dissoconium* with 102 ASVs (2.9% sequences). The ten most abundant genera were common phyllosphere inhabitants including *Alternaria*, *Dissoconium*, *Phaeosphaerea*, *Puccinia*, *Fusarium*, *Blumeria*, and *Aureobasidium*.

Alpha diversity and regression analyses

Our regression model — using MAP normalized around the mean precipitation (730.01 mm yr-1), LU, and their interaction as predictors — poorly predicted fungal richness (S_{Obs}) and explained only a small proportion of the variation. These analyses provided no evidence for fungal richness responses to MAP, LU, or their interaction (Table 11; Figure 14A). This result

did not change whether or not the potential outlier (LVN_N) was excluded from the analysis (Table 7). AIC comparisons suggest that plant predictors or geographic distance were not superior to MAP (Table 12) except in the case of FQI_{adj} which was a better predictor for ASV richness ($F_{3,12} = 1.28$, $R^2_{adj} = 0.054$, $P = 0.324$), however, none of these models performed well in predicting fungal richness overall.

Our regression models — using MAP normalized around the mean precipitation (730.01 mm yr-1), LU, and their interaction as predictors — predicted fungal diversity (H') and explained a large proportion of the variation in their communities (Table 11; Figure 14B). There was evidence for interaction between MAP and LU where fungal diversity increased with MAP in the native prairie remnants but did not significantly change with increasing MAP in post-agricultural sites. There was also evidence for a land-use main effect that indicated greater fungal diversity in native prairie remnants than post-agricultural sites (Table 11; Figure 14B). AIC comparisons suggest that replacing MAP with geographic distance or plant community metrics did not result in a superior model for predicting fungal diversity (Table 12).

Our regression models – using MAP normalized around the mean precipitation (730.01 mm yr-1), LU, and their interaction as predictors – predicted fungal community evenness (E_H) and explained much of its variation (Table 11; Figure 14C). Fungal evenness increased with MAP in native prairies (significantly for ASVs, but only marginally significantly for OTUs), but did not change in post-agricultural sites. There was also evidence for a land-use main effect indicating greater fungal evenness in native remnant prairies than post-agricultural sites (Table 11; Figure 14C). AIC comparisons suggest that replacing MAP with geographic distance or plant community metrics did not result in a superior model for predicting fungal evenness (Table 12).

Community analyses

We used PCoA and PERMANOVA to visualize and test for any community responses to MAP and land-use (Figure 15). In these analyses, we observed no evidence for interaction between MAP and land-use in fungal community composition (PERMANOVA: $F_{1,15} = 0.91$, $R^2 = 0.0557$, $P = 0.647$). However, fungal communities differed compositionally between the arid and mesic habitats (PERMANOVA: $F_{1,15} = 2.44$, $R^2 = 0.150$, $P = 0.001$). Similar to the richness and diversity analyses, there was no evidence for difference in community composition between native prairies remnants and post-agricultural sites (PERMANOVA: $F_{1,15} = 0.92$, $R^2 = 0.056$, $P = 0.637$). In addition to our PERMANOVA analyses, in which we simply divided the precipitation

gradient to arid and mesic habitats, we analyzed the PCoA axis scores for the ASV-inferred fungal communities using multiple linear regressions similar to those we used for community richness and diversity estimators. These models successfully predicted and explained a substantial proportion of the variation in the first but not the second PCoA axis of fungal communities (Table 11; Figure 14D-E). PCoA axis 1 scores linearly decreased with MAP with no evidence for either land-use effects or interaction between the MAP and land-use (Table 11; Figure 14D). In contrast to PCoA axis 1, there was no evidence for MAP, land-use, or interaction for PCoA axis 2 (Table 11; Figure 14E).

To further explore differences in community composition and its responses to environmental and anthropogenic factors, we used constrained ordinations, distance-based redundancy analyses, using main effects of MAP normalized around the mean precipitation (730.01 mm yr-1), MAT, longitude, LU, and first plant PCoA axis to explain variation in fungal communities. These analyses further confirmed that climate variables (MAP and MAT) had a greater influence on fungal community compositions than land-use. However, these environmental variables may be correlated as indicated by the similar direction of environmental vectors arrows in ordination space (Figure 16).

To also assess the heterogeneity in plant and fungal community composition, we tested community dispersion. Neither plant nor fungal communities differed in their dispersion between the arid and mesic habitats ($F_{1,15} = 1.585$, $P = 0.216$) or between native prairie remnants and post-agricultural sites ($F_{1,15} = 0.843$, $P = 0.349$).

Indicator taxon analyses of the 200 most abundant fungal ASVs identified 33 arid and 28 mesic indicator ASVs before FDR correction (Supplemental File 9). Indicators represented Phylum Ascomycota (30 arid and 22 mesic ASVs) and Basidiomycota (3 arid and 5 mesic ASVs). One mesic indicator represented unclassified fungi or could not be assigned beyond Kingdom Fungi. Fifteen arid and eight mesic indicators remained after FDR correction (arid: *Paraphaeosphaeria* sp., *Phaeosphaeria* sp., Phaeosphaeriaceae sp., *Saitozyma paraflava*, *Phaeopoacea* sp., *Cyphellophora* sp, *Blumeria* sp., Ascomycota sp., *Phaeoseptoriella zeae*, Didymellaceae sp., *Neostagonospora* sp., another *Blumeria* sp., *Dinemasporium bambusicola*, *Ascochyta hordei*, and *Alternaria* sp.; mesic: *Epicoccum sorghinum*, Capnodiales sp., *Codinaea* sp., *Phaeopoacea* sp., *Neoascochyta* sp., *Phaeosphaeria* sp., *Dissoconium* sp., *Symmetrospora gracilis*). Many of the most abundant indicators were plant pathogens or other plant-associated

fungi (Supplemental File 9). Among the most abundant fungal indicators for arid sites were two *Blumeria* sp., a member of the order Erysiphales (powdery mildews) which are obligate plant pathogens (Takamatsu 2013); *Phaeoseptoriella zeae*, a foliar pathogen of *Zea mays* (Crous et al., 2019, Tennakoon et al., 2020); and Neostagonospora sp. common pathogens of Carex (Quaedvlieg et al., 2013). Among the most abundant fungal indicators for the mesic sites was *Epicoccum sorghinum*, a common cereal crop pathogen (Oliveira et al., 2018) and *Dissoconium* sp. anamorph (teleomorph Mycosphaerella; Crous et al., 2007), a representative of a genus with many foliar pathogens (Li et al., 2012). Among those that were significant prior to FDR correction was a member of the family Herpotrichiellaceae, with many documented decomposers of plants or fungi (Untereiner and Malloch, 1999). Some mesic indicators that were significant in our OTU analyses before FDR correction such as *Puccinia andropogonis*, a common rust pathogen of the dominant grasses in the Great Plains (Szabo, 2006) and *Phyllozyma linderae* (basidionym *Sporobolus linderae*), a basidiomycetous phyllosphere yeast in the Phylum Pucciniomycotina, whose ecology remains elusive (Wang et al., 2015) were marginally significant in ASV analyses before FDR correction (Supplemental File 9).

Linkages between the plant and fungal communities

Our co-located sampling of plant and fungal communities was designed to permit testing whether the two communities correlate. Our Mantel tests indicated that the Bray-Curtis distance matrices characterizing the community dissimilarities among the plots were highly correlated between the plant and ASV-inferred fungal communities (R^2 = 0. 644, P = 0.001). Additionally, we utilized Procrustes analyses that compare two or more multidimensional shapes by translation, rotation and scaling the ordinations to maximize their superimposition (Figure 17). Corroborating the Mantel tests, these analyses highlighted the strong correlation between the plant and ASV-based fungal two-dimensional PCoA ordinations (R^2 = 0. 768, P = 0.001).

Tables and Figures

Table 11 Multiple linear regression model statistics for fungal Amplicon Sequence Variant (ASV) community diversity, richness evenness and compositional estimates predicted by land use history (LU) and mean annual precipitation (MAP) main effects and their interaction (LU x MAP) with native prairie as reference (0) compared to post-agricultural prairie (1). Statistically significant models and predictors (P<0.05) are bold-faced. Parameter estimate significances are denoted as 'ns' for not significant, '(*)' for

0.05≤P<0.10, '*' for 0.01≤P<0.05, '' for 0.001≤P<0.01, and '***' for P<0.001. Response variables with outliers were analyzed with and without the identified outliers. Models shown here include potential outliers, Table 7 provides model details with outliers removed.**

Response	Model	Predictor	Estimate±SE	|t-value|
ASV Richness (S_{Obs})[7]	$F_{3,12}$ =0.35^{ns}, R^2_{adj}=–0.15	**Intercept**	**1197.63±77.17**	**15.52*****
	AIC=223.15	LU	–46.38±109.13	–0.43^{ns}
		MAP	2.24x10^{-1}±3.69x10^{-1}	0.61^{ns}
		LU x MAP	3.61x10^{-2}±5.21x10^{-1}	0.07^{ns}
ASV Diversity (H')	**$F_{3,12}$ =10.15*, R^2_{adj}=0.647**	**Intercept**	**5.31±9.77x10^{-2}**	**54.32*****
	AIC=9.63	**LU**	**–5.76x10^{-1}±1.38x10^{-1}**	**–4.17****
		MAP	**1.55x10^{-3}±4.67x10^{-4}**	**3.33****
		LU x MAP	**–2.23x10^{-3}±6.59x10^{-4}**	**–3.38****
ASV Evenness (E_H)	**$F_{3,12}$ =6.23*, R^2_{adj}=0.511**	**Intercept**	**7.49x10^{-1}±1.66x10^{-2}**	**45.02*****
	AIC= –47.00	**LU**	**–7.46x10^{-2}±2.35x10^{-2}**	**–3.17****
		MAP	**2.03x10^{-4}±7.95x10^{-5}**	**2.55***
		LU x MAP	**–3.19x10^{-4}±1.12x10^{-4}**	**–2.84***
ASV PCoA Axis 1	**$F_{3,12}$ =48.40***, R^2_{adj}=0.905**	Intercept	2.06x10^{-2}±2.72x10^{-2}	0.76^{ns}
	AIC= –31.32	LU	–4.12x10^{-2}±3.84x10^{-2}	–1.07^{ns}
		MAP	**–1.10x10^{-3}±1.30x10^{-4}**	**–8.50*****
		LU x MAP	–8.42x10^{-6}±1.83x10^{-4}	0.05^{ns}
ASV PCoA Axis 2	$F_{3,12}$ =0.07^{ns}, R^2_{adj}=–0.229	Intercept	5.66x10^{-3}±7.88x10^{-2}	0.07^{ns}
	AIC= 2.75	LU	–1.10x10^{-2}±1.11x10^{-1}	–0.10^{ns}
		MAP	2.68x10^{-5}±3.76x10^{-4}	0.07^{ns}
		LU x MAP	–1.90x10^{-4}±5.31x10^{-4}	–0.36^{ns}

Table 12 Multiple linear regression model adjusted R-squared (R^2_{adj}) and Akaike's Information Criteria (AIC) for fungal community estimators predicted by land use history (LU) and listed predictor main effects and their interaction (LU x predictor) with native prairie as reference (0) compared to post-agricultural site (1). Superior models (ΔAIC > 2; Burnham and Anderson, 2004) are bold faced. If no model was superior, we used the model LU x MAP.

Predictor	ASV richness (S_{obs})	ASV Diversity (H')	ASV Evenness (E_H)
Plant Richness (S_{obs})	R^2_{adj} =–0.05; AIC=221.76	R^2_{adj} =0.47; AIC=16.20	R^2_{adj} =0.32; AIC=–41.80
Plant Diversity (H')	R^2_{adj} =–0.02; AIC=221.18	R^2_{adj} =0.43; AIC=17.16	R^2_{adj} =0.31; AIC=–41.59

[7] Contained a high outlier in LVN_N retained in this analysis

Plant Evenness (E_{var})	R^2_{adj} =–0.19; AIC=223.73	R^2_{adj} =0.28; AIC=20.95	R^2_{adj} =0.20; AIC=–39.11
Plant PCoA Axis 1	R^2_{adj} =–0.18; AIC=223.60	R^2_{adj} =0.51; AIC=14.83	R^2_{adj} =0.36; AIC=–42.72
Plant FQI_{adj}	**R^2_{adj} =0.05; AIC=220.03**	R^2_{adj} =0.52; AIC=14.69	R^2_{adj} =0.45; AIC=–45.16
Longitude (DD)	R^2_{adj} =–0.15; AIC=223.18	R^2_{adj} =0.61; AIC=11.38	R^2_{adj} =0.46; AIC=–45.53
MAP (mm yr^{-1})	R^2_{adj} =–0.15; AIC=223.15	**R^2_{adj}=0.65; AIC= 9.63**	**R^2_{adj}=0.51; AIC=–47.00**

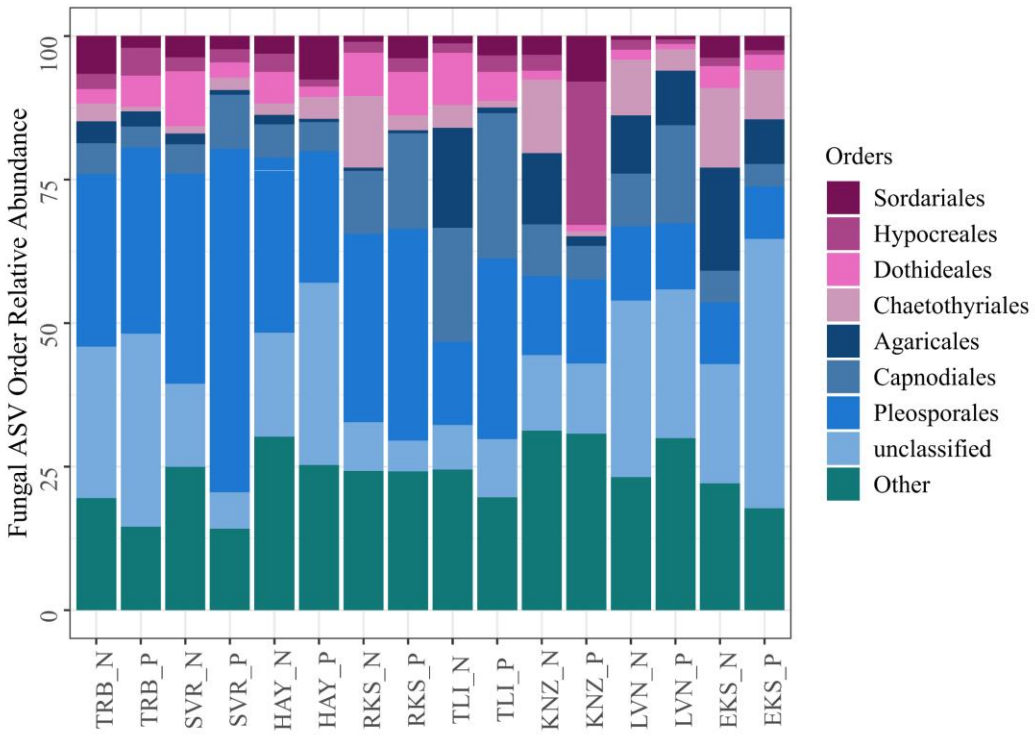

Figure 13 Relative abundance of fungal Amplicon Sequence Variants (ASVs) order assignment. Fungal orders representing less than 2% of the entire dataset are listed as "Other".

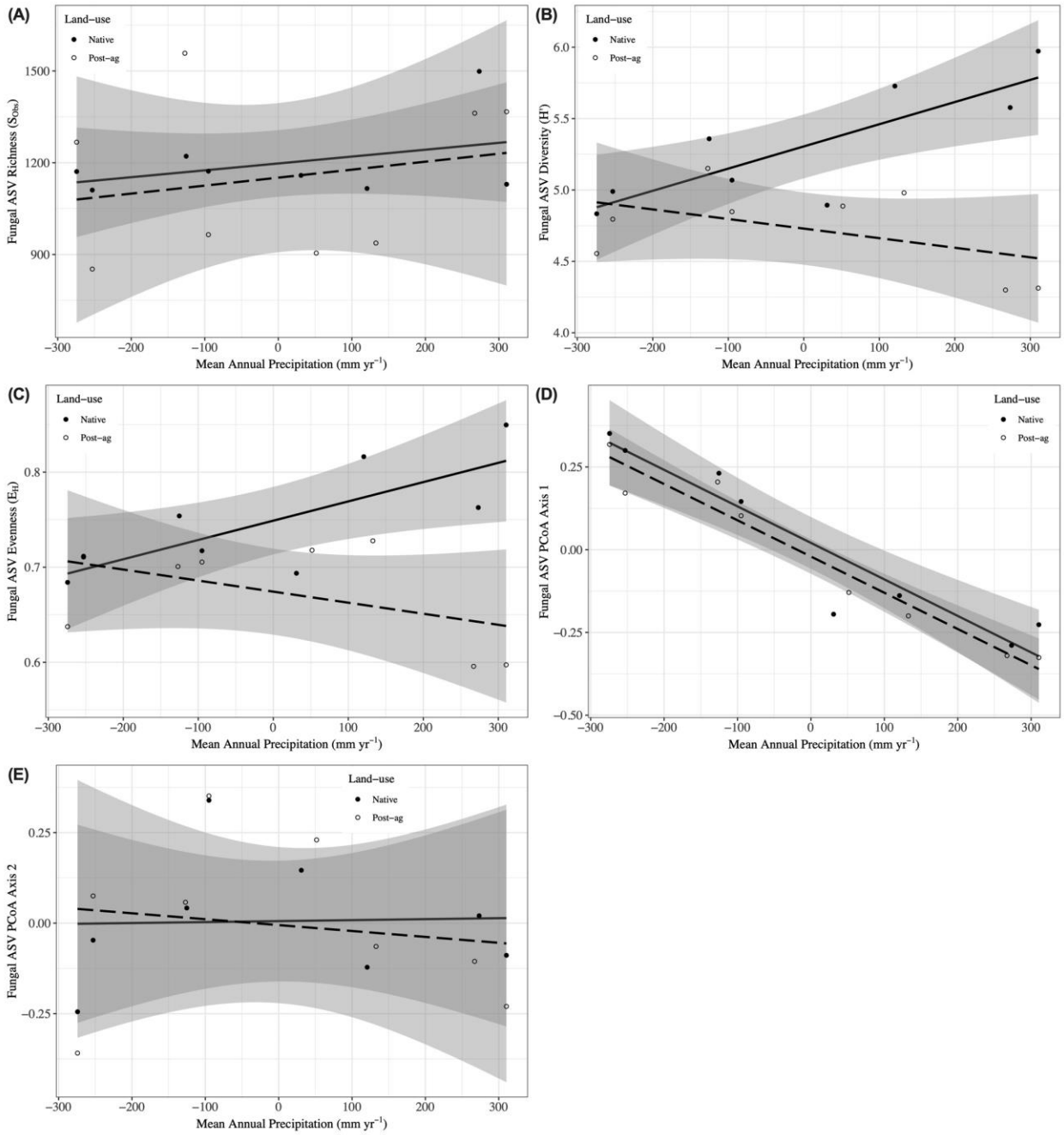

Figure 14 Fungal Amplicon Sequence Variant (ASV) responses to mean annual precipitation (MAP) in native prairie remnants (solid line and filled symbols) and post-agricultural sites (dashed line and open symbols). Models predict observed species richness (S_{Obs}) (A), Shannon diversity (H') (B), evenness (E_H) (C), PCoA Axis 1 scores (D), PCoA Axis 2 scores (E). The shaded areas represent 95% confidence intervals around the model predictions.

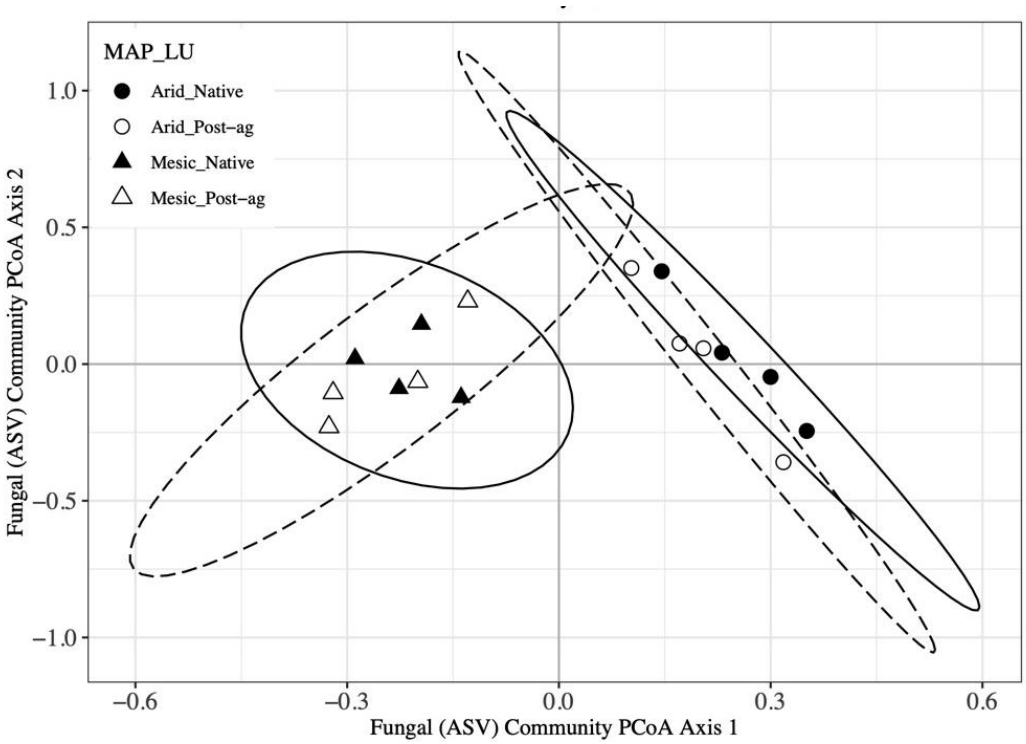

Figure 15 Principal Coordinates Analyses (PCoA) of fungal community composition using Amplicon Sequence Variants (ASVs) in native prairie remnants (solid line and filled symbols) and post-agricultural sites (dashed line and open symbols). Circles indicate the arid end of the precipitation gradient (455.7 – 634.9 mm yr^{-1}), whereas triangles indicate the mesic end (760.9 – 1040.5 mm yr-1). Lines indicate the 95% confidence intervals around PCoA centroid for each group in the PCoA ordination.

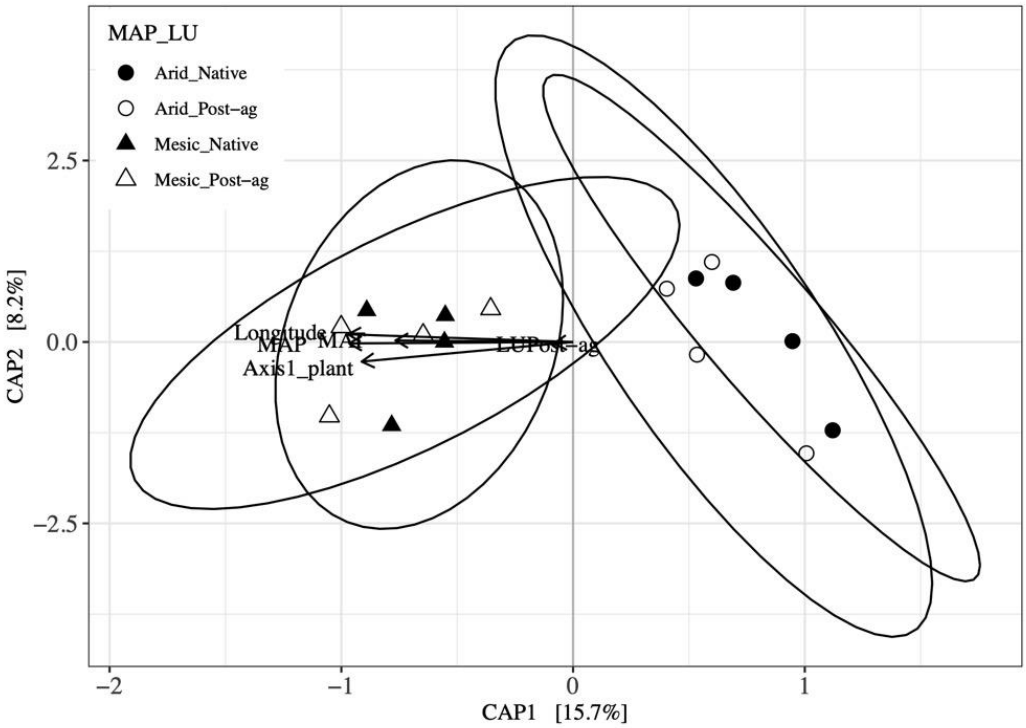

Figure 16 Distance Based Redundancy Analysis of the Bray-Curtis Distance of fungal Amplicon Sequence Variants (ASVs; $F_{5,10} = 1.42$, P = 0.001). Main effects including Longitude (ASV: $F_{1,10} = 1.38$, P = 0.045), Mean Annual Precipitation (MAP) normalized around the mean precipitation (730.01 mm yr^{-1}) (ASV: $F_{1,10} = 1.54$, P = 0.017), Mean Annual Temperature (MAT) (ASV: $F_{1,10} = 1.98$, P = 0.002) and land-use (LU) (ASV: $F_{1,10} = 0.94$, P = 0.595) and first plant PCoA axis (ASV: $F_{1,10} = 1.25$, P = 0.091) were used in the model. Arrows indicate the correlation of environmental variables with community composition.

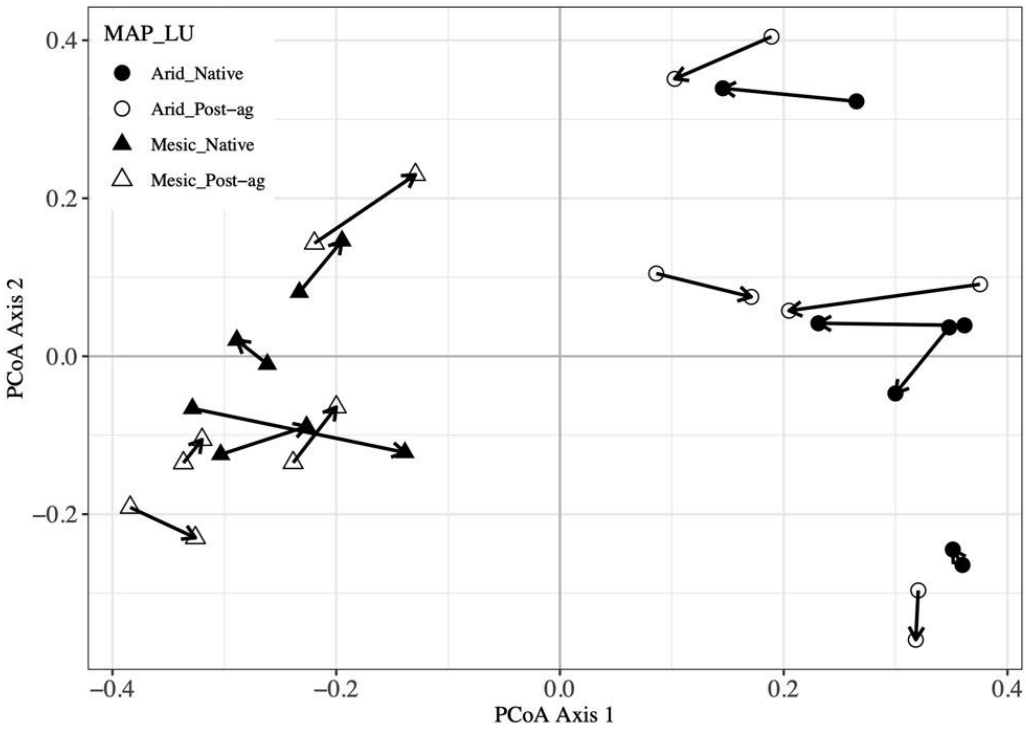

Figure 17 Procrustes analysis of plant community Principal Coordinate Analysis (PCoA) first and second ordination axes compared with Fungal Amplicon Sequence Variant (ASV) PCoA axes. Arrows point from plant community sample to the corresponding fungal community sample within a site.

Appendix C - Journal Permission to Reproduce

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Hannah,
This is definitely acceptable! Do you need a letter attached to an email with my proper signature or
is this email good enough?
Thanks
Erika
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Associate Professor, Aquatic ecology + Biology education
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I am a master's student at Kansas State University and am writng for permission to include in my
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MSc Student, Jumpponen Lab
Kansas State University
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