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Bison and burn timing shape arbuscular mycorrhizal diversity and community composition in tallgrass prairie restorations

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ABSTRACT

Understanding how restoration and management practices of tallgrass prairie ecosystems impact soil microbial communities is vital to mitigating biodiversity loss. However, the impact of management practices on arbuscular mycorrhizal fungal (AMF) communities remains a critical unknown. Both bison and fire are commonly used as management practices to mimic historical conditions. Here, we examine how bison presence and fire timing (spring, fall, and no burning) impact the structure of AMF communities in tallgrass prairies restorations ranging in age from 2 to 35 years. Additionally, these restorations were compared to two remnant prairies and two agricultural fields. Restoration age had little effect on AMF community composition and diversity, and remnant and agricultural units were not distinct from restorations, suggesting that the AMF community will likely be restored in conjunction with the active management of the plant community. However, burning led to ephemeral decreases in AMF evenness and diversity. Additionally, bison presence significantly altered AMF community composition, concurrently dampening otherwise strong positive relationships between common AMF abundances and soil C: N. Overall, active prairie management had a larger effect on AMF communities and diversity than prairie restoration age.

1. Introduction

Prior to European colonization, tallgrass prairies made up ~10 % of the landmass in North America (>65 million ha) (Samson and Knopf, 1994). These prairies have largely been lost due to conversion to agriculture and suppression of fire, resulting in huge losses in plant biodiversity (Samson and Knopf, 1994). To ameliorate this destruction, people are now actively restoring and managing tallgrass prairies by reseeding former agricultural lands with native tallgrass prairie plants and reintroducing fire and large herbivores (Mueller et al., 2021). However, belowground changes in biodiversity have been largely ignored (Markovchick et al., 2023), which may hinder plant-focused restoration efforts (Asmelash et al., 2016; Koziol et al., 2018). In particular, arbuscular mycorrhizal fungi (AMF) form associations with up to 90 % of plants (Begum et al., 2019) and play a pivotal role in plant success and community dynamics (van der Heijden et al., 1998). Given the recent interest in actively restoring mycorrhizal fungal communities to increase plant and fungal biodiversity and enhance ecosystem functioning (Averill et al., 2022; Koziol and Bever, 2017; Koziol et al., 2018), it is imperative to evaluate the extent to which such interventions are necessary.

AMF responses to restoration are highly variable. Soil microbial communities in remnant prairies and agricultural sites are often distinct from restored prairies (Cheeke et al., 2019; Fierer et al., 2013; Vogelsang et al., 2006), and, as such, it is often assumed that active inoculation of plants and soils with AMF spores is needed to recover AMF and plant communities (Koziol et al., 2018). While the inoculation of grassland restorations with native AMF can improve restoration success (Koziol and Bever, 2017, but see Hoeksema et al., 2010), it is likely not feasible for most grassland restorations (Koziol et al., 2018) as most restorations occur at large scales, and individually transplanting plants would quickly become labor-prohibitive. Chronosequence studies following agriculture conversion to tallgrass prairie and forest (Johnson et al., 1991; Van Der Heyde et al., 2018), restoration following gravel pit abandonment (Garcia De Leon et al., 2016), and conversion of mining sites to agriculture (Roy et al., 2017) suggest that AMF communities can passively recover over time. Additionally, AMF spore dispersal is abundant in highly-disturbed environments (Chaudhary et al., 2020), suggesting that AMF communities may have the ability to recover unaided. In contrast, there is also evidence that AMF communities fail to

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recover in forest restorations (Wall et al., 2020) and recover very slowly along chronosequences (Ezeokoli et al., 2020; Roy et al., 2023), which suggest the need for active intervention. Monitoring AMF diversity and community composition post-restoration is vital for understanding how they respond when not actively managed as there does not appear to be a universal restoration trajectory.

In addition to restoration age, management practices, such as bison presence and prescribed fire timing, may also impact AMF diversity and community composition. Bison alter tallgrass prairie plant communities by increasing the proportion of forbs (Elson and Hartnett, 2017), increasing overall plant diversity (McMillan et al., 2019; Ratajczak et al., 2022), and even impacting the local ecohydrology (O'Keefe and Nippert, 2017), all of which may lead to changes in AMF diversity and community composition. Similarly, the seasonality of prescribed fire greatly impacts tallgrass plant communities (Dickson et al., 2019; Novak et al., 2021; Roberton & Rebar 2022; Weir & Derekscasta, 2017), and AMF communities may respond in-kind as they are plant symbionts. Both bison and prescribed fire create a spatially heterogenous landscape wherein the plant community can vary significantly even when not physically far apart (Mueller et al., 2021), potentially increasing AMF diversity. Furthermore, bison presence and prescribed fire have been shown to alter free-living soil microbial communities (Hawkins, 2022; Neupane et al., 2023), and large herbivore presence has been repeatedly shown to alter both the impact and composition of AMF communities in other ecosystems (Allsup et al., 2022; Faghihinia et al., 2022). However, in Mediterranean forests, fire timing has been shown to not impact soil fungal communities (Vázquez-Veloso et al., 2022) as these communities seem to be adapted to frequent fire (Livne-Luzon et al., 2021). Given the well-documented effects of bison presence and prescribed fire timing on tallgrass prairie plant communities, as well as the impacts of fire and large herbivores on other ecosystems, it is critical to evaluate the effects of both management practices on AMF diversity and composition in tallgrass prairie restorations as these remain understudied.

In this study, we evaluated the impacts of tallgrass prairie restoration and management practices on AMF diversity and community composition. Specifically, we assessed the extent to which restoration age, bison presence, and prescribed fire seasonality affect AMF diversity and community composition at Nachusa Grasslands in northern Illinois, USA. Previous studies at Nachusa Grasslands have shown that bison presence and restoration age shape the bacterial communities (Barber et al., 2023). Additionally, management practices have a larger impact than plant community diversity on small mammal, snake, and beetle diversity at Nachusa Grasslands (Guiden et al., 2021). These previous studies suggest that it is important to evaluate the impacts of both restoration age and management practices on AMF communities. We hypothesized that AMF diversity would increase with restoration age, and AMF community composition in older restorations would resemble remnant units more closely than younger restorations due to the later successional stages of the older restorations. We also hypothesized that bison presence would impact AMF community composition and increase AMF diversity due to changes in plant community structure, trampling and waste inputs. Finally, we hypothesized that burn timing would not impact AMF communities, as tallgrass prairies plants are adapted to fire so it follows that their symbionts would be adapted to frequent fire as well. Additionally, the fire is low intensity and should not impact the root biomass of these perennial plants. Given that C:N and pH commonly influence microbial community variability at fine and large scales (Fierer et al., 2007; Lauber et al., 2008) and that previous work found that soil C:N and pH followed similar trends as bacterial diversity (Barber et al., 2023), we also assessed the effects of soil C:N and pH on the abundance of common AMF operational taxonomic units (OTUs) and the extent to which these relationships were mediated by restoration and management practices.

2. Methods

2.1. Study site

Nachusa Grasslands is owned and maintained by The Nature Conservancy and is located in north-central Illinois, USA (41.89 N, 89.31 W). Nachusa Grasslands contains tallgrass prairie restorations that range in age, and bison graze on some, but not all, of the restorations. The 1600 ha preserve is comprised of management units containing native tallgrass prairie restored from land that was previously intensively farmed and two small remnant prairies which have never been farmed. After the last agricultural harvest, units were seeded with dozens of native tallgrass prairie plants. The first restorations were initiated in 1987, and additional restorations were initiated as time and resources allowed. Restorations and remnants are maintained through the removal of invasive species, and prescribed fire is applied approximately every two years either in spring or fall. In 2014, a herd of American bison (*Bison bison*) were introduced to a portion of the nature preserve.

2.2. Experimental design

For this experiment, we sampled 15 restoration units, two remnant (never plowed) prairie units, and two agricultural fields in July from 2016 to 2021 (Fig. 1). The agricultural fields have a continuous crop rotation between corn and soybeans. The restoration units range in age with the oldest established in 1988 and the youngest established in 2021. We sampled five young units (0–10 years old), six middle units (10–15 years old), and four old units (greater than >15 years old). These categories are based on previous work at this site that showed strong separation based on restoration age at these thresholds in terms of both the bacterial and plant communities(Barber et al., 2023; Blackburn et al., 2020). The youngest restorations were started in 2020 and 2021. The 2020 restoration was sampled in 2020, as well as 2021 adding an additional 3 samples. The 2021 restoration was only sampled once, thus contributing only a single sample to the project. All other restorations, remnants, and agricultural fields were sampled all six years, leading to 12 samples from remnants, 12 samples from agriculture, and 81 samples from restored units. Collectively, 36 samples from restored units and remnant prairies were collected in the summer following spring burning, three samples were collected in summer following fall burning, and 45 samples were collected from sites unexposed to fire in the year preceding sampling. Seven of the restored units and one of the remnant units contain bison. Five randomly placed soil subsamples were taken per unit, throughout an approximately a 1 ha area, combined, and homogenized into a single composite soil sample. Samples were collected using a sterilized scoopula to approximately 5 cm soil depth. Plant litter was removed prior to sampling, and sampling directly in a plant's root biomass was avoided, however given the density of roots in tallgrass prairies, some roots were inevitably sampled. Samples were stored on ice and transported back to the lab where they were stored at -80° C until further analysis.

2.3. DNA analysis

DNA was extracted from homogenized soil samples using 0.1–0.2 g of soil with the DNeasy PowerSoil DNA Isolation Kit following manufacturer instructions (Qiagen, Venlo, Netherlands). AMF diversity and composition were assessed using the primer set with Illumina adapters WANDA (5′-CAGCCGCGGTAATTCCAGCT-3′) and ALM2 (5′-GAACCCAAACACTTTGGTTTCC-3′) (Dumbrell et al., 2011). The PCR mix consisted of 0.3 μl of each primer, 12.5 μl of Platinum SuperFi Mastermix (ThermoFisher Waltham, Massachusetts), 8.5 μl of water and 2.5 μl of template DNA. The PCR reaction consisted of an initial denaturation step for two minutes at 98° C, followed by 28 cycles of 98° C for 30 s, 54° C for 30 s, 72° C for 30 s, finishing with a final extension of 72° C for four

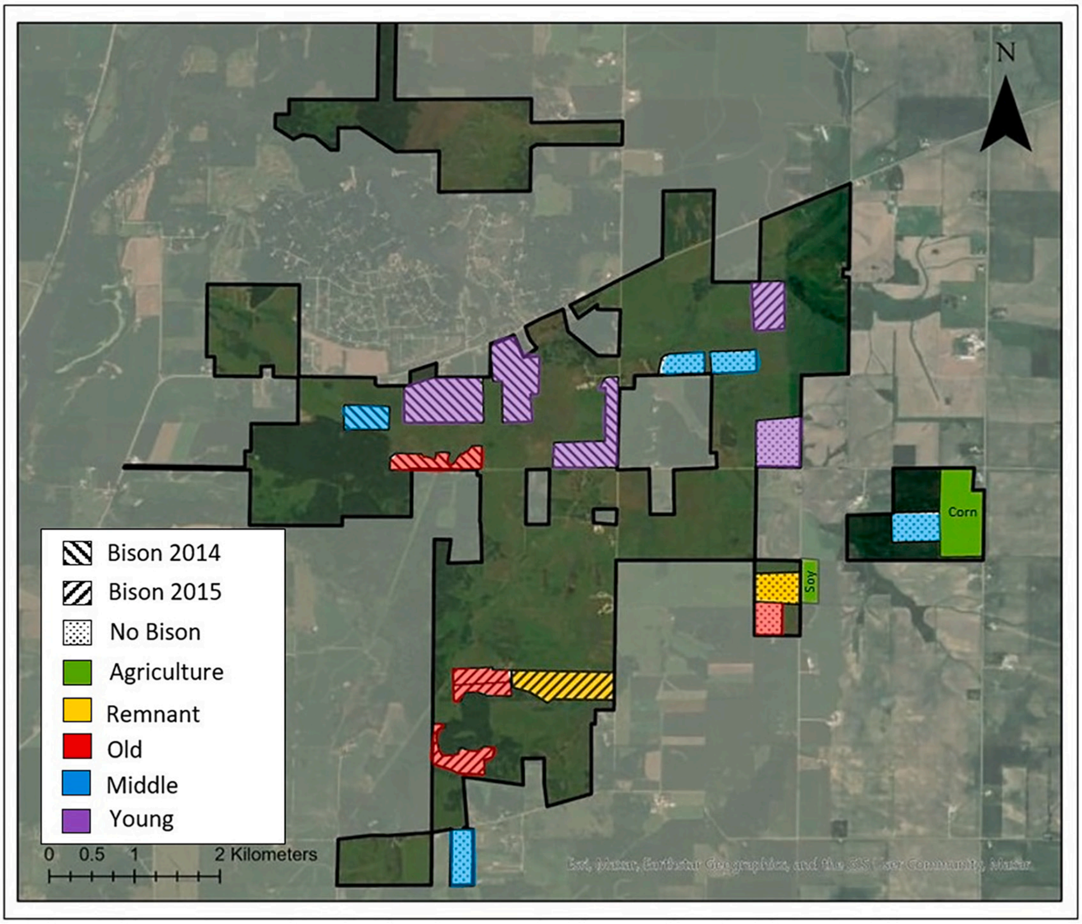

Fig. 1. A map of the Nachusa Grasslands. Remnant units (never plowed) are in light orange and agricultral crops (continuous row cropping rotation of corn and soybeans) are in green. Old restorations are in red, middle aged are in blue and young restorations purple. Units sampled with bison are filled in with the lined pattern whereas units sampled without bison have the dotted pattern.

minutes. After purification samples were barcoded using Fluidigm primers (San Francisco, CA) following Illumina protocols (Illumina, San Diego, CA). Samples were then sent to Rush University (Chicago, IL) and sequenced on an Illumina MiSeq (Illumina, San Diego, CA). Negative controls were included in both the initial PCR and sequencing.

2.4. Bioinformatics

A total of 105 samples (12 total remnant samples, 12 total agricultural samples, and 81 restored samples) were sequenced resulting in 6,526,651 reads with an average of 17,339 reads per sample. Nine samples were removed from downstream analysis due to low sequencing depth, resulting in 96 samples for subsequent analyses. Sequence data was processed using QIIME2 (Bolyen et al., 2019). Primers were removed using cutadapt (Martin, 2011) and then sorted into amplicon sequence variants (ASVs) using DADA2 (Callahan et al., 2016). ASVs were then clustered into 97 % similarity operational taxonomic units (OTUs) using closed reference clustering with maarjAM database (Öpik et al., 2010). OTUs that occurred less than two times in the dataset were removed, resulting in 521 OTUs.

2.5. Statistical analysis

We used a series of mixed linear models to evaluate the effects of restoration age (young, middle, old, or remnant), bison presence (absent or present), and prescribed fire timing in the year prior to sampling (none, fall, spring) on AMF richness, Pielou's evenness, and diversity (Simpson's, Shannon's, Inverse Simpson, and Fisher indices). Agricultural fields were not included in these analyses because these fields were never burned or exposed to bison. We included unit as a random effect to account for our repeated sampling. Because a small subset of OTUs were dominant community members (Fig. 2B, SI Table 4), we split our OTU dataset into a high abundance (common) dataset (29 OTUs) and a low abundance (rare) dataset (492 OTUs). While not all 29 common OTUs were present in all years or at all units, each OTU represented at least 1 % of the total abundance of all OTUs. We ran the model described above on the overall, common, and rare OTU community datasets. Sampling year was not included in this model to examine the management effects that were noticeable despite interannual variation. We also ran two additional sets of models to evaluate the effects of restoration age including agricultural fields (agriculture, young, middle, old, or remnant) or sample collection year (2016, 2017, 2018, 2019, 2020, 2021) on AMF diversity metrics, again including unit as a random effect. We conducted Shapiro-Wilk Normality tests on residuals to assess normality. Results were considered significant if they had *P*-values <0.05. When we found a significant main effect of restoration age, prescribed fire timing, or year, we conducted a Tukey HSD post-hoc test to identify differences among groups.

We evaluated the effects of restoration age, bison presence, fire timing, and sample collection year on AMF community composition using PCoA ordinations, PERMANOVAs, and differential abundance analyses. We conducted PCoA ordinations and PERMANOVAs on all three OTU datasets, both with and without agriculture. All ordinations and PERMANOVAs were conducted on Bray-Curtis distance matrices of

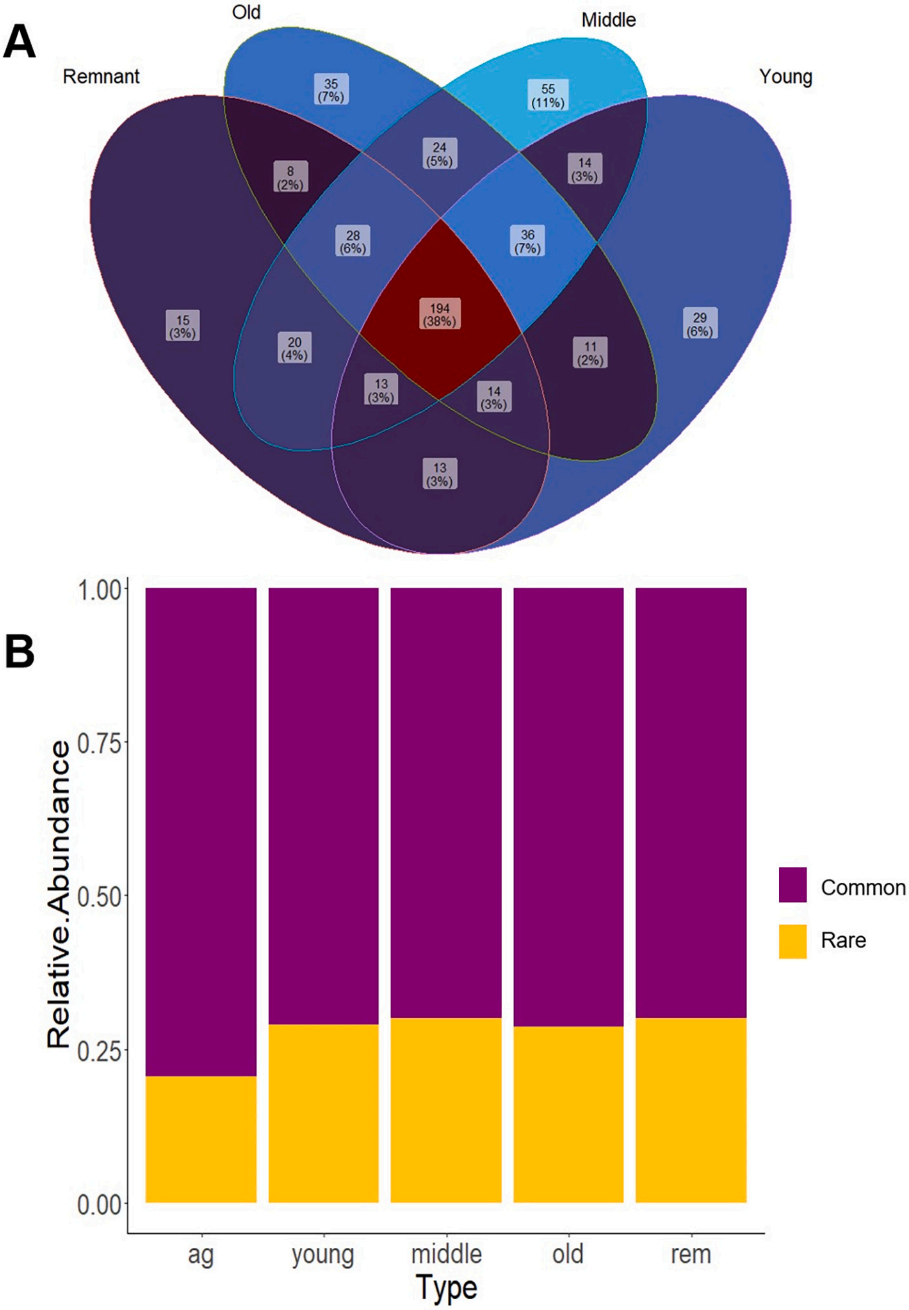

Fig. 2. Venn diagram (A) of the shared OTUs between restoration ages, young (0−10) years since restoration, middle aged (10–15 years since restoration) and old (> 15 years since restoration). Relative abundance (B) of Common (purple) and Rare (yellow) AMF communities by unit type (agriculture, young, middle aged, old and remnant).

each dataset (total, common, and rare). The factors included in the PERMANOVAs were bison presence, burn status, restoration age, and sample collection year and no strata were employed. In order to examine changes in specific OTU abundances, we conducted differential abundance analyses on the common dataset. The effects of each factor (bison presence, prescribed fire timing, unit type, and restoration age) were analyzed separately due to constraints of the DESeq2 model (only one factor can be analyzed at a time).

To assess the extent to which soil C:N and pH drove changes in the abundance of common OTUs, we used the MicrobiomeSeq package (Torondel et al., 2016) to correlate the total relative abundances of common OTUs with environmental variables using a Pearson correlation. Environmental variables included in the analysis were C:N ratio, percent C and N, and soil pH. Soil pH, carbon, and nitrogen were measured previously (Barber et al., 2023). Soil C:N and pH increased after initial restoration, but both C:N and pH began to decline once a restoration passed twenty years of age (Barber et al., 2023).

All analyses were conducted in R version 4.1.3 (R core Team). Mixed linear models were fit using the lmer function the lme4 package (Bates et al., 2015), the lmerTest package was used to derive lmer *P*-values (Kuznetsova et al., 2017),and the lsmeans package was used for post-hoc tests (Lenth, 2016). R^2 values were calculated using the function *r2beta* in the r2glmm package (Edwards et al., 2008). Diversity measures were calculated and ordinations and PERMANOVAs were conducted using the vegan package (Oksanen et al., 2007). Differential abundance analyses were performed on the abundant subset using the DESeq2 package (Love et al., 2014). Figures were created using ggplot2 (ggplot2: Elegant Graphics for Data Analysis, 2016).

3. Results

3.1. *Effects of restoration on AMF diversity*

Restoration age did not impact AMF diversity (Table 1, S1). Restoration age had no effect on overall AMF richness, evenness, or any diversity metric ($F_{3,76} \leq 1.66$, $P \geq 0.18$, $R^2 \geq 0.02$). We similarly found no effects of restoration age on the richness, evenness, or diversity of the common or rare OTUs ($F_{3,76} \leq 1.82$, $P \geq 0.15$). Surprisingly, AMF diversity also did not vary among agricultural units, restored units of varying ages, and remnant units ($F_{4,88} \leq 2.14$, $P \geq 0.08$; $R^2 \geq 0.01$ Fig. 3A, B, C; Table 1).

Bison presence also had little effect on AMF diversity (Table 1). As with restoration age, bison presence had no effect on overall AMF richness, evenness, or any diversity metric ($F_{1,76} \leq 1.93$, $P \geq 0.17$, $R^2 \geq 0.001$; SI Fig. 2G, H, I). While bison presence tended to decrease common OTU richness ($F_{1,76} = 3.80$, $P = 0.05$, $R^2 = 0.001$, Table 1, Fig. 3H), no other metric was impacted by the presence of bison in the common OTU community ($F_{1,76} \leq 2.77$, $P \geq 0.12$, $R^2 \geq 0.001$ Table 1, Fig. 3G, H, I). Similarly, bison presence had no effect on AMF richness, evenness, or any diversity metric for the rare OTU community ($F_{2,76} \leq 1.36$, $P \geq 0.25$ Table 1, SI Fig. 3G, H, I).

In contrast, burn timing influenced the evenness and diversity of AMF (Table 1). For the overall AMF community, burning impacted Peilou's evenness ($F_{2,76} = 3.37$, $P = 0.04$ $R^2 = 0.158$ SI Fig. 2D, E, F); units burned in the fall had lower evenness than units that were unburned in the year prior to sampling or units burned in the spring. Burn timing also altered AMF diversity (Simpson, $F_{76,2} = 0.01$, $P = 0.04$, $R^2 = 0.113$, Table 1), with units burned in the fall prior to sampling having lower Inverse Simpson diversity than unburned units and units burned in the fall tending to have lower diversity than units burned in the spring. For the common subset, units burned in the fall also had lower Simpson's diversity than units that were not burned in the year leading up to sampling ($P = 0.02$, Table 1, Fig. 3F). Burn timing also altered AMF diversity in the common OTUs subset (Shannon and Inverse Simpson, $F_{2,76} \geq 4.74$, $P \leq 0.02$, R^2 0.075, 0.11, Table 1, Fig. 3D, E, F), with units burned in the fall prior to sampling having lower Inverse Simpson diversity than unburned units (Fig. 3D) and units burned in the fall tending to have lower diversity than units burned in the spring (Fig. 3D). It should be noted that the sample size for fall burning was small ($n = 3$) however, we still see trends in lowered diversity for spring burning ($n = 36$). However, burn timing had no effect on any other metric of overall or common AMF diversity ($F_{76,2} \leq 2.27$, $P \geq 0.11$, $R^2 \geq 0.006$ Table 1), and burn timing did not impact evenness or any diversity metric in the rare OTU community ($F_{76,2} \leq 1.40$, $P \geq 0.25$ Table 1, SI Fig. 3D, E, F). Furthermore, burn timing had no effect on the richness of the overall, common, or rare OTU communities ($F_{76,2} \leq 2.31$, $P \geq 0.10$; Table 1).

Year also impacted all diversity metrics ($F \geq 0.45$, $P \leq 0.05$; R^2 0.1–0.263, SI Table 1), with the notable exception of evenness ($F \leq 1.12$, $R^2 \geq 0.035$, $P \geq 0.10$, SI, Table 1) and the Simpson Index for the rare and the entire community ($F \leq 1.12$, $R^2 \geq 0.015$, $P \geq 0.35$, SI Table 1).

3.2. *Effects of restoration on AMF Community composition*

AMF community composition did not vary with restoration age within the overall, common, or rare OTU communities ($F_{76,3} \leq 0.93$, $R^2 \geq 0.02$, $P \geq 0.76$; Table 2). Among restored units, middle-aged restorations had the highest number of OTUs of any restoration age category with 384 OTUs whereas young restorations had the fewest at 323 (Fig. 2A). All of the age categories of restored units had more OTUs than the remnant units, which had a total of 304 OTUs (Fig. 2A). Within the restorations, all ages shared 47 % of OTUs. Old and middle-aged units shared a higher proportion (11 %) of OTUs than middle aged and young units (5 %) or young and old units (5 %). However, the proportion of OTUs shared between restored units and remnant units did not increase with restoration age; restored units of each age class shared 59 % of their total OTUs with remnant units.

Overall, common, and rare AMF community compositions also did not vary among agricultural units, restored units of varying ages, and remnant units ($F_{84,4} \leq 1.10$, $R^2 \geq 0.02$, $P \geq 0.26$; Table 2; SI Fig. 4). Agriculture fields had the lowest number of total OTUs at 245, while restored units had the highest number at 493 (SI Fig. 1). Restored units also had the highest number of unique OTUs, with 161 OTUs only found in restored units. There were 102, OTUs shared between restored units and remnant units. This is much higher than the proportion of OTUs shared between remnants and agriculture (1) or restorations and agriculture (44). Additionally, rare OTUs made up a larger proportion of the

Table 1

Diversity indices (richness, evenness, Simpson, Shannon, Inverse Simpson and Fisher) for restored and remnant units. Age category included four levels: remnant, old, middle and young. Agricultural samples were not included in these analyses as these units were never burned nor had bison present. For diversity indices that include agricultural samples see SI Table 4. Unit was included as a random term. Significant results have been bolded.

All OTUs	Richness	Evenness	Simpson	Shannon	Inverse Simpson	Fisher
Age category	$F_{3,76} = 0.57$, $R^2 =$ 0.047, $P = 0.64$	$F_{3,76} = 0.94$, R2 = 0.036, $P = 0.4$	$F_{3,76} = 1.66$, R2 = 0.136, $P = 0.18$	$F_{3,76} = 0.98$, R2 = 0.079, $P = 0.41$	$F_{3,76} = 1.07$, R2 = 0.057, $P = 0.37$	$F_{3,76} = 0.80$, R2 = 0.063, $P = 0.50$
Bison presence	$F_{1,76} = 0.004$, R2 = 0, $P = 0.95$	$F_{1,76} = 0.07$, R2 = 0.001 $P = 0.79$	$F_{1,76} = 1.93$, R2 = 0.029, $P = 0.17$	$F_{1,76} = 0.05$, R2 = 0.001 $P = 0.82$	$F_{1,76} = 0.43$, R2 = 0.006, $P = 0.52$	$F_{1,76} = 0.04$, R2 = 0.001, $P = 0.84$
Burn timing	$F_{2,76} = 0.41$, 0.001, $P = 0.66$	$F_{2,76} = 3.37$ R2 = 0.158, $\boldsymbol{P = 0.04}$	$F_{2,76} = 0.01$, R2 = 0.113, $\boldsymbol{P = 0.04}$	$F_{2,76} = 2.48$, R2 = 0.1, $P = 0.09$	$F_{2,76} = 1.31$, R2 = 0.062 $P = 0.28$	$F_{2,76} = 0.39$, R2 = 0.016, $P = 0.68$
Common OTUs						
Age category	$F_{3,76} = 1.82$, R2 = 0.092, $P = 0.15$	$F_{3,76} = 0.30$, R2 = 0.022, P = 0.82	$F_{3,76} = 1.99$, R2 = 0.177, $P = 0.18$	$F_{3,76} = 1.59$, R2 = 0.122, $P = 0.2$	$F_{3,76} = 1.28$, R2 = 0.104, $P = 0.29$	$F_{3,76} = 1.12$, R2 = 0.072, $P = 0.35$
Bison presence	$F_{1,76} = 3.8$, R2 = 0.055, $\boldsymbol{P = 0.05}$	$F_{1,76} = 0.18$, R2 = 0.003 $P = 0.67$	$F_{1,76} = 2.77$, R2 = 0.044, $P = 0.12$	$F_{1,76} = 2.29$, R2 = 0.034, $P = 0.13$	$F_{1,76} = 1.27$, R2 = 0.19, $P = 0.26$	$F_{1,76} = 2$, R2 = 0.03, $P = 0.16$
Burn timing	$F_{2,76} = 2.3$, R2 = 0.012, $P = 0.11$	$F_{2,76} = 0.\ 5$, R2 = 0.006, $P = 0.61$	$F_{2,76} = 4.74$, R2 = 0.121, $P = 0.11$	$F_{2,76} = 4.75$, R2 = 0.11, $\boldsymbol{P = 0.01}$	$F_{2,76} = 3.99$, R2 = 0.075, $\boldsymbol{P = 0.02}$	$F_{2,76} = 2.43$, R2 = 0.045, $P = 0.09$
Rare OTUs						
Age category	$F_{3,76} = 0.35$, R2 = 0.051 $P = 0.79$	$F_{3,76} = 1.49$, R2 = 0.156, $P = 0.27$	$F_{3,76} = 1.63$, R2 = 0.134, $P = 0.19$	$F_{3,76} = 1.68$, R2 = 0.171, $P = 0.18$	$F_{3,76} = 1.78$, R2 = 0.107, $P = 0.16$	$F_{3,76} = 0.86$, R2 = 0.064, $P = 0.47$
Bison presence	$F_{1,76} = 0.08$, R2 = 0.001, $P = 0.78$	$F_{1,76} = 0.73$, R2 = 0.023, $P = 0.41$	$F_{1,76} = 1.36$, R2 = 0.02 $P = 0.25$	$F_{1,76} = 0.47$, R2 = 0.007 $P = 0.5$	$F_{1,76} = 0.23$, R2 = 0.004, $P = 0.63$	$F_{1,76} = 0.07$, R2 = 0.001, P = 0.79
Burn timing	$F_{2,76} = 0.15$, R2 = 0.001, $P = 0.86$	$F_{2,76} = 0.02$, R2 = 0.001, $P = 0.98$	$F_{2,76} = 0.81$, R2 = 0.046, $P = 0.45$	$F_{2,76} = 0.95$, R2 = 0.045, $P = 0.39$	$F_{2,76} = 1.4$, R2 = 0.051, $P = 0.25$	$F_{2,76} = 0.22$, R2 = 0.009, $P = 0.8$

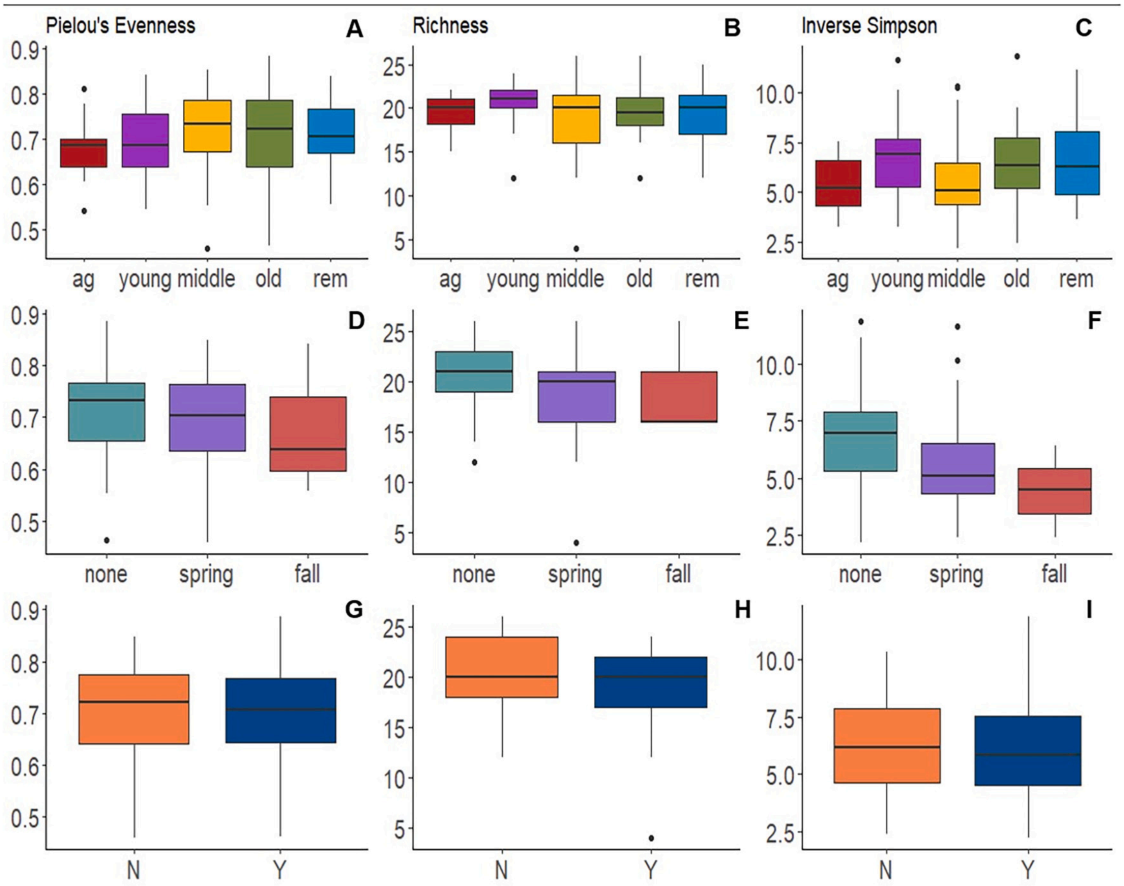

Fig. 3. Pielou's evenness for common OTUs (A, D, G), Richness (B, E, H) and Inverse Simpson (C, F, I) by restoration age (A, B, C), burn timing (D, E, F) and bison presence (yes or no, G, H, I). Fall burning had the fewest samples ($n = 3$), followed by spring burning ($n = 36$) and no burning having the most samples ($n = 45$).

AMF community in restored and remnant units than in agricultural fields (Fig. 3B).

Conversely, overall AMF community composition was affected by bison presence ($F_{72,1} = 1.91$, $R^2 = 0.02$, $P = 0.036$; Table 2, SI Fig. 6). This variation in community composition was driven by bison impacts on common OTUs ($F = 1.92$, $R^2 = 0.02$, $P = 0.03$; Table 2, SI Fig. 6B); the rare OTU community did not respond to bison presence (Table 2, SI Fig. 6C). Bison presence significantly increased the abundance of six common OTUs by 1.3–7.66 log2 fold change ($P \leq 0.01$; Table SI 2; Fig. SI 8). The presence of bison increased the total number of OTUs from 405 in units without bison to 439 in units with bison. However, the overall proportion of shared OTUs between units with and without bison was high at 66 %.

Much like restoration age, burn timing did not impact overall, common, or rare AMF community compositions ($F_{72,2} \leq 1.09$, $P \geq 0.27$; SI Table 2, SI Fig. 5). Fall burning had the lowest number of OTUs at 153 OTUs, units that were not burned that year had the highest number of OTUs (408 OTUs), and units burned in the spring had the highest number of OTUs at 422 with 84 unique OTUs. Nonetheless, fall burned units and unburned units shared 66 % of their total OTUs.

While community composition did not vary among units differing in restoration age or burn timing, common OTU differential abundances were impacted by restoration age and burn timing (SI Table 2;SI Fig. 7). Middle aged units had significantly lower abundances of six common OTUs ($P \leq 0.01$; log2 fold change ≥ -2.13, SI Table 2). Old restoration units had increased abundances of two common OTUs ($P < 0.01$; log2 fold change ≥ -2.38, SI Table 2). No common OTUs were increased in young restorations nor remnants. Fall burning significantly decreased the abundances of five common OTUs ($P \leq 0.04$; log2 fold change ≥ -3.48, SI Table 2). Similarly, if a unit was not burned in the year prior to sampling, that increased of the abundances of three common OTUs ($P \leq 0.02$; Table S2; log2 fold change ≥ 5.28, SI Table 2). Surprisingly, spring burns did not impact the abundances of any common OTUs.

Finally, AMF community composition across all OTU datasets varied by year sampled ($R^2 = 0.13$, $P = 0.001$; Table 2) with 2018 and 2020 consistently separating from one another.

3.3. *Influencers of AMF Community composition*

Burning and bison both significantly lowered the soil pH by less than a tenth of a point (SI Fig. 9) but neither bison nor burning impacted the soil C:N ratio at these sites (SI Fig. 10). The entire community composition was not impacted by soil C:N or pH ($F_{71,1} \leq 0.67$, $R^2 = 0.01$, $P \geq 0.814$; Table S4). Additionally, neither the common nor the rare community compositions were impacted by the slight changes in C:N ratios or pH ($F_{71,1} \leq 1.01$, $R^2 = 0.01$, $P \geq 0.41$; SI Table 3). However, the abundances of nine of the common OTUs were positively correlated with soil C:N in the units where bison were not present ($r \leq 0.36$, $P \geq 0.01$; Fig. 4). When bison were present, there was a negative correlation between the few common OTUs that significantly differed in abundance ($r \leq -0.32$, $P \geq 0.01$; Fig. 4). These patterns were not reflected by correlations between common OTU abundances and soil C or N concentrations (Fig. 4). Additionally, the abundance of *Paraglomus*_sp. _VTX00281 was positively correlated with pH when bison were absent ($r = 0.47$, $p = 0.002$; Fig. 4) whereas *Claroideoglomus*_sp._VTX00057 was negatively correlated with soil pH when bison were present ($r = -0.40$, $p = 0.005$; Fig. 4). The abundances of four common OTUs were positively correlated with soil pH in old restorations ($r \leq 0.42$, $P \geq 0.006$) while two different common OTUs were positively correlated with soil pH in young restorations ($r \leq 0.43$, $P \geq 0.03$).

Table 2
PERMANOVA table of all sample types (agriculture, restorations, remnants) by Age Category (agriculture, remnants and for restored units: old, middle and young). For PERMANOVAs that included bison presence and burn timing, agricultural samples were removed as these units were never burned nor had bison present.

With agriculture					
All OTUs	Degrees of freedom	Sums of squares	R2	F value	P
Age category	4	1.11	0.04	0.98	0.50
Year	5	3.39	0.13	2.72	**0.00**
Residual	84	23.73	0.83		
Common abundance	Degrees of freedom	Sums of squares	R2	F value	P
Age category	4	1.12	0.04	1.10	0.26
Year	5	3.85	0.15	3.02	**0.00**
Residual	84	21.38	0.81		
Rare abundance	Degrees of freedom	Sums of squares	R2	F value	P
Age category	4	1.29	0.04	0.91	0.77
Year	5	3.79	0.11	2.14	**0.00**
Residual	84	29.75	0.85		

No agriculture					
All OTUs	Degrees of freedom	Sums of squares	R2	F value	P
Bison	1	0.5339	0.01995	1.7363	0.036
Burn	2	1.1744	0.04389	1.2731	0.101
Age category	3	0.7484	0.02797	0.8113	0.852
Year	5	0.6241	0.02332	2.0295	**0.013**
Residual	72	23.6775	0.88487		
Common abundance	Degrees of freedom	Sums of squares	R2	F value	P
Bison	1	0.495	0.02076	1.9195	**0.03**
Burn	2	0.5901	0.02475	1.1442	0.276
Age category	3	0.6434	0.02699	0.9317	0.763
Year	5	3.5463	0.14874	2.7504	**0.001**
Residual	72	18.567	0.77876		
Rare abundance	Degrees of freedom	Sums of squares	R2	F value	P
Bison	1	0.4818	0.01543	1.3481	0.096
Burn	2	0.7802	0.02498	1.0914	0.273
Age category	3	0.795	0.02546	0.7414	0.981
Year	5	3.4338	0.10997	1.9214	**0.001**
Residual	72	25.7349	0.82416		

4. Discussion

Establishing native plant communities on former agricultural lands and applying prescribed fire are common practices in tallgrass prairie restoration. Additionally, some restoration areas have reintroduced bison as a way to mimic historic conditions and improve plant recovery (Zak et al., 2022; Truett et al., 2001). Here, we evaluated the effects of these plant-focused restoration and management practices on AMF diversity and communities. We hypothesized that AMF community composition and diversity would change with restoration age and respond to bison presence but not be impacted by prescribed fire timing. While restoration age had little effect on AMF diversity or community composition, bison presence altered AMF community composition and the relative abundances of common OTUs, concurrently dampening the relationship between the abundances of common AMF OTUs and soil C:N. Additionally, prescribed fire decreased AMF evenness, and diversity. However, diversity quickly rebounded in years without prescribed fire. Finally, all responses were driven by shifts in the common AMF community, suggesting that a small subset of AMF OTUs drive ecosystem-wide responses and recover quickly. Though more-common tallgrass prairie restoration and management practices may minimally impact AMF diversity and community composition, bison presence was found to have the strongest effect on AMF communities in our study system.

4.1. Bison altered AMF community composition

Previous studies from Nachusa Grasslands and other tallgrass prairies have shown that bison presence alters bacterial, saprotrophic fungal, and AMF spore community composition (Barber et al., 2023; Hopkins and Bennett, 2023; Neupane et al., 2023). Our results complement these findings; in this study we found that bison presence impacted AMF community composition and OTU abundances, likely via bison-induced changes in the relationship between AMF and soil C:N. Bison likely alter AMF communities in two ways: by acting as a spore dispersal vector and by altering the structure of the plant communities. Bison may physically disperse AMF through their dung and by wallowing. AMF spores have been found in bison dung in Yellowstone National Park, suggesting that bison dung can act as a dispersal mechanism for AMF (Lekberg et al., 2011; Murray et al., 2010;Hawkins, 2022; Lekberg et al., 2011; Murray et al., 2010). In our study, the abundance of *Claroideoglomus* sp. VTX00056, an aerial spore disperser (Chaudhary et al., 2020), was depauperate in bison units, which could suggest that bison enhance the movement and establishment of non-aerial spores. Bison may also alter AMF communities via changes in the plant community, which in turn could alter the soil C:N ratio. Previous work at Nachusa Grasslands has shown that bison presence increased variability in plant community composition (Blackburn et al., 2020), and bison presence at other sites increased the proportion of forbs (Elson and Hartnett, 2017) and altered plant community richness (Collins and Calabrese, 2012; Manning et al., 2017; McMillan et al., 2019; Winter et al., 2015). Additionally, grazing has been shown to later the C and N allocation to belowground plant biomass(Schuman et al., 1999), which in turn could alter the AMF found in units with bison. Given that AMF are key symbionts of plants, any change in the plant community is likely coupled with changes in the AMF community (Chen et al., 2023). As such, some combination of bison-driven changes in AMF dispersal patterns and plant community shifts are likely driving our observed shifts in AMF community composition. Future work should look more closely at the relationship between changes in the plant community coupled with changes in the AMF community.

In addition to documenting bison-induced shifts in AMF community composition, our study highlights a potential driver of this pattern: bison alter the relationships between common AMF abundances and soil C:N. In units without bison, 8 of the 29 common OTUs were positively correlated with soil C:N while only 3 of the common OTUs were negatively correlated with soil C:N in the presence of bison. Changes in the plant community and bison defecation patterns could drive this dynamic. Bison decrease grass dominance in tallgrass prairies, increasing forb relative abundance (Z Ratajczak et al., 2022). Forbs tend to have lower tissue C:N than grasses, which may drive our observe shifts in AMF relationships with soil C:N. While the AMF community composition was not significantly impacted by C:N changes, it could be that the small changes in the stoichiometric ratio driven by bison presence is enough to alter relationships between soil C:N and the abundances of certain common OTUs. Similarly, any bison-induced changes in other nutrients could also shift relationships between AMF abundances and C:N; for instance, bison waste is rich in phosphorus and may impact the relationship between AMF communities and soil C:N. Regardless of mechanisms, bison alter the dynamic between common AMF abundances and soil C:N. AMF communities are highly variable and ecosystem-dependent under grazing (Barto and Rillig, 2010; van der Heyde et al., 2017, 2019). As such, future studies should examine the interplay between bison presence, AMF communities, and soil nutrient fluxes in depth to disentangle these relationships.

4.2. Fall prescribed fire induced short-term decreases in AMF evenness

As plant communities in tallgrass prairies are adapted to frequent fire regardless of restoration age (Blackburn et al., 2020; Manning et al., 2017; Rowland-Schaefer et al., 2022), we hypothesized that AMF

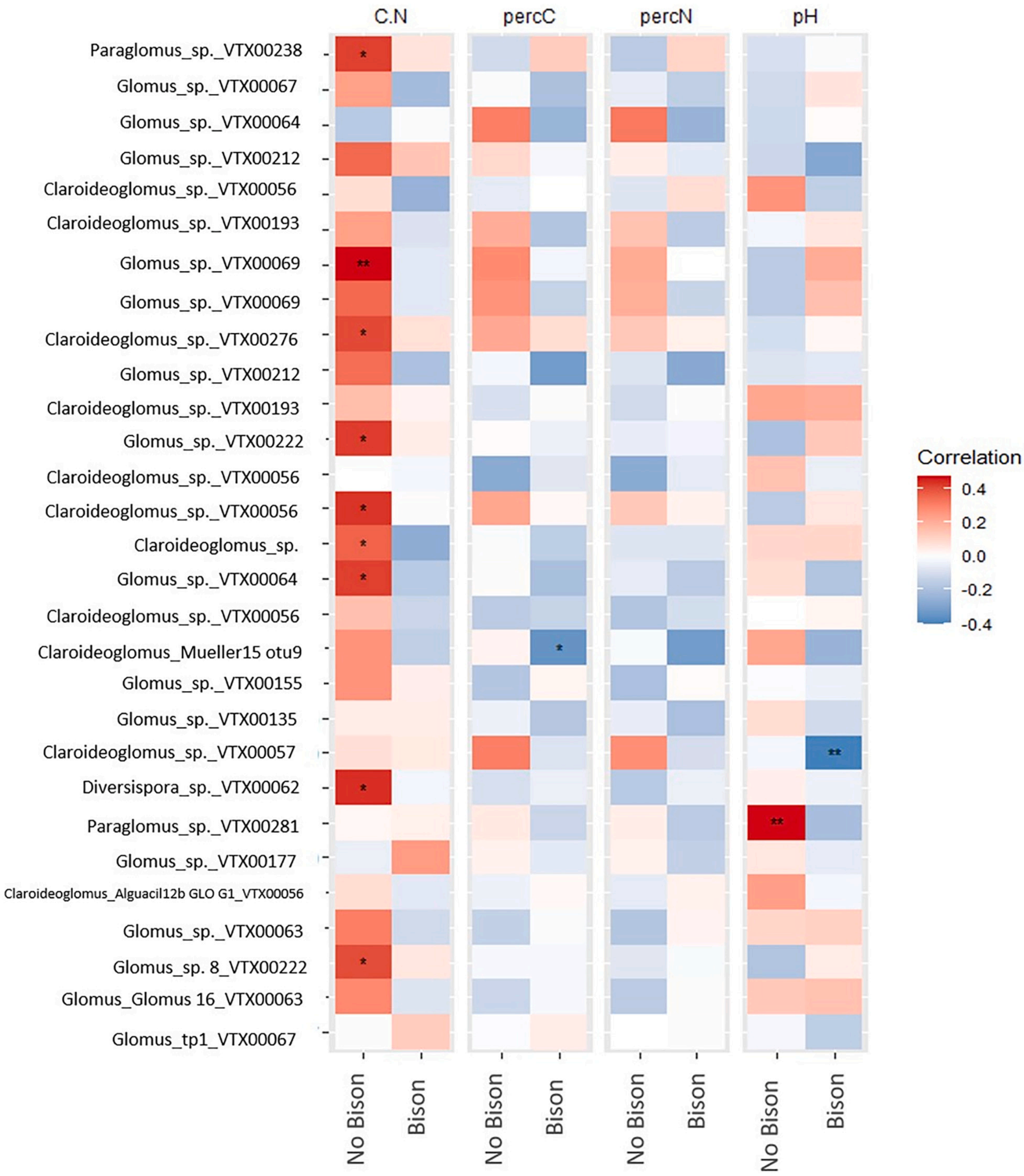

Fig. 4. The correlation (Pearson) between the abundance of Common OTUs and environmental variables (soil C:N, percent soil carbon, percent soil nitrogen and soil pH) by Bison access. Asterisks mark significant correlations ($p > 0.05$) of the abundance of the OTU with environmental variables.

communities would also exhibit muted responses to prescribed fire timing. In support of our hypothesis, prescribed fire timing did not affect AMF community composition. While units burned in the fall had lower AMF evenness with subsequent lower AMF diversity than units that were unburned in the year prior to sampling, our results suggest that decreases in evenness were ephemeral as burn timing in a given unit changed across sampling years. These evenness and diversity effects largely manifested in the common AMF community, and fall burning increased the relative abundance of 5 common OTUs, likely driving down evenness. In an examination of AMF spore communities at Nachusa Grasslands, prescribed fire increased the proportion of darker AMF spore types at Nachusa Grasslands, an adaptation to more open soil and higher ultraviolet stress after fire (Hopkins and Bennett, 2023). This effect faded when a unit was unburned. Both our study and the Hopkins and Bennett (2023) suggest that while prescribed fire does lower AMF diversity these impacts are transitory, as evenness and diversity recovery in the following year as seen in the unburned units. However, it should be noted that our study had limited fall burning samples ($n = 3$) but spring burning ($n = 36$) also decreases diversity. As long-term spring or fall burning can create unique plant communities (Novak et al., 2021), the effects of consistent spring or fall prescribed fire on AMF communities and diversity merits further study.

4.3. Restoration age did not impact AMF diversity or community composition

As AMF are prolific dispersers (Chaudhary et al., 2020), we hypothesized that AMF communities would increase in diversity and exhibit shifts in community composition as restoration age increased. In contrast with our expectations, we found no effects of restoration age on AMF diversity or community composition. Our results are also contrary to previous work at Nachusa Grasslands, which found that the plant community (Blackburn et al., 2020), the bacterial communities (Barber et al., 2023), and the reproducing AMF population (Hopkins and Bennett, 2023) shift with restoration age. Interestingly though, Barber et al. (2023) did find that middle aged restorations had the highest bacterial diversity which was similar to our finding that, while not significantly different, middle aged restorations had the highest number of AMF OTUs. While Hopkins and Bennett (2023) did find a significant effect of restoration age on the spore community, this study only represented the AMF spores, or the reproductive parts, of the AMF community, which likely does not respond in the same way as the community as a whole. Furthermore, previous work on AMF succession in tallgrass prairies found that AMF communities rapidly recovered one year following restoration and newer restorations did not vary from older restorations, which we could be occurring at our study site as well. Limited AMF responses to restoration age may have also been driven by the lasting effects of planting year conditions (Groves et al., 2020) and high temporal variability in AMF communities and diversity, both of which merit further study. Our results suggest that restored prairies will exhibit limited changes in AMF community composition or diversity over the course of succession.

Surprisingly, we also detected no differences in AMF diversity or community composition among remnant units, restored units, and agricultural fields. This result conflicts with a previous study showing that remnant and disturbed prairies have distinct AMF communities and that abundant OTUs in Eastern tallgrass prairie remnants, like those at Nachusa Grasslands, are particularly sensitive to disturbance (House & Bever, 2018). Our unexpected results are likely the result of our unbalanced experimental design and the low number of abundant OTUs. We sampled only two remnant units and two agricultural fields while 17 restored units sampled. While restored and remnant units had substantial overlap in OTUs, remnants and agricultural fields had nearly no overlap in OTUs, and restored units and agricultural fields shared only 8 % of their OTUs. Additionally, agricultural fields contained a higher proportion of common OTUs than restored or remnant sites. The low number of abundant OTUs and the unbalanced nature of units sampled increases the chance of type II errors or failing to detect significance (Banerjee et al., 2009). The lack of response seen could be driven by the low overall species richness seen in the AMF community compared to other microbial and plant communities (Barber et al., 2023), because as the species number increases, so does the likelihood of finding statistically significant differences (Type I error). Additionally, neither the ITS nor the LSU of the AMF ribosome are universal markers, and available reference databases for taxonomic identification of AMF remain limited (Delavaux et al., 2021, 2022), both of which could bias our results. Finally, the lack of differences could be caused by N deposition from neighboring agricultural fields as N deposition has been shown to alter AMF community composition (Treseder et al., 2018). Despite the lack of clear differences among agricultural field, restored units, and remnants, our work suggests that AMF communities will recover quickly following agriculture but remain somewhat distinct from remnants.

5. Conclusions

Management practices played a larger role in shaping AMF community composition and diversity than restoration age. Restoration age reflects the effects of unaided succession while both bison grazing and prescribed fire are ongoing disturbances. As such, regardless of age, restorations managed in a similar fashion harbored similar AMF communities, while both bison presence and fire timing altered AMF community composition and diversity, with the bison presence having a larger impact. Prescribed fire had an ephemeral “pulse” effect, likely selecting for a few fire-tolerant AMF species that were most abundant before less fire-tolerant AMF populations rebounded. In contrast, bison presence represents a “push” disturbance creating alternative plant communities, dispersal pathways, and shifting plant-AMF-soil relationships, potentially with long-term impacts on AMF community structure and functioning. Our findings complement a previous study at Nachusa Grasslands that found that the effects of management strategies on animal communities were six times stronger on average than the effects of plant biodiversity (Guiden et al., 2021) and help to fill the knowledge gap of how AMF communities respond to tallgrass prairie restoration. At a landscape-level, AMF management does not appear to be necessary meaning that the AMF communities will be restored in conjunction with the plant community. However, if a land manager is interested in a particular rare or conservative plant, previous work suggests that these plants may benefit from AMF inoculation (Koziol et al., 2022),. Future work should compare Nachusa Grasslands with similar sites to examine how the intensity of presence and the frequency of fire impact AMF communities. Additionally, future work should examine more closely how both bison presence and fire impact soil chemistry and function in tallgrass prairie restorations.

CRediT authorship contribution statement

Jennifer K. Bell: Writing – original draft, Visualization, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Wesley D. Swingley:** Writing – review & editing, Conceptualization. **Meghan G. Midgley:** Writing – review & editing, Supervision.

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Declaration of competing interest

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2025.105895.

Data availability

Sequencing data is available at the National Center for Biotechnology Information (NCBI) at PRJNA986176. Code is available at github.com/jbell364. All other data is available upon request.

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