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Land management drives dynamic changes to microbial function through edaphic factors and soil biota

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ABSTRACT

Land management for conservation alters the abiotic and biotic components that underly belowground ecosystem health and function. We know that prescribed burning and grazing influence soil characteristics, nutrients, and biota individually, but rarely have these management effects been explored holistically, affecting an interacting belowground system. Since most belowground functions (e.g., nutrient cycling) arise from feedbacks among many soil factors, a better understanding of system-level responses to distinct management practices, rather than individual component responses, can help us better predict these ecosystem functions. In a late successional tallgrass prairie ecosystem, we contrasted how prescribed fire and mowing altered nutrient cycles through changes to the abiotic soil environment, microbial community structure, and microbial enzyme functions. Individual soil factors responded rapidly to both fire and mowing, and remained different from pre-treatment values. However, as a system, many relationships among soil factors that were present before management and lost directly after management, returned 1 month after management. This shows the system-level resilience to management supported by the long evolutionary history between grasslands, fire, and grazing, and illustrates the importance of understanding management effects from a holistic perspective. Since global disturbance regimes and anthropological influence are predicted to change in the future, understanding how belowground components respond to change as a system can help land managers and ecologists alike conserve endangered ecosystems.

1. Introduction

Human conservation activities (e.g., land management) alter the abiotic and biotic components that underly ecosystem health and function. Despite the importance of land management to ecosystem health, there is a dearth of knowledge on how management influences ecosystem dynamics through belowground pathways (Heneghan et al., 2008). This gap is concerning given that interactions between nutrients, soil characteristics, and soil biota (e.g., bacteria and fungi) influence entire ecosystems (Graham et al., 2016; Otwell et al., 2018). Considerable work has established management effects (e.g., tree harvesting, prescribed fire, and grazing/mowing) on individual belowground components and processes including soil characteristics (Burke et al., 1997), microbial communities (Bardgett and van der Putten, 2014; Kivlin et al., 2020), and nutrient cycles (Cole et al., 2021). It is less clear, however, whether management effects on individual soil components influence the relationships between these components (i.e., belowground systems; Heneghan et al., 2008; Eisenhauer et al., 2015; Otwell et al., 2018; Crowther et al., 2019; but see Roy and Bagchi, 2022). The system interactions give rise to belowground functions, like nutrient cycling and plant-microbe interactions, which underly the health and stability of ecosystems. In light of increasing anthropological effects on ecosystems (Liu and Wimberly, 2016; Balch et al., 2017; Riggio et al., 2020), it is important that we understand system-level responses to disturbance in order to preserve ecosystem health.

Approximately 40% of Earth's terrestrial ecosystems are maintained by frequent (often human managed) disturbances like fire and grazing (Archibald et al., 2018). Human-managed disturbances can maintain ecosystem productivity (Walker, 1999), prevent wildfires (Roos et al., 2020), and preserve biodiversity (Whelan, 1995), including through

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their effects on belowground components such as soil microbes, nutrients (e.g., N and P; Raison, 1979; Butler et al., 2018; Yuan et al., 2019; He et al., 2020), and soil characteristics (e.g., C, pH, and moisture; Certini, 2005; McSherry and Ritchie, 2013; Alcañiz et al., 2018). For example, the long evolutionary history of grazing and fire in grassland ecosystems shows a tight relationship between modern management and soil components (Ford, 2009). This relationship makes these ecosystems ideal for studying land management effects on soil systems and ecosystem health. Both land managers and ecologists are particularly interested in soil nutrient cycling responses to recurrent disturbance because they influence post-disturbance ecosystem dynamics like primary production and recovery time. Disturbance type and intensity modify soil nutrient cycles (Raison, 1979; Butler et al., 2018), both directly through alterations to nutrient pools and fluxes, and indirectly through changes to microbial communities and soil properties that drive nutrient cycles (Rumpel et al., 2015). For example, fire drives flushes (or volatilization at higher severities) of nutrients through the combustion of plant matter, while grazing intensity can determine plant allocation of carbon and nitrogen into or away from rhizosphere microbes. Understanding the relative strength of these pathways on nutrient cycling and how management alters these paths is an important first step in understanding disturbance effects on ecosystem health through belowground processes.

Soil nutrient cycles are complex systems formed from interactions between edaphic properties, soil biota, and biotic functions like extracellular enzyme production. Each is known to independently respond to fire and grazing (or mowing) (Johnson and Curtis, 2001; Certini, 2005; Knelman et al., 2017; Butler et al., 2018; Chuan et al., 2020; He et al., 2020), and through these effects, management practices are thought to shape the soil environment. Despite knowing that these systems depend on one another, we do not know whether management alters the underlying relationships among these properties. For example, soil fungi produce extracellular enzymes that breakdown recalcitrant forms of carbon, nitrogen, and phosphorous (Dick, 1994; Alkorta et al., 2003; Valášková et al., 2007; Eichlerová et al., 2015). Enzyme production, however, is sensitive to both soil properties (e.g., pH, C, and moisture; Šnajdr et al., 2008; Moorhead et al., 2013) and fungal community composition (Eichlerová et al., 2015; Mašínová et al., 2018). These communities, in turn, are shaped by disturbance itself and the changes to the soil environmental, and these effects may be particularly strong for taxa that tightly track the soil environment through roles as mutualists (Johnson et al., 2015) and decomposers (Manzoni et al., 2010; Semenova-Nelsen et al., 2019; Hopkins et al., 2020). Addressing management effects as a system, therefore, can give managers and ecologists alike a clearer picture of management consequences and system shifts that may change the trajectory of ecosystem recovery. Finally, disturbance effects vary with time (Chen et al., 2003; Farrell et al., 2011; Burns et al., 2013; Hopkins et al., 2021) and depth (Bolton et al., 1993; De Barros et al., 2020; Upton et al., 2020), so system-level assessments of nutrient cycling responses must also account for the time since disturbance and location in the soil profile. Only by understanding the dynamic responses of this linked system can we accurately quantify ecosystem health and predict ecosystem resilience to future changes in disturbance regimes.

Our research explores how prescribed fire and mowing impact the edaphic properties, soil fungal communities, and microbial functions of an intact, late successional tallgrass prairie ecosystem. Prescribed fire and grazing (or mowing) are commonly used in prairie systems to prevent woody colonization and conversion to forests. In this system, we assessed soil characteristics, soil fungal communities, and hydrolytic enzyme activity prior to and over time following either burning or mowing. We hypothesized that fungal community composition would be influenced by soil characteristics, soil depth, and sampling time, and that hydrolytic enzyme activity would be associated with fungal community composition. We further hypothesized that belowground systems would respond management treatments differently due to differences between fire and mowing effects on soil properties. We predicted that 1) both management activities would impact soil properties and fungal communities directly (Fig. 1 paths a-b), 2) soil properties would also structure fungal communities (Fig. 1 path c), 3) changes to soil enzymes would largely occur through changes to fungal communities and substrate availability (Fig. 1 paths d-e), and finally, 4) at each time point the relationships among these pathways would change depending on disturbance type and with depth. Our data show that soil properties, fungal community composition, and function (i.e., hydrolytic enzyme activity) all rapidly respond to management and effects grow stronger over time, but interactions among these variables that shift with management display significant resiliency after only one month.

2. Methods (1929 words)

2.1. Site description

We conducted our study at (38° 10' N; −95° 16' W; Fig. A.1) the Anderson County Prairie Preserve (Anderson County, Kansas), a nearly 1,500-acre prairie preserve with active fire, grazing, and mowing management. The Nature Conservancy-owned site represents the largest intact, remnant Kansas prairie east of the Flint Hills. Our experiment occurred in tract 13, a remnant tallgrass prairie, that has been historically managed with annual to biennial low-intensity, prescribed fire in the Spring or Fall, as well as haying during non-fire years since at least the mid-1990 s. Surface soils at this site are part of the Clareson-Rock outcrop complex (USDA NRCS, 2022). The site is characterized by diverse graminoid and forb vegetation, dominated by *Andropogon gerardii* (grass), *Baptisia australis* (forb), *Tradescantia occidentalis* (forb), and *Schizachyrium scoparium* (grass) (Kansas Biological Survey, 2010). Average annual temperatures for the site range from 7 °C to 19 °C. Average annual precipitation is 970.3 mm, with the majority occurring between April and September.

2.2. Plot set-up

Experimental plots (5 m^2) were established prior to management treatments in October 2019. We created two columns of seven plots each (14 total) oriented north to south, with a 10 m fire break established between the columns. Plot corners were marked with 1 m tall PVC poles and, within each column plots were separated by 20 m buffer zones. This setup allowed for 7 pairs of plots (Fig. A.1). The latitude, longitude, and elevation of each plot was marked with GPS to later account for spatial effects.

2.3. Management treatments

To establish mowed and burned management treatments, the western column of plots was mowed using a ZTrak™ mower on October 8th, 2019. To mimic the effects of grazing, mowed plant litter was removed from each plot with a leaf blower. The prescribed fire took place on October 9th, 2019. Just prior to ignition, winds out the southeast were estimated at 10mph, the air temperature was 20 °C, and humidity was 49%. At 11 am, a backing fire was ignited with a drip torch along the northern edge of the plot, and then flanking and head fires were ignited. Only the eastern column of plots was burned during the prescribed fire. During the fire, average flame heights were 0.25–0.5 m, with some flare ups to 1 m. The fire line moved quickly, and the plots were completely burned by 12:30 pm. The fire left a heavily blackened surface ash layer with sporadic white-grey ash spots. Fuel consumption was approximately 60% based on visual assessment.

2.4. Soil sampling

Soil samples were collected two weeks prior to mowing and fire

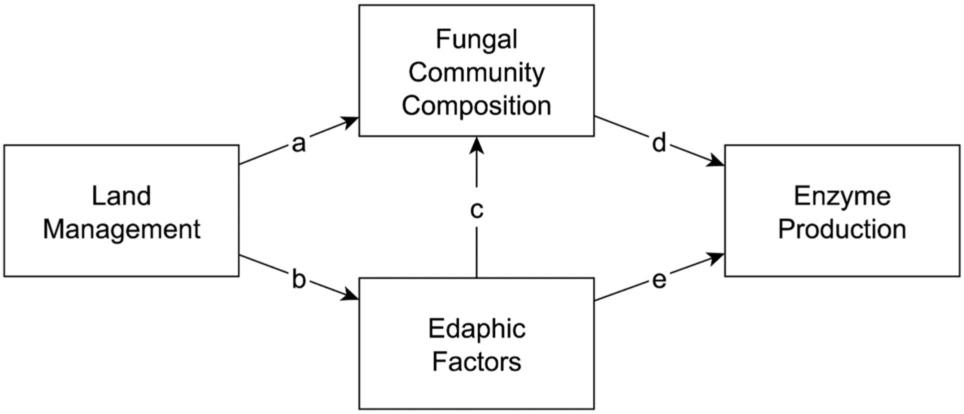

Fig. 1. : Hypothesized pathways for land management effects on soil fungal community structure and function. We hypothesized that land management would impact soil properties (Fig. 1 path a) and fungal community composition (Fig. 1 path b) directly, and that changes to edaphic factors would also structure soil fungal communities (Fig. 1 path c). We also hypothesized that changes to fungal community composition (Fig. 1 path d) and edaphic factors (Fig. 1 path e) would drive changes to soil enzyme profiles. Finally, we also expected that management effects on belowground systems would vary across sampling times and soil depths.

management, as well as 2 weeks and 1-month post-management. A 2.5 cm diameter soil hammer was used containing acrylic sample retention sleeves. At each sampling time, two soil cores were collected from the center of each plot, and the retention sleeves were deposited into sterile bags, and kept on ice until processing at the University of Kansas within six hours of sampling. To avoid inter-sample contamination, the soil hammer was sterilized using soap and water between plots. Upon return to the lab, each soil sample was divided into two sections, 0–5 cm and 5–10 cm. For each plot, samples for each depth were combined and homogenized, then subsamples were taken for downstream DNA and hydrolytic enzyme assays. This produced a total of 84 soil samples (2 management treatments, 3 time periods, 2 soil depths, 7 replicate plots; $n = 84$). Subsamples for DNA and enzyme analyses were stored at -80 °C, and the remaining soil, to be used for nutrient and edaphic analyses were stored at 4 °C for less than 2 weeks.

2.5. Soil analysis

Soil nutrient and edaphic analyses were completed at the Kansas State University Soil Testing Lab. Soil phosphorus was quantified using the Mehlich-3 method (Mehlich, 1984) on a Lachat Quickchem 8000 (Lachat Instruments, Loveland, USA). Total soil carbon and nitrogen were measured on a LECO TruSpec CN Carbon/Nitrogen combustion analyzer (LECO Corporation, St. Joseph, USA). Carbon-to-nitrogen ratio was then calculated by dividing total carbon by total nitrogen. Available ammonium ($NH4+$) and nitrate ($NO3-$) were extracted using 2 M KCl on 2 g of soil, and then a cadmium reduction for nitrate and colorimetric procedures, followed by analysis for ion quantification (Brown, 1998).

Soil pH was measured using a pH probe in a 1:1 soil:DI water solution. For each sample, the average pH was determined by taking the mean of three separate measurements. To measure Gravimetric water content (GWC), an approximately 5 g subsample of soil was weighed ("wet weight"), dried at 100 °C for at least three days, and re-weighed. GWC was calculated as the mass lost as a proportion of the wet weight. Following GWC quantification, soil organic carbon content (OrgC) was determined by placing the original 5 g soil subsamples in a muffle furnace at 550 °C for 1 h. OrgC was calculated as the mass lost as a proportion of the dry, pre-furnace weight.

2.6. Enzyme assays

Soil subsamples for enzyme analysis were thawed and enzyme activities quantified with fluorometric assays described in (German et al., 2012; Stone et al., 2012). Enzyme activities for β-1,4-glucosidase (BGase), β-1,4-N-acetylglucosaminidase (NAGase), and Acid Phosphatase (APase) were measured using 4-methylumbelliferyl beta-D-glucopyranoside, 4-methylumbelliferyl N-acetyl-beta-D-glucosaminide, and 4-methylumbelliferyl phosphate substrates respectively. All substrates were used to create 400 μM solutions using DI water. 50 mM sodium acetate solution (pH 6.5) and 10 μM 4-methylumbelliferone solution (MUB) were used for buffer and standards respectively. Approximately 1 g of wet soil from each soil sample was mixed with 125 ml of sodium acetate buffer using an emulsion blender for 30 s to create a soil slurry. Then, the buffer, MUB, substrate, and soil slurry solutions were added to 96 well microplates (1 plate per sample). This plate set-up (Fig. A.2) allowed for soil, sterile, and substrate controls, 3 enzyme assays with 12 analytical replicates, as well as quench corrections for each enzyme assayed. Plates were then covered with aluminum foil and incubated at 25 °C for no more than 18 h. Following incubation, fluorescence was measured with a microplate reader using an excitation wavelength of 360 nm and an emission wavelength of 460 nm to calculate nmol activity h-1 g soil-1. All enzyme activities were corrected for soil moisture content (GWC above).

2.7. DNA extraction and PCR

DNA was extracted from 0.25 g of the DNA soil subsample using Machery-Nagel NucleoSpin® Soil kits (Machery-Nagel, Düren, Germany) following the manufacturer's protocol. A single step PCR was then used to amplify the ITS2 rDNA region with the fITS7 (forward; Ihrmark et al., 2012) and ITS4 (reverse; White et al., 1990) universal fungal primer pair. For the PCR reactions, solutions of 0.8 μL of DNA, 8 μL of 5x Q5® buffer (New England Biosystems, Ipswich, Massachusetts), 0.8 μL of dNTPS (10 mM), 2 μL of each primer (10 mM), 0.4 μL of Q5® High-Fidelity DNA polymerase (New England Biosystems), 8 μL of enhancer (New England Biosystems), and 17.8 μL of ddH2O were used for each reaction (40 μL total). The PCR set-up followed Semenova-Nelsen et al., 2019, with an initial denaturation step at 98 °C for 30 s, followed by 25 cycles of 98 °C for 10 s, 57 °C for 30 s, and 72 °C for 30 s, and a final extension step at 72 °C for 2 min, then held at 4 °C. Products for all PCRs were checked on agarose gels to ensure amplification and cleaned using Agencourt AMPure XP magnetic beads (Beckman Coulter, Indianapolis, Indiana).

2.8. Library preparation and sequencing

Illumina MiSeq Nextera protocol was used to sequence fungal community samples. First, a second PCR reaction was used to ligate unique, 12 bp sequence barcodes (Nextera indices, Illumina, San Diego, California) to each individual sample. The second PCR parameters were similar to the first, however, 5 μL of the primary PCR amplicon was used instead of 8 μL of the original DNA template, and the number of PCR cycles was reduced to 8. Barcoded amplicons were purified using Agencourt beads (see above), and DNA concentrations were checked using a Qubit 2.0 (LifeTechnologies, Carlsbad, California). Samples were then pooled in equimolar concentrations into a single library and sequenced using an Illumina MiSeq (Illumina, San Diego, California) with 300 bp paired-end reads and V3 chemistry at the Kansas State Integrated Genomics Center. Sequence data is deposited in the GenBank Sequence Read Archive (SRA) PRJNA906953.

2.9. Bioinformatics

Raw sequencing data were analyzed using Qiime2 version 2019.10 following methods outline in Bolyen et al. (2019). Quality and barcode filtering resulted in approximately 4.5 M reads for the 84 samples. Unique barcodes were trimmed from paired reads using cutadapt (Martin, 2011), then combined using the dada2 tool (Callahan et al., 2016). The UNITE fungal ITS reference database v8 "dynamic" (Abarenkov et al., 2010, accessed Feb. 2019) was used to train a Naive Bayes classifier, which defined amplicon sequence variants (ASVs) and assigned them probable taxonomic identities. ASVs with less than five reads were removed to reduce sequencing artefacts. Normalization procedures are detailed below. Bioinformatics scripts are included in the appendix.

2.10. Statistical analysis

All analyses were completed in R v. 4.0.2 (R Core Team, 2022). To prepare the fungal community ASV table for compositional data analysis, zeroes were replaced using the cmultRepl function with the Bayesian Laplace method in the zCompositions package (Palarea-Albaladejo and Martín-Fernández, 2015). Then the fungal community data was transformed using a centered log-ratio transformation via the clr function in the compositions package (van den Boogaart and Tolosana-Delgado, 2008). Following transformation, a dissimilarity matrix was created using the Aitchison's distance with the Vegan package's dist function (Oksanen et al., 2013). Note that Aitchison's distance is defined as the Euclidean distance following a centered log-ratio transformation (Calle, 2019). Since use of Aitchison's distance allows for compositional data to be analyzed with linear methods, a principal components analysis (PCA) was used to create an ordination for the fungal community data.

To test for differences in fungal community structure between management treatments (burned and mowed), sampling times (pre, 2 weeks post, and 1 month post), and sample depths (0–5 cm and 5–10 cm), PERMANOVAs that accounted for locational effects were applied using the adonis function. The location term was included first in the PERMANOVA model since the adonis function uses sequential sums of squares. When a PERMANOVA denoted significant main effects, the pairwise.perm.manova function from the RVAideMemoire package (Hervé, 2021) was used to explore intra-treatment differences. Additionally, diversity metrics (inverse Simpson and Shannon's diversity metrics) for the fungal community data were calculated using the diversity function. To identify indicator taxa for burned and mowed treatments, the ALDEx2 package (Fernandes et al., 2013) was used to detect differential ASV expression between post-treatment burned and mowed groups. For ALDEx2 analyses, p-values were adjusted using the Benjamini-Hochberg or "fdr" method.

Multivariate analyses of variance (MANOVA) with marginal sums of squares were used to test for differences in fungal diversity metrics, soil characteristics, and enzyme activity between management treatments, soil layers, and sampling times using the manova and joint_tests function (emmeans package; Lenth, 2018). When MANOVAs denoted significant treatment effects, pairwise contrasts were applied using the contrast function.

Following tests for the individual components, we then explored mechanisms underlying management driven shifts to microbial roles in ecosystems using structural equation modeling (SEM) with the piecewiseSEM package (Lefcheck, 2016). Based on existing literature, we hypothesized a meta-model with pathways linking management treatments to microbial enzyme production that included pathways through fungal community structure and changes to soil properties (Fig. 1; Table A.1). Specifically, we hypothesized that management treatment (burned vs. mowed) would alter fungal community structure due to direct effects of management (Fig. 1 – path a), management driven changes to soil characteristics (Fig. 1 – path b). Note that burned plots were coded as a 1 and mowed as a 0, meaning that a positive correlation between management and other variables means that the positive interaction was higher in burned relative to mowed plots. We further hypothesized that management driven changes to fungal communities (Fig. 1 – path d), soil characteristics (Fig. 1 – path e), as well as direct management effects would alter hydrolytic enzyme production. For a description of model variables and initial model set-up see Table 1 and A.1. After developing initial hypotheses for model structure, a goodness of fit guided approach (AIC, BIC, and Fisher's C statistics) was used to determine model modifications.

3. Results (1617 words)

3.1. Fungal community data

A total of 5031 ASVs were identified, with 90% representing 9 fungal phyla (including 4 basal lineages and 1 subphylum), 37 classes, 87 orders, 193 families, and 395 genera. Only one ASV was not classified as a fungus, and was removed from downstream analyses, all other ASVs were retained. 498 ASVs were only identified to the kingdom level (Fungi). Fungal communities were dominated by four classes: the Ascomycota classes Sordariomycetes (32%), Dothideomycetes (18%), and Eurotiomycetes (8%), as well as the Basidiomycota class Agaricomycetes (17%). The five most abundant ASVs were an unidentified taxon in the genus *Staphylotrichum*, an unidentified taxon in the genus *Periconia*, *Hygrocybe acutoconica*, an unidentified taxon in the family Nectriaceae, and *Fusarium redolens*.

3.2. Treatment effects on fungal communities

Fungal community composition varied between sampling times, land management treatments, and soil depths (Fig. 2a-b). Fungal communities exhibited compositional turnover between successive sampling

Table 1
Variable descriptions for structural equation models. Cont. = continuous, Categ. = categorical, Perc. = percent.

Variable	Type	Coding	Transformation	Mean	s.d.
PCA 1	Cont.	na	Aitchison	na	na
PCA 2	Cont.	na	Aitchison	na	na
Management treatment	Categ.	0 = mowed, 1 = burned	none	mowed is reference	na
Soil depth	Categ.	0 = 0–5 cm, 1 = 5–10 cm	none	0–5 cm is reference	na
Total nitrogen	Perc.	na	none	0.34	0.03
Inorganic phosphorus	Cont.	na	none	1.37	1.5
NH_4^+	Cont.	na	none	3.7	1.2
NO_3^-	Cont.	na	none	0.4	0.24
Gravimetric water content (GWC)	Perc.	na	none	0.31	0.07
Soil pH	Cont.	na	none	6.9	0.18
Organic carbon	Perc.	na	none	0.12	0.02
C:N ratio	Cont.	na	none	0.12	1
Inverse Simpson metric	Cont.	na	natural log	4.05	0.77
Bgase	Cont.	na	natural log	5.1	0.55
NAGase	Cont.	na	natural log	5.6	0.47
APase	Cont.	na	natural log	6.9	0.37

categorical, Perc. = percent.

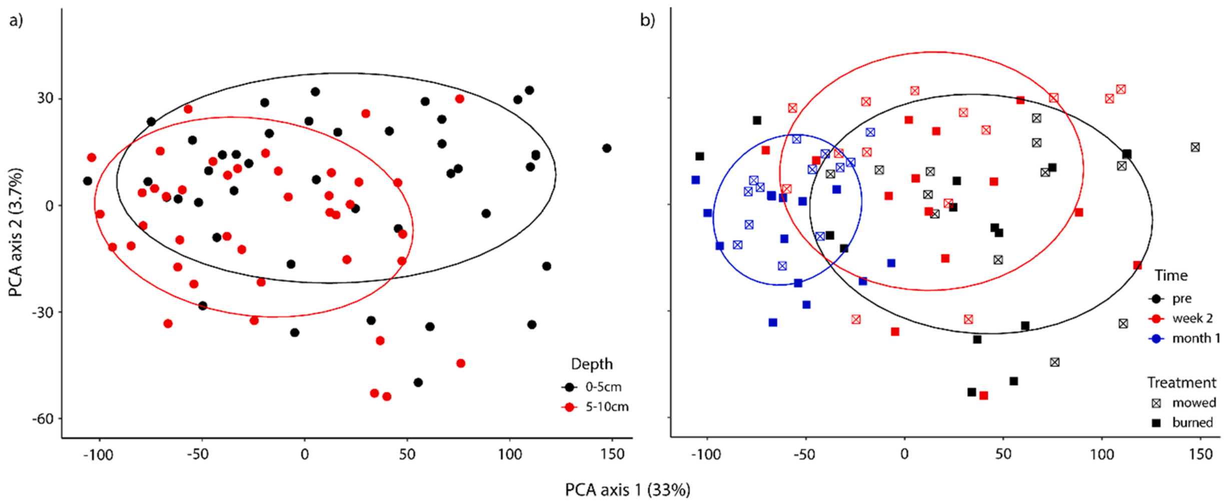

Fig. 2. Principal components analysis ordinations for fungal community structure responses to experimental treatments. Ellipses represent one standard deviation from the centroid of each a) treatment group and b) sampling time. Fungal community structure varied between a) soil depths and b) between management treatments across sampling times.

times ($F_{1,82}$ = 2.43, p < 0.001, R^2 = 5%; Table 2 & A2) and distinct variation between management treatments ($F_{2,82}$ = 2.01, p < 0.001, R^2 = 2%). Further, the fungal communities in burned and mowed communities remained compositionally distinct following management activity ($F_{2,82}$ = 1.16, p = 0.01, R^2 = 3%). Some of this variation in community composition between management treatments may be due to locational differences, as fungal communities in burned and mowed plots differed prior to fire and mowing treatments. Fungal community composition also differed between soil depths ($F_{1,82}$ = 2.64, p < 0.001, R^2 = 3%), however this influence was independent of management ($F_{1,82}$ = 0.96, p = 0.68) and sampling time effects ($F_{1,82}$ = 0.89, p = 0.99).

Fungal diversity varied between soil depths, but not land management treatments or sampling times ($F_{1,65}$ = 16.91, p = 0.0001; Table A.3). Fungal community diversity was higher in the 0–5 cm layer versus the 5–10 cm layer.

Indicator species for land management treatments reflected management driven differences that favored fungi able to survive in post-burn/mowed environments (Table 3). In mowed plots, an unidentified taxa in the family Didymosphaeriaceae, *Penidiella aggregata*, and *Periconia homothallica* were more abundant relative to burned plots. Members of Didymosphaeriaceae are generally saprophytic, with some being mycoparasites. *P. aggregata* is a member of the Teratosphaeriaceae family, which contains several plant pathogens and genera known to survive in harsh environments. *P. homothallica* comes from a genus that are functionally diverse, but often found as soil saprotrophs or plant pathogens. The *Periconia* genus includes indicators of other non-burned and mowed tallgrass prairies (Hopkins et al., 2021) as well as fire-adapted taxa in other systems (Semenova-Nelsen et al., 2019; Fox et al., 2022). In the burned plots, the only indicator species was an unidentified member of Ascomycota. In summary, fungal community composition differed due to land management, sampling time, and soil depth, however, these changes were primarily compositional in nature rather than diversity related.

Table 2
PERMANOVA model table for treatment effects of soil fungal community composition.

Effect	d.f.	SS	Mean Squares	F-Statistic	R^2	p-value
plot	6	39,772	6628.70	1.95	0.13	< 0.001 ***
management treatment	1	6829	6828.70	2.01	0.02	< 0.001 ***
sampling time	2	16,489	8244.70	2.43	0.05	< 0.001 ***
soil layer	1	8962	8961.70	2.64	0.03	< 0.001 ***
treatment x time	2	7916	3957.90	1.16	0.03	0.010 **
treatment x layer	1	3248	3248.30	0.96	0.01	0.68
layer x time	2	6050	3025.20	0.89	0.02	0.99
treatment x time x layer	2	5552	2775.90	0.82	0.02	1
residuals	65	220,930	3398.90		0.70	
total	82	315,749			1	

*p ≤ 0.1, **p ≤ 0.05, ***p ≤ 0.001

3.3. Treatment effects on soil characteristics

Overall soil nutrient and edaphic profiles varied between land management treatments ($F_{1,66}$ =3.73, p = 0.0579; Fig. 3; Table 4), sampling times ($F_{2,66}$ =26.25, p < 0.0001), and soil depths ($F_{1,66}$ =65.39, p < 0.0001). Specifically, the overall post-management soil environment differed from pre-management conditions, in addition to further differences between burned and mowed treatments and soil depths. Individual soil characteristics varied between management treatments ($F_{7,66}$ =4.46, p = 0.0004), however, these differences were modified by sampling time ($F_{14,66}$ =83.7, p < 0.0001) and soil depth ($F_{7,66}$ =50.55, p < 0.0001), as well as a 3-way interaction between these variables ($F_{14,66}$ =3.18, p < 0.0001). Gravimetric water content (GWC; $F_{2,66}$ =8.42, p < 0.0001; Table A.4), total nitrogen ($F_{2,66}$ =11.059, p < 0.0001; Table A.5), ammonium ($F_{2,66}$ =4.272, p = 0.0426; Table A.6), nitrate (marginal effect; $F_{2,66}$ =2.39, p = 0.099; Table A.7), and inorganic phosphorous ($F_{2,66}$ =16.27, p < 0.0001; Table A.8) varied between management treatments, however, this effect was often dependent on sampling time and soil depth. From pre to post treatment, both management treatments drove increases in GWC and ammonium levels, but this increase was greater in mowed relative to burned plots across both soil layers. Total nitrogen and inorganic phosphorous also varied between management treatments, with mowed plot nitrogen levels increasing at 2 weeks-post relative to pre-management levels, and burned plot nitrogen levels increasing 1 month-post relative to pre-management levels. Total phosphorous however only increased in the upper layers of burned plots and did not change in mowed plots following land management. Nitrate levels also increased in mowed plots relative to pre-management conditions, but only in the upper soil layer 2 weeks following mowing.

Soil pH ($F_{2,66}$ =3.82, p = 0.0269; Table A.9), C:N ratios ($F_{2,66}$ =52.1, p < 0.0001; Table A.10), and organic carbon (marginal effect; $F_{2,66}$

Table 3
Indicator species output for post-management burned and mowed soil fungal communities. Diff.btw is the median difference between groups on a log base 2 scale. Diff.win is the largest median variation within group. Effect is the effect size of diff.btw/diff.win and describes whether inter- vs. intra-group variance is larger. Overlap describes confusion in assigning an observation to either group. Wi.ep is the expected value of the Wilcoxon test p-value. Wi.eBH is the expected value of the Benjamini-Hochberg corrected p-value.

Group	Taxon	Diff.btw	Diff.win	Effect	Overlap	Wi.ep	Wi.eBH
mowed	Didymosphaeriaceae sp.	3.9	5.3	0.7	0.19	< 0.001 ***	0.04 **
	Penidiella aggregata	4.2	4.9	0.77	0.19	< 0.001 ***	0.04 **
	Periconia homothallica	4.7	5.6	0.78	0.22	< 0.001 ***	0.05 **
burned	Ascomycota sp.	-3.94	5.5	-0.6	0.22	0.001 ***	0.11

*: $p \leq 0.1$, **: $p \leq 0.05$, ***: $p \leq 0.001$

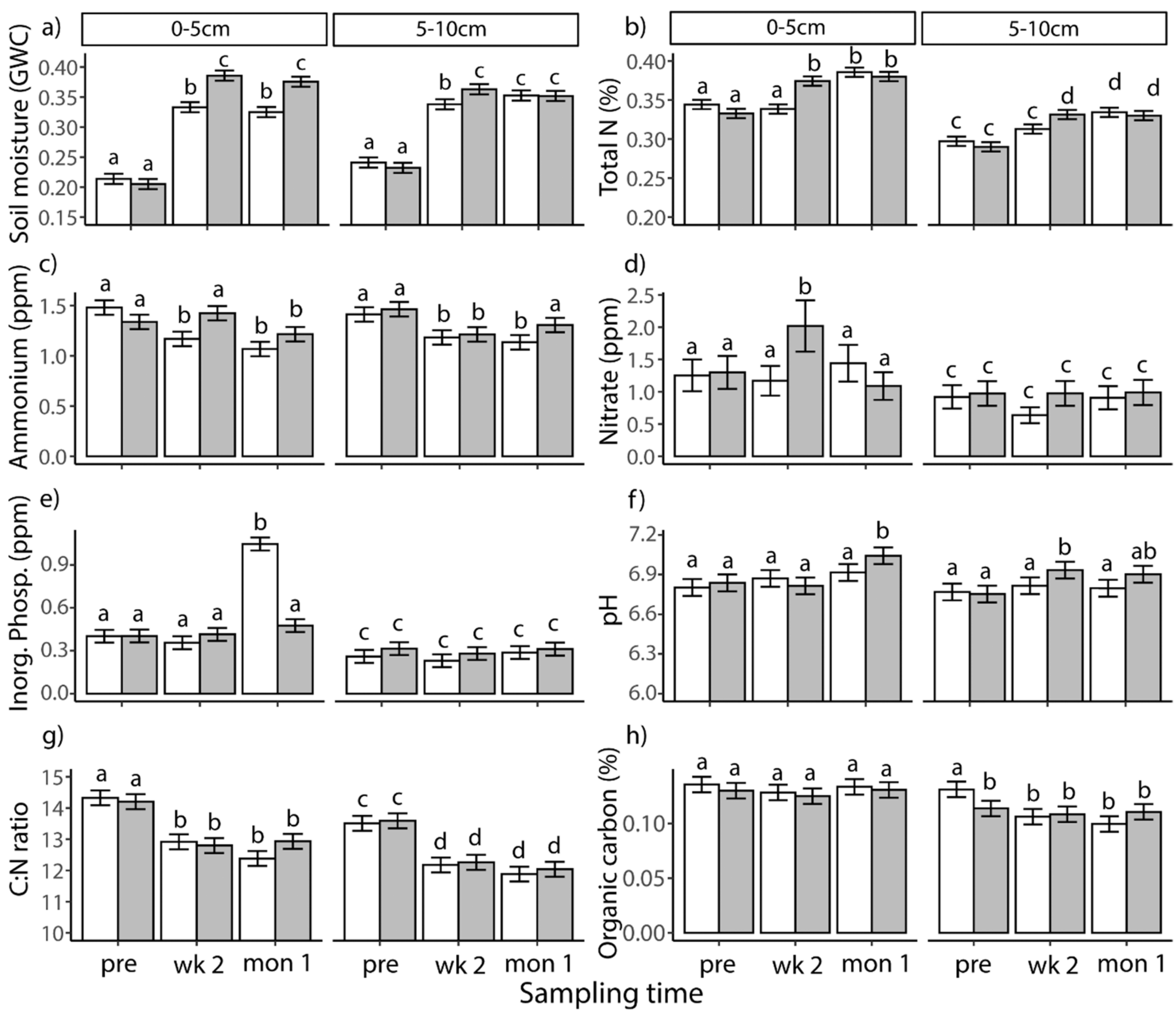

Fig. 3. Soil characteristic responses to experimental treatments. Bars denote the estimated marginal mean and error bars represent the mean plus or minus one standard error. White bars represent burned plots and grey bars represent mowed plots. Lower case letters denote statistically significant differences between treatments at the land management*sampling time*soil depth level. A) soil moisture, b) total nitrogen, c) ammonium, d) nitrate, and e) inorganic phosphorous responses to land management varied between burned and mowed plots. Soil characteristics also varied across sampling times, with f) pH and g) C:N ratios increasing post-management and h) organic carbon decreasing post-management at deeper soil depths.

$=2.62$, $p = 0.08$; Table A.11) primarily across sampling times, and these effects were modified by management treatment and soil layer. Soil pH increased following mowing; however, this was limited to the upper soil layer. C:N ratios also increased following burning and mowing, but this effect was similar across soil layers and management treatments. Organic carbon decreased slightly following management activity, but this decrease was primarily limited to the lower soil layer of burned plots. In summary, soil characteristics varied across sampling times, and the direction of these changes were determined by management treatment and soil depth.

3.4. *Treatment effects on enzyme activity*

Hydrolytic enzyme profiles primarily varied between sampling times ($F_{2,70} = 84.5$, $p < 0.0001$; Fig. 4; Table 5). Following both fire and mowing, enzyme activities increased at 2 weeks and 1-month post-management. Individual enzyme activities also varied across soil layers ($F_{2,70} = 44.7$, $p < 0.0001$; Table A.12–14). Specifically, BGase

Table 4
MANOVA model table for treatment effects on soil characteristics.

Model term	d.f.1	d.f.2	F-ratio	p-value
Management treatment	1	66	3.73	0.0579 *
Soil layer	1	66	65.39	< 0.0001 ***
Sampling time	2	66	26.25	< 0.0001 ***
Plot	6	66	3.35	0.0061 **
Repeated measure	7	66	36,195.53	< 0.0001 ***
Treatment x layer	1	66	0.76	0.39
Treatment x time	2	66	1.61	0.21
Treatment x rep. meas.	7	66	4.46	0.0004 ***
Layer x time	2	66	0.50	0.61
Layer x rep. meas.	7	66	50.55	< 0.0001 ***
Time x rep. meas.	14	66	83.7	< 0.0001 ***
Plot x rep. meas.	42	66	4.42	< 0.0001 ***
Treatment x layer x time	2	66	0.22	0.8069
Treatment x layer x rep. meas.	7	66	3.12	0.0066 ***
Treatment x time x rep. meas.	14	66	5.19	< 0.0001 ***
Layer x time x. rep. meas.	14	66	5.63	< 0.0001 ***
Treat. x layer x time x rep. meas.	14	66	3.18	0.0008 ***

*: $p \leq 0.1$, **: $p \leq 0.05$, ***: $p \leq 0.001$.

activity was higher in the 0–5 cm layer, NAGase activity was higher in the 5–10 cm layer, and APase activity did not vary between soil layers. In summary, hydrolytic enzyme activity increased following management and varied between soil layers.

3.5. SEM model fitting

We began with highly saturated SEMs for each sampling time (pre, 2 weeks post, and 1 month post management) that were based on our hypothesized pathways. Through several iterations for each SEM, poorly supported paths in the model were pruned using increasingly stringent p-value thresholds (e.g., $p > 0.7$, 0.5), and Shipley's tests for independence were used to assess the inclusion of unconsidered model paths (if they were also supported in the literature). Overall model fit statistics were consulted after each step (Table A.15) using AIC and BIC values. The final models were well supported (Pre-management: Fishers's C = 113.261, p = 0.297; 2 weeks post: Fisher's C = 115.935, p = 0.769; 1 month post: Fisher's C = 120.716, p = 0.978), and did not require further adjustment. Final SEM coefficients for model paths are presented in appendix Table A.16–18. See appendix for a detailed description of model fitting procedures.

3.6. SEM - pre-management model

Prior to land management, soil depth and soil characteristics were linked to fungal community composition which in turn predicted hydrolytic enzyme activity (Figs. 5–7, A.3; Table A.16). Total nitrogen content (−0.934), soil depth (−1.37), and GWC (0.583) were the primary predictors of fungal community PCA axis 1 (R2 here), while PCA

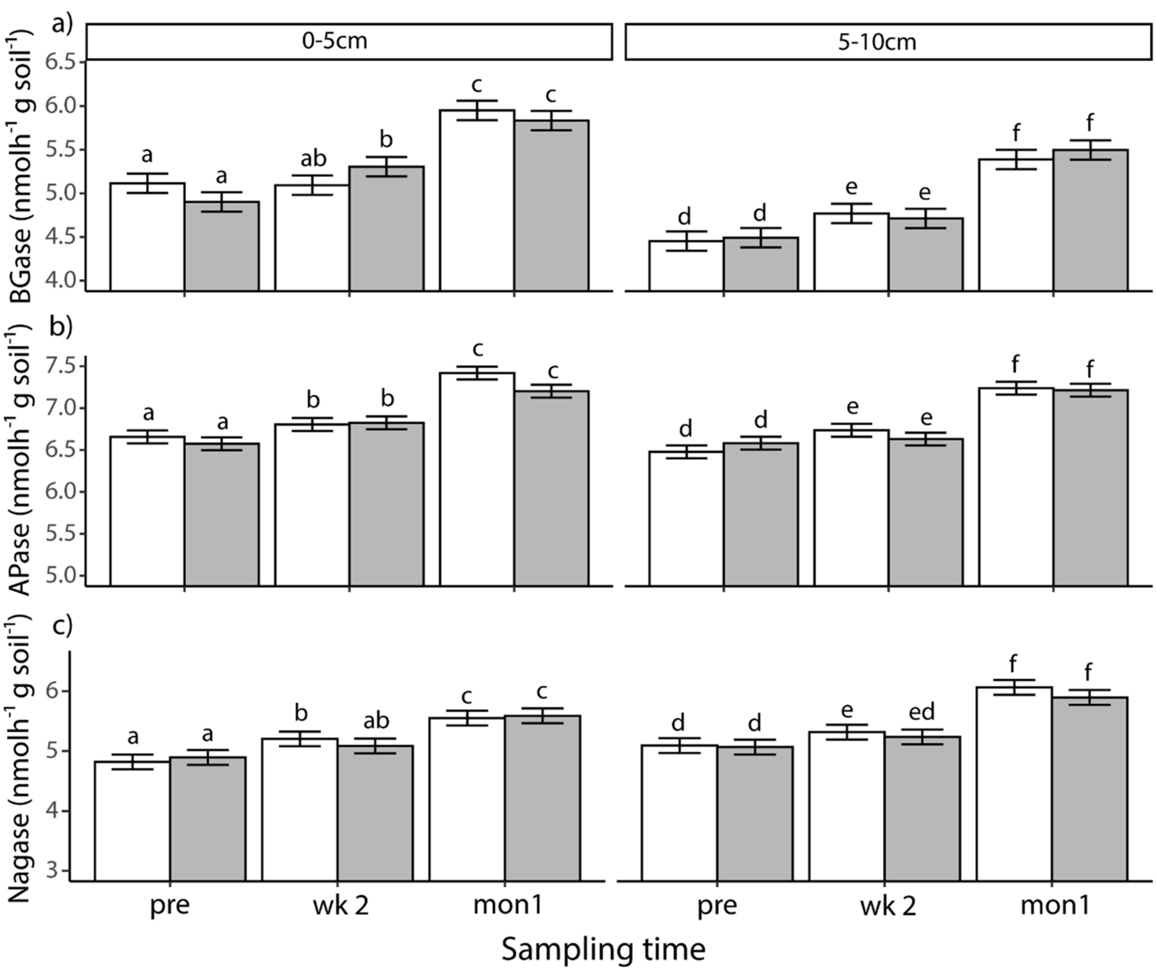

Fig. 4. Hydrolytic enzyme responses to experimental treatments. Bars denote the estimated marginal mean and error bars represent the mean plus or minus one standard error. Lower case letters denote statistically significant differences between treatments at the land management*sampling time*soil depth level. White bars represent burned plots and grey bars represent mowed plots. Activity of a) BGase, b) APase, and c) NAGase increased following land management. Changes in BGase and NAGase activities differed between land management treatments early after management however, BGase only increasing 2 weeks post-management in mowed plots, and NAGase increasingly only in burned plots 2 weeks post-management.

Table 5
MANOVA model table for treatment effects on hydrolytic enzyme activity.

Model term	d.f.1	d.f.2	F-ratio	p-value
Management treatment	1	70	0.258	0.6129
Soil layer	1	70	1.027	0.3143
Sampling time	2	70	84.494	< 0.0001 **
Plot	1	70	0.006	0.9402
Organic carbon	1	70	2.917	0.0921.
Repeated measure	2	70	1999.951	< 0.0001 **
Treatment x layer	1	70	0.02	0.8883
Treatment x time	2	70	0.213	0.8088
Treatment x rep. meas.	2	70	0.787	0.4593
Layer x time	2	70	0.674	0.5127
Layer x rep. meas.	2	70	44.687	< 0.0001 **
Time x rep. meas.	4	70	7.73	< 0.0001 **
Plot x rep. meas.	2	70	1.822	0.1693
Organic carbon x rep. meas.	2	70	1.077	0.3462
Treatment x layer x time	2	70	0.563	0.572
Treatment x layer x rep. meas.	2	70	1.128	0.3294
Treatment x time x rep. meas.	4	70	1.955	0.1109
Layer x time x. rep. meas.	4	70	1.061	0.3822
Treat. x layer x time x rep. meas.	4	70	1.325	0.2693

$p \leq 0.1$, *: $p \leq 0.05$, **: $p \leq 0.001$.

axis 2 was primarily associated with GWC (−0.876), soil depth (0.258), and C:N ratios (0.229). Fungal community diversity increased with GWC (0.776) and decreased with soil depth (−0.791). Fungal community composition (PCA axis 2) in turn predicted BGase (−0.611) and APase (−0.516) activities. Soil characteristics were also directly associated with soil enzyme activity, with higher C:N ratios (BGase: 0.261, APase: 0.274), organic carbon levels (APase: 0.363), and GWC (NAGase: 0.687) increasing enzyme activity, and higher inorganic phosphorous levels decreasing enzyme activity (NAGase: −0.49). In summary, soil fungal community composition varied due to soil depth and characteristics, and was associated with carbon and phosphorous acquiring enzyme activity.

3.7. SEM - 2 weeks post-management model

Soon after land management, soil characteristics and fungal community composition differed between burned and mowed sites, and these changes were correlated with altered enzyme activity (Figs. 5–7, A.4; Table A.17). Relative to mowed plots, fire reduced GWC (−0.513), nitrate (−0.302), ammonium (−0.337), and total nitrogen levels (−0.491). Management treatments (burn vs. mow) also directly drove shifts in fungal community composition (−0.513), but also influenced fungal community composition indirectly through changes to soil characteristics. Specifically, relative to mowed plots, reduced GWC (0.391), and total nitrogen (−0.233) altered fungal community composition, while lower ammonium in burned plots reduced fungal diversity (−0.167). Management effects on fungal community composition also influenced enzyme activity, with shifts in community composition (0.473) and decreased diversity (−0.08) leading to lower APase activity in burned plots. Additionally, fungal community composition was not correlated with BGase activity, but it was associated with NAGase activity (PCA 1: −0.52). Enzyme activity was also higher in mowed plots (BGase: −0.159, APase: −0.153), and negatively associated with fire driven reductions in nitrate (BGase: −0.159, APase: −0.153), GWC (BGase: −0.272, APase: −0.435), and ammonium (NAGase: −0.189). To summarize, land management treatments produced distinct fungal community compositions both directly and through changes to soil characteristics, and these changes altered fungal enzyme activity.

3.8. SEM - 1-month post-management model

Management driven differences in fungal community composition persisted 1 month after management and were associated with altered enzyme activity (Fig. 7a-c, A.5; Table A.18). Direct, management driven differences in fungal community composition (burn vs. mow) reflected larger differences than at 2 weeks (−0.971). Management indirectly altered fungal community composition due to reduced GWC (PCA 1: −0.157, PCA 2: 0.209) and ammonium (PCA 2: 0.222) in burned relative to mowed plots. Further, management associated changes in GWC, and fungal community composition reduced BGase activity in burned relative to mowed plots (−0.072). Management also influenced enzyme activity through non-fungal pathways. In burned plots, direct, unmeasured effects of fire were correlated with increased APase activity (0.261), while in mowed plots, a reduction in nitrate availability was associated with higher NAGase activity (−0.152). In summary, while

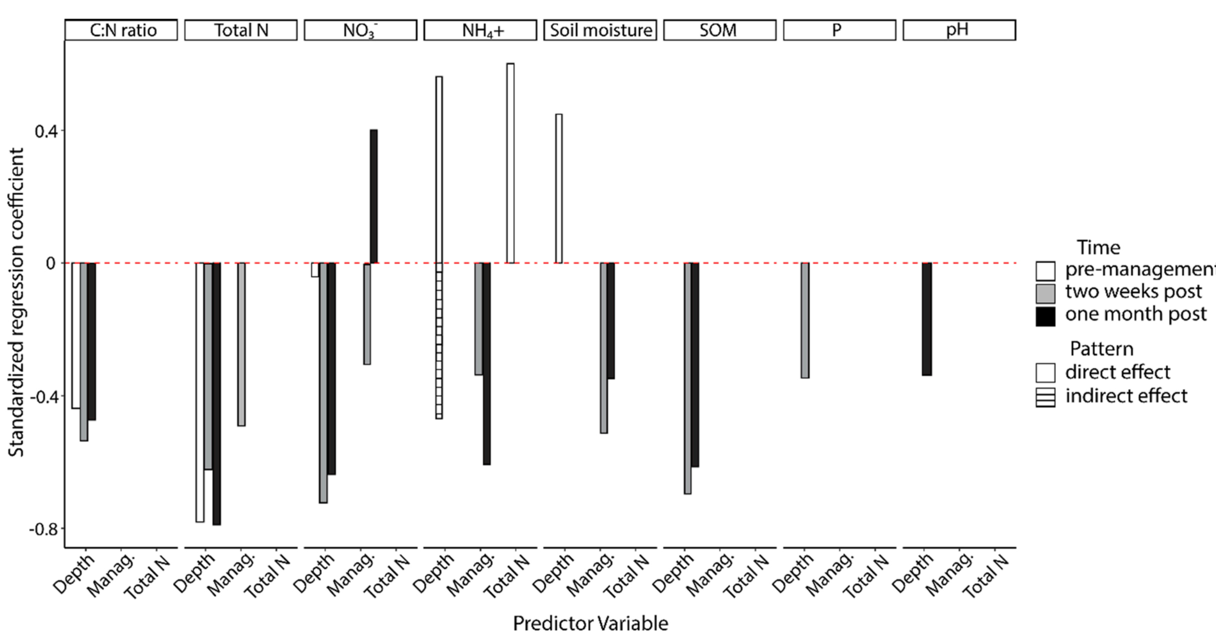

Fig. 5. Soil depth and management effects on soil characteristics from pre, two weeks, and 1 month post management SEM models. Direct effects (hollow bars) represent paths linking the response and predictor variable, while indirect effects (bars with lines) represent paths between variables that are mediated by one or more variables. Manag. = land management.

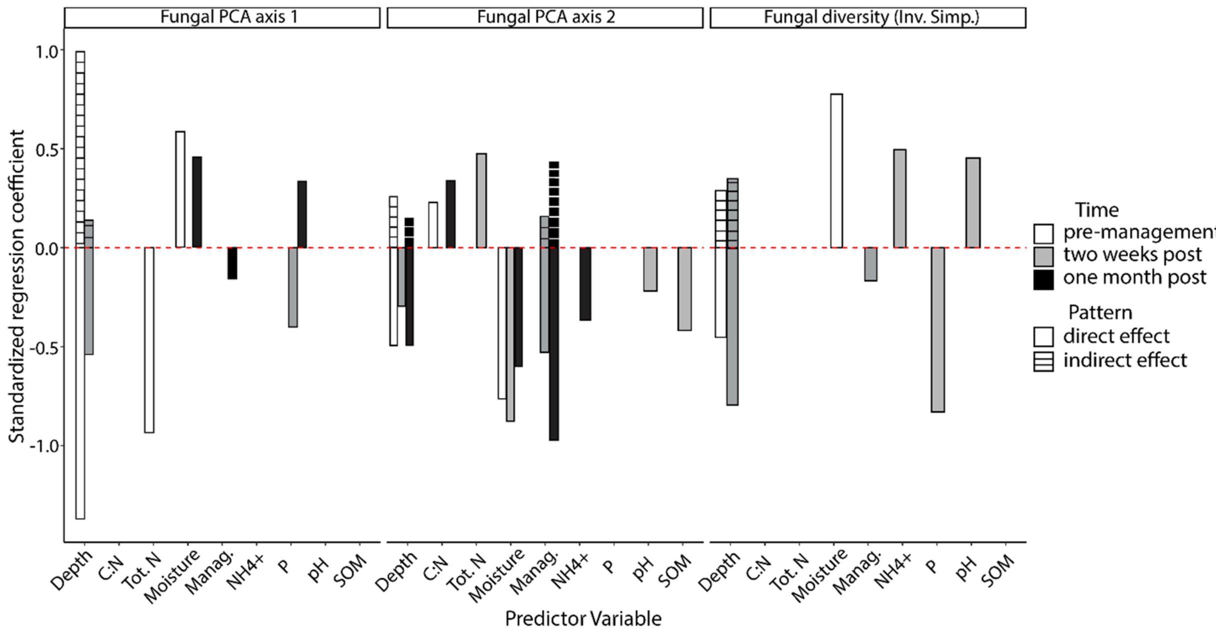

Fig. 6. Soil and management effects on fungal community composition and diversity from pre, two weeks, and 1 month post management SEM models. Direct effects (hollow bars) represent paths linking the response and predictor variable, while indirect effects (bars with lines) represent paths between variables that are mediated by one or more variables. Manag. = land management.

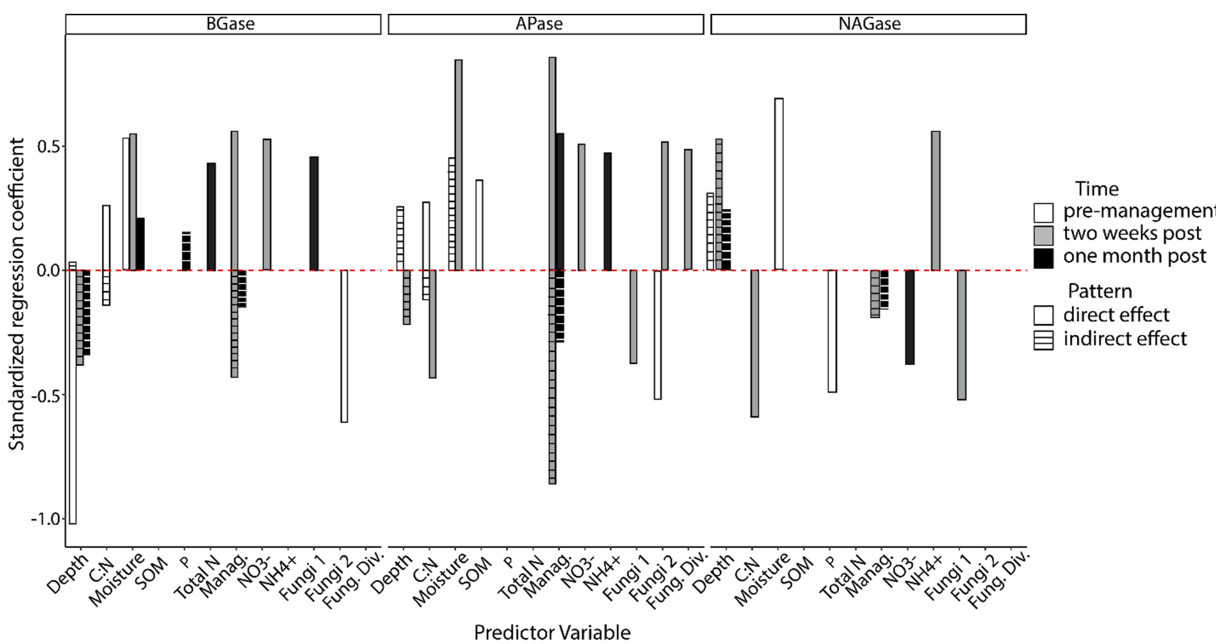

Fig. 7. Soil characteristic, fungal, and management effects on hydrolytic enzyme production from pre, two weeks, and 1 month post management SEM models. Direct effects (hollow bars) represent paths linking the response and predictor variable, while indirect effects (bars with lines) represent paths between variables that are mediated by one or more variables. Manag. = land management.

management effects on soil characteristics and fungal community composition persisted 1-month post-management, fungi were again associated with carbon acquiring enzyme production as in the pre-management model.

4. Discussion

Land management altered nutrient cycles through cascading effects on soil characteristics and fungal communities. This confirms our hypotheses that management activity would directly influence soil characteristics and fungal communities, and that management treatment-based differences in soil characteristics would contribute to differences in fungal community composition. Further, we confirm that downstream effects of land management on nutrient cycle associated functions (i.e., hydrolytic enzyme activity) would respond to changes in soil characteristics and biota. These findings support other studies that have tested management effects on individual soil properties(Knelman et al., 2017; Alcañiz et al., 2018; Chuan et al., 2020; He et al., 2020). By looking at the system-level though, we were able to see the more complex direct and indirect paths for these responses (Figs. 5–7, A.3–5).

Hydrolytic enzyme profiles, for example, did not show particularly strong associations with land management alone, but did when fungal community composition and soil characteristics were considered. While soil characteristics and fungal communities displayed strong responses to land management, these changes also varied between soil depths and with time, supporting the dynamic spatial and temporal responses to management. Following management, the belowground components of this tallgrass prairie ecosystem showed a rapid ability to respond to changes in soil characteristics and nutrient availability (specifically N), while also displaying longer term resilience (in terms of microbial function) when the initial management driven nutrient flushes went away. This result is supported by work in other disturbance mediated systems (Chuan et al., 2020; Hopkins et al., 2020, 2021), and provides mechanisms for how belowground processes respond to management disturbance. The broad similarities in belowground responses to both management activities in this study also contained some distinct differences in responses to fire and mowing.

In both burned and mowed plots, management effects varied with soil depth and developed over time to form unique belowground responses to changes in the soil environment. Relative to mowing, fire made soils drier and drove a loss of ammonium that in addition to the direct effects of fire, contributed to differences in fungal community composition and reduced diversity in burned plots. In turn, these changes were correlated with lower N (NAGase) and P (APase) acquiring enzyme activity following fire as compared to nearby mowed plots. Work in other fire recurrent ecosystems has found similar effects of fire on soil characteristics and other microbial functions early after fire (Ficken and Wright, 2017; Semenova-Nelsen et al., 2019; Hopkins et al., 2020, 2021), where initial post-fire changes in the soil environment drive changes in microbial functions like decomposition that gradually fade with time. Belowground responses following mowing differed however, in that soils in mowed plots were wetter and saw increases in nitrate and nitrogen availability. The wetter soils and increased N availability in turn led to the formation of fungal communities distinct from burned plots that were ultimately associated with increased N and P acquiring enzyme activity. This verifies work in other systems where grazing alters nutrient stoichiometry (He et al., 2020) and enzyme activity (Chuan et al., 2020), and provides a mechanism explaining how grazing driven changes in nutrient availability and microbial communities mediates functional changes. By exploring management effects on belowground processes as a system, we were able to illustrate the complex mechanisms through which soil components respond to changes in their environment. Far from being static however, belowground responses to management varied both with time and soil depth.

Land management's effect on belowground processes displayed dynamic patterns across time that were modified by soil depth. Depending on depth (0–5 cm and 5–10 cm), soil characteristics and nutrient availability responded differently to land management treatments. In the upper soil layer, management driven changes were associated with altered nutrient availability (N and P) and soil moisture, while changes in the lower soil layer reflected loss of organic carbon following management disturbance potentially due to decreases in aboveground plant biomass. These effects were not static however, as management effects on belowground processes also varied across time. While soil characteristics (Farrell et al., 2011), nutrients (Chen et al., 2003), and microbial communities (Averill et al., 2019) display natural seasonal variation, management treatments likely interacted with this variation to produce different responses between burned and mowed treatments. Despite different belowground responses to burning and mowing, differences between management treatments began to decrease by one month as N and P acquisition became less important for fungi relative to BGase production. This illustrates the evolutionary importance of fire and grazing in grasslands (Ford, 2009; McSherry and Ritchie, 2013; Rumpel et al., 2015), as this belowground system was able to quickly respond to management disturbance and then revert to pre-management conditions (i.e., resiliency). The close relationship between disturbance and belowground processes in grasslands and savannas may also help explain their persistence, as well as how positive feedbacks between recurrent, low intensity fire and grazing promote the belowground processes that underly grassland ecosystems (Alcañiz et al., 2018; Neary and Leonard, 2020). The importance of time and depth here also illustrates the necessity of exploring spatial and temporal heterogeneity when looking holistically at land management effects belowground.

A holistic view of land management allows not only for an improved understanding of ecosystem responses to management, but can also aid in predicting how changes to management regimes will affect ecosystems. Since belowground ecosystem components respond as a dynamic system rather than individual parts, management efforts going forward should develop management goals that reflect the system, rather than individual pieces (reviewed in Heneghan et al., 2008). Changes to management or disturbance regimes (e.g., low fire frequency, high severity fire, overgrazing, or removal of grazers) that have long-term impacts on the relationships among system components likely reflect a transition into an alternate stable state (Shlisky et al., 2007; Keeley and Pausas, 2019; Mantero et al., 2020). For example, if prescribed fire is used too frequently (i.e., short fire return intervals) this could reduce nutrient availability, produce unfavorable nutrient stoichiometry for decomposition, and ultimately change plant fuel load dynamics (Ficken and Wright, 2017; Butler et al., 2019; Semenova-Nelsen et al., 2019; Hopkins et al., 2020). These changes in management regimes could have profound influences on carbon (Johnson and Curtis, 2001; Certini, 2005; Yuan et al., 2019) and nutrient cycles (Toberman et al., 2014; Butler et al., 2018; He et al., 2020) that scale to impact the bioregion and globe (Archibald et al., 2018; Pausas and Bond, 2020). While this work highlights the importance of viewing management from a holistic perspective, further integration of unconsidered below- and aboveground components is necessary.

Ecosystems are comprised of above- and belowground components whose interactions drive their underlying ecological processes. In this study we illustrated how the interplay between soil characteristics, nutrients, and fungi, influence nutrient cycles and microbial activity. Future work should consider the contribution of other soil biota that contribute to nutrient cycles and belowground processes (e.g., bacteria and archaea) in belowground systems. This may explain some of the direct effects of management on soil enzymes since bacteria and archaea also contribute to nutrient cycle and other belowground processes (Graham et al., 2016; Fierer, 2017; Otwell et al., 2018; Anthony et al., 2020). Both groups help shape nutrient cycles and impact aboveground components like plant communities (Mendes et al., 2013; Bauer et al., 2015) which govern ecosystem productivity (van der Heijden et al., 2008; Schnitzer et al., 2011). In addition to considering interactions between above- and belowground components, future work would also benefit from inclusion of untreated (no management) controls to assess how natural seasonal variation influences the system of belowground relationships. Quantifying this baseline may be particularly important as global change alters components (i.e., climate) beyond normal limits.

In summary, we show that land management has cascading effects on nutrient cycles that are mediated by changes to abiotic and biotic soil components. Further, we illustrate how management technique (burning vs. mowing) has different effects on belowground systems that are not apparent when viewed individually. When viewed as a system, belowground relationships respond to land management distinctly at different time points, and their resilience is likely a product of the close evolutionary relationship between these disturbances (and human management) in grassland and savanna ecosystems. Given the cascading and dynamic effects of management on belowground systems, it is crucial that land managers and ecologists alike utilize a systems approach to restoration and preservation of ecosystems. Since approximately 40% of Earth's terrestrial systems rely on recurrent fire and grazing for maintenance (McSherry and Ritchie, 2013; Archibald et al., 2018), a better understanding of how management influences the below- and aboveground components of ecosystems can help us respond to expected changes in global disturbance regimes and other anthropogenic influences.

CRediT authorship contribution statement

Jacob R. Hopkins, Tatiana A. Semenova-Nelsen, and Benjamin A. Sikes conceived and designed the experiment. Jacob R. Hopkins and Tatiana A. Semenova-Nelsen performed the experiment. Jacob R. Hopkins analyzed the data. Jacob R. Hopkins and Benjamin A. Sikes wrote the manuscript. Tatiana A. Semenova-Nelsen provided editorial advice.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Jacob R. Hopkins reports financial support was provided by Mycological Society of America. Jacob R. Hopkins reports financial support was provided by University of Kansas Field Station. Jacob R. Hopkins reports financial support was provided by National Science Foundation. Benjamin A. Sikes reports financial support was provided by National Science Foundation.

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.pedobi.2022.150859.

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