



Effects of land-use intensity, grazing and fire disturbances on soil bacterial and fungal communities in subtropical wetlands

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ABSTRACT

Bacteria and fungi are primary components in wetland soil microbial communities and provide essential ecosystem functions and services. Understanding responses of bacterial and fungal communities to multiple drivers of environmental change and their interactions is crucial for wetland conservation and management, particularly for those embedded in agricultural landscapes. Yet little is known about effect of agricultural land use and wetland management on soil microbial communities in subtropical seasonal wetlands. Here, we used a long-term whole-ecosystem wetland experiment to examine individual and interactive effects of upland land-use intensification, livestock grazing, and prescribed fire on soil bacteria and fungi. We asked: (1) How do land-use intensification, grazing and fire disturbances interact to alter taxonomic composition and functional potential of wetland soil bacterial and fungal communities? (2) To what extent would these management and disturbance effects on wetland microbial communities manifest through alterations in soil properties? Our results showed that both microbial taxonomic and functional composition are responsive to agricultural land use and wetland management. Upland land-use intensification was the strongest driver (as compared to fire and grazing) in shaping bacterial and fungal community composition. Specifically, land-use intensification increased functional richness of both bacteria and fungi, whereas grazing and fire only interactively affected bacterial functional richness. In addition, responses of bacterial and fungal species diversity to wetland management varied, where grazing and fire reduced fungal species diversity in wetlands embedded in low-intensity managed pastures, but none of these management practices altered bacterial species diversity. Further, we found that pH and secondary nutrients (i.e., Ca and Mg) availability were the most important soil properties that explain how agricultural land use and wetland management drive the composition of bacterial and fungal communities. Our findings suggest that integration of lime application into intensified land uses to neutralize soil pH could facilitate maintenance of microbial diversity and associated functions. Our results highlight the need to comprehensively assess management impacts on soil microorganisms, rather than using a single or few indicators, due to inconsistent responses of bacterial and fungal communities, as well as their varied taxonomic and functional responses.

1. Introduction

Geographically isolated wetlands are disproportionately valuable landscape elements that provide critical functions such as biodiversity support, flood mitigation, carbon sequestration, sediment and nutrient retention (Cohen et al., 2016; Marton et al., 2015; Rains et al., 2016). Ecosystem functions and services delivered by geographically isolated wetlands are particularly important in North American landscapes,

where wetlands occupy a significant proportion of agricultural landscapes (O'Connell et al., 2013; Tiner, 2003). Therefore, these wetlands serve as important linkages between agricultural production, natural communities, and rural livelihoods (Swain et al., 2013). However, these wetlands are facing constant threats from anthropogenic activities, including upland intensified land uses, altered hydrology and disturbance regimes, and introduced livestock grazing (McCauley et al., 2015; Tiner, 2003). For example, upland land-use intensification (e.g.,

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fertilization, extensive drainage) often results in increased nutrient runoff and eutrophication, greater hydrologic variability, and biodiversity loss in wetlands (Capece et al., 2007; Jansen et al., 2019; Shukla et al., 2019, Fig. S1). Grazing can also alter wetland plant community composition via dietary preferences and trampling effects from livestock (Boughton et al., 2010; Jones et al., 2011, Fig. S1), which further modify soil properties by increasing soil compaction and incorporating surface litter into mineral soils (Ho et al., 2018, Fig. S1). Furthermore, wetlands embedded in agricultural grasslands are often affected by prescribed fire, which is a common practice to prevent woody plant encroachment and reduce fuel accumulation (Boughton et al., 2016).

Prior research has well documented the effects of land use and agricultural management practices on wetlands from the perspective of regional hydrology, biogeochemical cycling, plant and animal communities (Boughton et al., 2016; DeLucia et al., 2019; Ho et al., 2018; Jansen et al., 2019; Marty, 2005; Upadhyay et al., 2019a; Upadhyay et al., 2019b). Nevertheless, it remains less clear on how those anthropogenic activities could interactively alter wetland microbial communities, which control the majority of biological processes in soils, regulate biogeochemical cycling of carbon and key nutrients, and thus play vital roles in climate mitigation, pollution removal, and overall stability of wetlands (Chandra et al., 2020; Upadhyay et al., 2019b). In particular, very little work has examined how multiple anthropogenic drivers, including land-use intensification, grazing and fire disturbances, could affect wetland microbial communities, and whether there are any interactions (e.g., synergistic or antagonistic) among these drivers. Such knowledge is especially scarce for subtropical and tropical wetlands that are in general among the least studied and underrepresented compared to other biographic regions (e.g., temperate) (Veach et al., 2021). Understanding the responses of wetland soil microorganisms to different agricultural management is critical to inform sustainable wetland management and conserve ecosystem functions and services provided by wetlands.

Bacteria and fungi are the predominant microbial groups mediating biogeochemical cycling of carbon (C), nitrogen (N), phosphorus (P), and sulfur (S) in sediments and soils, where they function either as decomposers (e.g., heterotrophs) or primary producers (e.g., autotrophs) (Lamers et al., 2012). Different microbial taxonomic groups and their metabolic activities collectively determine the overall wetland functioning in terms of organic matter decomposition, N fixation, nitrification, denitrification, methane oxidation, etc. (Iqbal et al., 2019; Yarwood, 2018). Previous studies have revealed that land-use intensification and grazing profoundly altered the composition of soil microbial communities in diverse grassland and forest ecosystems (Jangid et al., 2011; Praeg et al., 2020; Xu et al., 2017; Yang et al., 2013). For example, as a result of fertilization, increases in soil nutrients and plant biomass may result in greater mineral nutrients and organic carbon input, which can shift soil microbial community composition, such as by inhibiting growth of mycorrhizal fungi (Leff et al., 2015, Fig. S1), and stimulating growth of other heterotrophic groups (Soong et al., 2020). In addition, prescribed fire might lead to reduction of microbial biomass and associated enzyme activities through mineral nutrient release and increase of soil pH (Fontúrbel et al., 2012; Switzer et al., 2012), although the magnitude of these effects has been found to differ in bacterial and fungal communities (Sun et al., 2011). Hence, the specific composition and structure of bacterial and fungal communities are believed to reflect the changes in soil redox potential, nutrient status, and even soil-plant interactions, thus acting as sentinels for the influence of anthropogenic environmental changes (Leff et al., 2015; Y. Y. Liu et al., 2021; L. Liu et al., 2021; Wagg et al., 2018). Nevertheless, studies on effects of land-use intensification, grazing and fire disturbances have thus far primarily focused on terrestrial ecosystems such as forests and grasslands (Yang et al., 2021; Zhou et al., 2020). Similar effects and mechanisms might govern wetland microbial communities but those as yet remain elusive.

In addition, it was suggested that microbial functional responses to

disturbances and other transient environmental changes might be subtle or different from taxonomical responses because of the resistance, resilience, and functional redundancy of microbial communities (Allison and Martiny, 2008). Further, seasonal wetlands, as transitional ecosystems between aquatic and terrestrial systems, may support high variability of microbial communities under a wide range of environmental conditions and thus reduce their functional sensitivity to human disturbances (Waldrop and Firestone, 2006). It is therefore of interest to evaluate both taxonomic composition and functional potential of the soil microbiome, and their responses to human disturbance and land management, especially in subtropical seasonal wetlands with distinct intra-annual variabilities in water levels due to seasonal climatic conditions. Improved knowledge on the microbial functional responses to anthropogenic environmental changes will better predict the consequences of agricultural land management for ecosystem functions and services in the subtropical and tropical wetlands.

To improve understanding of how subtropical wetland soil microbiomes are affected by land-use intensification, livestock grazing, and fire disturbances, we investigated geographically isolated wetlands embedded in managed grazing lands in south-central Florida, USA. We analyzed bacterial and fungal community composition using high-throughput sequencing of bacterial 16 S ribosomal RNA (16 S rRNA) and fungal ribosomal internal transcribed spacer (ITS) genes (Schirmer et al., 2015). We also mapped ecologically relevant functions of identified bacteria and fungi based on two large, annotated functional gene databases, FAPROTAX and FunGuild (Louca et al., 2016; Nguyen et al., 2016), to examine potential microbial functional responses, which were widely used to assign potential functions of soil microbial communities in numerous recent studies (e.g., Fahey et al., 2020; Morillo et al., 2022; Wang et al., 2021; Xu et al., 2021). Our specific research questions are: (1) How do land-use intensification, grazing and fire disturbances interact to alter taxonomic composition and functional potential of wetland soil bacterial and fungal communities? (2) To what extent would these management and disturbance effects on wetland microbial communities manifest through alterations in soil properties? Our main hypothesis is that land-use intensification, grazing and fire would induce shifts in soil microbial taxonomic composition, but depending on the changes in microbial communities, these shifts may or may not lead to significant changes in functional potential within those communities. We also expect that land-use intensification and grazing may exert stronger effects on microbial communities than prescribed fire, because intensification and grazing are press disturbances that exert long-term effects on soil properties, whereas fire is considered as a pulse disturbance whose effects tend to be transient, temporary, and recoverable. Findings from our research are intended to improve current understanding of microbial responses to individual and interactive effects of major global change drivers in subtropical wetland ecosystems and foster their conservation and sustainable management.

2. Material and methods

2.1. Study area

Our study was conducted at the Buck Island Ranch (BIR, 27°09'N 81°11'W) of the Archbold Biological Station in south-central Florida. BIR is a full-scale commercial cow-calf ranch operation and a part of USDA funded Long-Term Agroecosystem Research (LTAR) Network serving as a living laboratory for agroecology research. The wetland soils within the ranch are primarily mucky fine sands with high organic matter content. The climate of the region is humid subtropical with distinct wet (i.e., May-October) and dry (i.e., November-April) seasons. Mean annual temperature is 22.5 °C and precipitation is 1360 mm, of which 75% falls during the wet season. Combination of wet climate and high water table results in more than 600 isolated seasonal wetlands distributed across the landscape (i.e., comprising 15% area of BIR), which typifies the integrated grassland-wetland mosaic for this region

(Gathumbi et al., 2005). Hence, wetlands embedded within grazing lands are heavily influenced by surrounding upland grassland management practices (Boughton et al., 2016, 2011; DeLucia et al., 2019; Gomez-Casanovas et al., 2020; Medley et al., 2015). BIR is characterized by two dominant agricultural land-use intensities: (1) intensively managed (IM) and (2) semi-natural (SN) pastures (Fig. 1). Intensively managed pastures are heavily drained, planted with non-native forage grasses, intensively cattle-grazed during wet seasons, regularly limed, N-fertilized every 1–2 years, and until 1987 fertilized with P and K. In contrast, SN pastures are less drained, never fertilized or limed, sparsely planted with forage grasses, and moderately grazed during dry seasons (Boughton et al., 2011).

2.2. Experimental design and soil sampling

To investigate interactive effects of three environmental change drivers (i.e., land-use intensity, grazing, and prescribed fire) on wetland soil microbial communities, we used a long-term whole-ecosystem

experiment that consists of 40 seasonal wetlands with eight treatment combinations ($2 \times 2 \times 2$ complete factorial design) in a randomized block design (5 blocks). Details of the original experimental design and treatments can be found in Boughton et al., (2016) and Guo et al. (2021). In brief, 40 wetlands with similar size (0.5 – 1.5 ha) and hydroperiods (2 – 10 months) randomly distributed across the entire ranch were selected (Fig. 1). Water depth of these wetlands ranged from 27 to 49 cm on average (Jansen et al., 2019). Half of the wetlands were located within IM pastures and the remaining half in SN pastures. Wetlands in SN pastures have more diverse plant species and are dominated by native perennial species, such as *Panicum hemitomon*, *Rhynchospora inundata*, *Diodia virginiana*, *Sagittaria lancifolia*, *Pontedaria cordata*. Wetlands in IM pastures have relatively low plant richness and are dominant by exotic and annual species, such as *Juncus effusus* var. *solutus*, *Persicaria punctata*, and *Pontedaria cordata* (Boughton et al., 2011). Further, water in IM wetlands tended to have higher conductivity and higher N and P levels than water in SN wetlands (Jansen et al., 2019). For the ungrazed treatment, fences were installed in 2007 in 10 wetlands in IM and SN

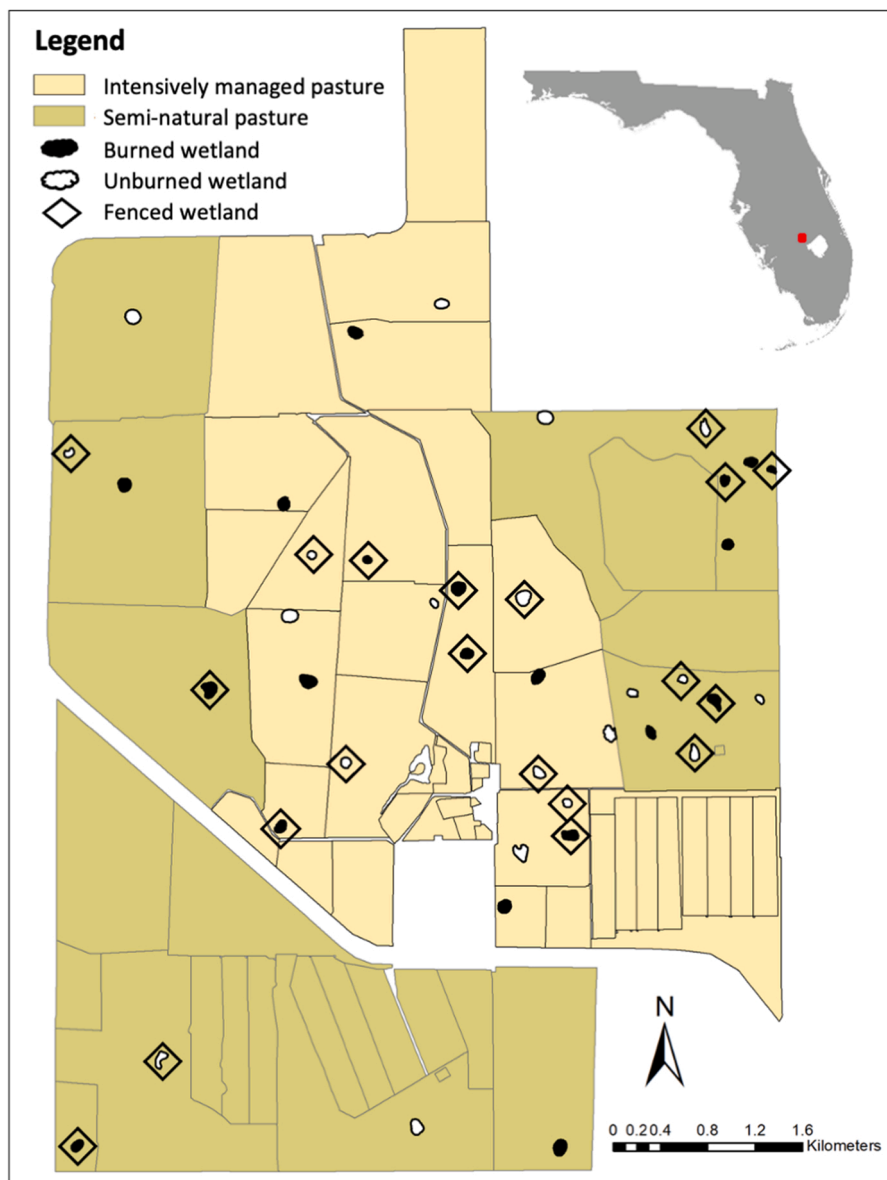


Fig. 1. Map of the 40 experimental wetlands at Archbold Biological Station's Buck Island Ranch. Intensively managed (IM) pastures are shaded light yellow, and semi-natural (SN) pastures are shown in green. Fenced (ungrazed) wetlands are surrounded by black diamond-shaped enclosure whereas grazed wetlands are shown without diamond-shaped enclosure. Burned wetlands are filled with black color and unburned wetlands are filled with white color.

pastures, respectively, to exclude cattle grazing. In grazed wetlands, grazing was imposed in cattle foraging activities at typical local stocking rates. Vegetation in grazed wetlands tended to show greater abundance of short graminoids and forbs, including *Luziola fluitans*, *Eleocharis vivipara* and *Bacopa caroliniana*, than fenced wetlands, where were not disturbed by grazing activities and allowed growth of taller hydrophytes such as *P. hemitomom* and *Sacciolepis striata* (Boughton et al., 2011; 2016). For prescribed fire, burn treatment was imposed to 5 random wetlands in each combination of land-use intensity \times grazing treatments during the dry season of 2008, 2011, and 2013–2014, 2017–2018, and 2020 that mimicked local fire return intervals. To implement the fire treatment, standard drip torch and overlapping strip lines were used to ignite fires and ensure that $> 80\%$ of wetland was burned. Fire has been shown to interact with grazing to increase abundance of shrubs in ungrazed wetlands but exert no effect on shrubs in grazed wetlands (Boughton et al., 2016). In addition, fire caused a pulse release of P to wetland water but a reduction of N due to volatilization of organic materials (Jansen et al., 2019).

Soil samples were collected in March and June 2019 to capture distinctive seasonal variations. At each sampling effort, three soil cores (5-cm depth \times 10-cm diameter) per wetland were collected along a hydrological gradient of the edge, middle and center point of the wetlands. A detailed sampling protocol can be found in (Guo et al., 2021). Replicate soil cores collected from the same wetland were then mixed together and homogenized thoroughly to represent the overall condition of the entire wetland. At the time of soil sampling, soil temperature was measured using a soil temperature probe (Traceable®, Fisherbrand) with mean wetland soil temperature of 18.97 °C in March and 25.10 °C in June. Subsamples for microbial analyses were prepared and immediately placed on dry ice for transportation, and stored at -80 °C until DNA extraction.

2.3. Soil physiochemical property measurement

Soil subsamples for physiochemical analyses were stored in plastic bags at 4 °C. Samples were sieved through a 2-mm mesh in lab and used to determine water content, pH, organic matter content, total C and N content, and Mehlich-3 (plant-available) P, K, Ca, and Mg content. Soil water content was measured gravimetrically by weight loss after oven drying at 105 °C for 72 h. Soil pH was measured in a 1:1 soil/distilled water solution using a hand-held HANNA pH meter (Hanna Instruments, CA) (Thomas, 1996). Soil organic matter was measured by mass loss after 4-hr high-temperature oxidation in 450 °C muffle furnace (Schulte and Hopkins, 2015). Soil total C and N content were measured using LECO CN628C/N analyzer (LECO Corporation, MI). Plant-available P, K, Ca and Mg were extracted with Mehlich-3 solution (Mehlich, 1984), and analyzed using a Perkin Elmer Avio 200 inductively coupled plasma atomic emission spectrometer (Perkin-Elmer, CT).

2.4. DNA extraction, sequencing, and taxonomic assignment

Genomic DNA of soil microbes was extracted from 0.25 g of each soil sample using DNeasy PowerSoil extraction kit (Qiagen, Norway). The V3-V4 region of bacterial 16 S rRNA and fungal ITS4 genes were amplified using a two-step polymerase chain reaction (PCR) protocol according to Chen et al. (2021) and primer sets of 341 F (5'-CC TACGGGNGGCWGCAG-3') / 806 R (5'-GGACTACHVGGGTWTCTAA T-3') and ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') / ITS4 (5'-TCCT CCGCTTATTGATATGC-3'), respectively. Quality and quantity of DNA, and size of PCR products was verified using a UV/VIS spectrophotometer (NanoDrop™, ThermoFisher Scientific, Waltham, MA, USA) and 1.7% (w/v) agarose gel electrophoresis. All 160 barcoded PCR products were pooled and sequenced at Duke Center for Genomic and Computational Biology (GCB, Durham, NC, USA) in a single library using Illumina Miseq v3 kits (paired-end 2 \times 300 bp, Illumina Inc., San Diego, CA, USA). The raw sequences were deposited in the public NCBI

Sequence Read Archive (SRA) database under the BioProject number PRJNA824538.

The amplicon sequencing data were processed in QIIME2 (version 2020.2). After denoising by DADA2 (Callahan et al., 2016) and quality filtering 1735,406 high-quality 16 S rRNA gene, and 561,066 ITS gene reads were obtained. For taxonomic identification quality-filtered and non-chimeric sequences were aligned to the SILVA version 132 (Quast et al., 2013) and UNITE version 8.0 (Kõljalg et al., 2013) databases, respectively. Operational taxonomic units (OTUs) were determined at the 99% similarity level of the nucleotide sequences. Non-bacterial and non-fungal OTUs were removed for downstream analyses. Bacterial and fungal functional annotations were assigned according to FAPROTAX (Louca et al., 2016) and FunGuild (Nguyen et al., 2016) databases, respectively, which have been proved as promising and robust tools for ecological relevant function predictions of bacterial and fungal taxa derived from 16 S and ITS rRNA amplicon sequencing (Nilsson et al., 2019; Sansupa et al., 2021). Specifically, the tables of frequency of bacterial and fungal taxa at genus level were used as inputs and converted into putative functional tables by FAPROTAX and FunGuild databases, respectively. For beta diversity analysis the OTU tables were randomly rarefied to 6000 and 600 reads per sample for bacterial 16 S rRNA and fungal ITS genes, respectively, according to the rarefaction curves (Fig. S2).

2.5. Statistical analyses

Taxonomical composition and functional potential of bacterial and fungal communities were assessed using non-metric multidimensional scaling (NMDS) based on Bray-Curtis dissimilarity matrix via the “metaMDS” function from the *vegan* package (Oksanen et al., 2020) in R (version 4.1.2). Differences in community composition in OTU or functional groups across wetland treatments (i.e., land-use intensity, cattle grazing, prescribed fire, and their interactions) were evaluated using permutational multivariate analysis of variance (PERMANOVA) with 999 permutations with the “adonis” function of the *vegan* package (Oksanen et al., 2020). We also applied NMDS analysis based on weighted and unweighted UniFrac distances to account phylogenetic information for bacterial and fungal OTU composition (Table S4), and found the PERMANOVA results were overall consistent with those calculated from Bray-Curtis distance.

Alpha diversity of microbial communities was characterized by assessing richness, Shannon diversity index, and Pielou’s evenness at the levels of both OTU and functional group. All diversity indices were calculated using the “diversity” function of the *vegan* package in R. To further test how land-use intensity, grazing, and fire treatments interacted to alter the alpha diversity of microbial communities, we used linear mixed-effects models, in which factorial wetland treatments and their interactions were set as fixed factors, and block and sampling time as random factors, using the “lmer” function of the *lme4* package (Bates et al., 2014). We checked the normality and homogeneity of model assumptions by visual inspection of model residuals, and applied log or square root transformation to response variables when necessary to ensure that all model assumptions were met in the analyses. Type III sums of squares (SS) were used to calculate *F* and *p* value to determine the significance of fixed factors. Pairwise contrasts and Tukey’s test were conducted for post-hoc analysis to compare means where significant interactions were detected, using the *lsmeans* package (Lenth, 2016).

Further, effects of wetland treatments on relative abundance of bacterial and fungal OTU and functional groups were examined by the analysis of composition of microbiomes (AMCOM) (Mandal et al., 2015). Taxonomic and functional groups were filtered for abundance (account at least 0.1% of total reads) and prevalence (present in at least 10% of samples). We performed ANCOM-II using the R implementation of Lin (2021) that was designed to process data with excess zeros while controlling false discovery rate (FDR). Moreover, ANCOM-II allows to adjust for the effects of covariates and accounting random effects, such as the

block and sampling time effects in our study. Differentially abundant groups were selected if their W values surpassed the 0.7 threshold.

Finally, the relationship of microbial alpha diversity and relative abundances of dominant microbial phyla and functional groups with soil physiochemical properties was analyzed using Spearman's rank-order correlation. Due to strong associations (correlation coefficients > 0.9) between soil total C, total N, and OM, we did not include soil total C and total N into all the correlation analyses. A significance level at $p < 0.05$ was used unless otherwise specified.

3. Results

3.1. Wetland microbial taxonomic and functional groups

In total, 27 bacterial phyla (100% of quality-filtered sequences), 72 classes (97.9%), 156 orders (93.9%), 206 families (74.6%) and 257 genera (54.4%) were identified across all 40 wetland soil samples. The most abundant bacterial phyla across all wetland soil samples were Proteobacteria (31.6%), Acidobacteria (16.8%), Actinobacteria (12.3%), Chloroflexi (10.2%), and Firmicutes (8.3%) (Fig. S3). At the class level, the bacterial communities were dominated by Alphaproteobacteria (16.4%), Acidobacteriia (14.5%), Gammaproteobacteria (8.6%), Deltaproteobacteria (6.6%), and Bacilli (6.4%) (Fig. S4). In the bacterial dataset, 21.3% of OTUs were identified as having known putative functions based on the FAPROTAX database. Among the total 37 identified bacterial functional groups, the most abundant ones were chemoheterotrophs (32.0%), aerobic chemoheterotrophs (31.1%), and cellulolytic bacteria (17.0%). A complete list of bacterial functional groups is shown in Table S1.

For fungal communities, the numbers of identified phyla, classes, orders, families, and genera were 6, (65.3% of quality-filtered sequences) 12 (46.4%), 22 (43.5%), 42 (39.7%), and 57 (35.0%), respectively. The most abundant fungal phyla were Ascomycota (32.7%), followed by Basidiomycota (22.3%) and Rozellomycota (9.0%) (Fig. S5). At the class level, Tremellomycetes (20.7%), Dothideomycetes (11.2%), and Sordariomycetes (9.5%) were most frequent (Fig. S6). In the fungal dataset, 15.5% of OTUs were identified as having at least one known guild in the FUNGuild database. Among the total 22 identified fungal guilds, the most frequent guilds were plant pathogen (12.4%), undefined saprotroph (11.6%), dung saprotroph (10.6%), and soil saprotroph (9.5%). A complete list of fungal guilds is shown in Table S2.

3.2. Treatment effects on wetland soil microbial composition

Among all wetland treatments, land-use intensification was the strongest driver of bacterial and fungal community composition according to both taxonomic and functional groupings (Table 1).

Table 1

Results of PERMANOVA on Bray-Curtis distance of bacterial OTU, fungal OTU, bacterial functional, and fungal functional communities. Bold values indicate significant coefficients. * indicates $p \leq 0.05$, ** indicates $p \leq 0.01$, *** indicates $p \leq 0.001$.

	Treatment	DF	OTUs composition				Functional groups composition		
			Sum of sq	Pseudo-F	P	Sum of sq	Pseudo-F	P	
Bacteria	Land-use intensity (L)	1	0.552	1.15	0.042 *	0.210	7.38	< 0.001 * **	
	Grazing (G)	1	0.456	0.95	0.715	0.038	1.33	0.237	
	Fire (F)	1	0.460	0.96	0.635	0.009	0.33	0.878	
	L × G	1	0.464	0.97	0.616	0.015	0.52	0.702	
	L × F	1	0.522	1.09	0.121	0.022	0.77	0.494	
	G × F	1	0.566	1.18	0.035 *	0.030	1.07	0.332	
	L × G × F	1	0.535	1.12	0.109	0.028	0.97	0.362	
	Fungi	Land-use intensity (L)	1	0.616	1.24	0.045 *	1.041	2.51	0.010 * **
	Grazing (G)	1	0.474	0.95	0.625	0.349	0.84	0.566	
Fire (F)	1	0.489	0.98	0.373	0.324	0.78	0.638		
L × G	1	0.461	0.93	0.796	0.381	0.92	0.502		
L × F	1	0.445	0.90	0.982	0.898	2.16	0.016 *		
G × F	1	0.495	1.00	0.349	0.322	0.78	0.607		
L × G × F	1	0.446	0.90	0.980	0.179	0.43	0.922		

Specifically, land-use intensification exerted individual effects on bacterial OTU composition (Fig. 2A, $p < 0.05$), bacterial functional group composition (Fig. 2C, $p < 0.001$), and fungal OTU composition (Fig. 2B, $p < 0.05$). In addition, land-use intensification also showed interactive effects with prescribed fire to alter fungal functional group composition (Fig. 2D; $p < 0.005$). Finally, cattle grazing and prescribed fire interactively affected bacterial OTU composition (Fig. 2A, $p < 0.05$).

3.3. Treatment effects on wetland microbial alpha diversity

Bacterial OTU alpha diversity was not affected by any of the treatments (Table 2). Fungal OTU richness and Shannon's diversity were interactively affected by land-use intensity, cattle grazing, and prescribed fire (Table 2). Specifically, in SN and unburned wetlands, grazing treatment reduced fungal richness by 53.6% (Fig. 2A; $p < 0.01$) and reduced Shannon diversity by 50.7% (Fig. 2B; $p < 0.01$). In SN and ungrazed wetlands, fire treatment resulted in a 53.2% lower fungal richness (Fig. 2A; $p < 0.01$) and a 51.3% lower fungal Shannon diversity (Fig. 2A; $p < 0.01$).

Although bacterial OTU alpha diversity did not respond to wetland treatments (Table 2), bacterial functional diversity metrics were affected individually by land-use intensity, and interactively by grazing and fire (Table 3). Specifically, land-use intensification increased bacterial functional richness by 22.1% (Fig. 4A; $p < 0.01$) and bacterial functional Shannon diversity by 13.4% (Fig. 4B; $p < 0.01$). In addition, in unburned wetlands, grazing decreased bacterial functional richness by 16.8% (Fig. 5; $p < 0.05$). In ungrazed wetlands, fire led to a 22.3% lower bacterial functional richness (Fig. 5; $p < 0.01$). Fungal functional richness showed a similar response to land-use intensification as the bacterial functional richness where IM wetlands had 50.7% more fungal guilds than SN wetlands (Fig. 4C; $p < 0.05$).

3.4. Treatment effects on relative abundance of wetland microbial groups

The relative abundance of bacterial taxa was affected by wetland treatments, including 4/27 phyla, 7/72 classes, 12/156 orders, 15/206 families, and 9/257 genera. For example, at the phylum level, the relative abundance of Bacteroidetes was > two times higher in IM wetlands than in SN wetlands (Fig. S7A), while the relative abundance of Acidobacteria was lower in IM wetlands (Fig. S7A). Two major bacterial phyla responded to livestock grazing, with relative abundance of Actinobacteria being higher under grazing (Fig. S7B) and that of Proteobacteria relatively higher without grazing (Fig. S7B). No differences in relative abundance of bacterial phyla were detected in the fire treatment. At the class level, IM wetlands contained a greater relative abundance of Bacteroidia, but lower relative abundances of Acidobacteriia, Ktedonobacteria, and Verrucomicrobiae than SN wetlands

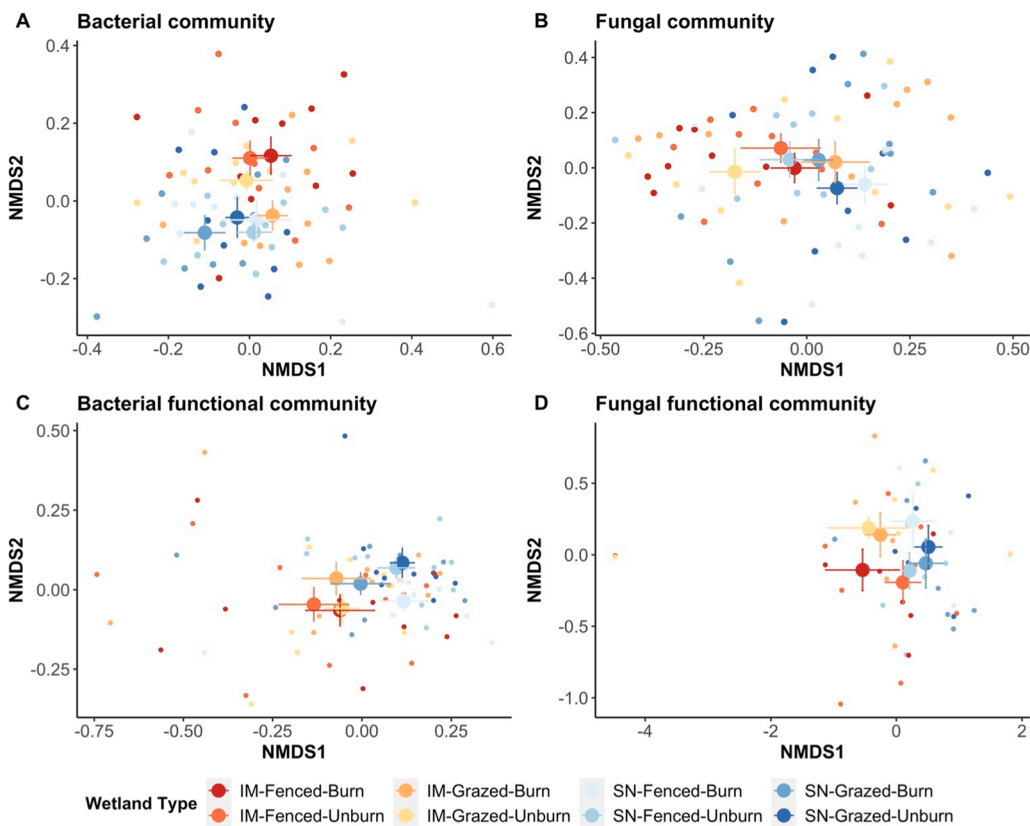


Fig. 2. Nonmetric multidimensional scaling ordinations of bacterial (A), fungal (B), bacterial functional (C), and fungal functional (D) community dissimilarities using Bray-Curtis distance matrix (A: stress = 0.188, B: stress < 0.05, C: stress = 0.142, and D: stress = 0.135) among land-use intensity, grazing and fire treatments. Each small point indicates one wetland soil sample. Larger circle points with error bars indicate the mean \pm SE for each combination of treatment (N = 10; i.e., 5 replicates for each wetland type \times 2 sampling times).

(Fig. S8A). In addition, cattle grazing reduced the relative abundances of Alphaproteobacteria and Gammaproteobacteria (Fig. S8B), and prescribed fire tended to reduce the relative abundance of Clostridia (Fig. S8C).

The relative abundance of only one fungal taxon was affected by land-use intensity, (1/12 classes, 1/22 orders, 1/42 families, and 1/57 genera). The relative abundance of fungal class Sordariomycetes, order Sordariales, families Lasiosphaeriaceae, genus *Arniium* were greater in SN wetlands than in IM wetlands (Fig. S9).

No bacterial or fungal functional groups significantly differed in their relative abundance under the cattle grazing or prescribed fire treatments ($p > 0.05$). On the other hand, the relative abundance of three out of 37 bacterial functional groups and one out of 22 fungal guilds were affected by land-use intensity. For example, the relative abundance of aerobic chemoheterotrophs, cellulolytic bacteria, and dung saprotrophs were greater in SN wetlands than in IM wetlands (Fig. 6), while nitrogen-fixing bacteria had higher relative abundance in IM wetlands (Fig. 6).

3.5. Relationship of wetland microbial community features with soil properties

To assess potential indirect pathways of treatment effects via alterations in soil physiochemical properties (as treatments have been shown to affect soil properties; Table S3), we further analyzed relationships of wetland microbial community with selected soil variables. Our results showed that soil pH, which was responsive to three interactive treatments (Table S3), appeared to be the most strongly correlated factor among all soil properties with microbial alpha-diversity, where Shannon's diversity and evenness of fungal OTUs and bacterial functional groups were all positively correlated with soil pH (Fig. 7). Soil Ca and Mg content, which were responsive to individual upland land-use intensification and cattle grazing (Table S3), showed positive correlations with microbial functional diversity, while soil P, K, OM, and water contents showed negative correlations with microbial OTU diversity

(Fig. 7).

Soil properties were also significantly correlated with the relative abundance of certain dominant bacterial and fungal phyla (Fig. 8). For example, the relative abundance of Bacteroidetes was highly positively correlated with soil Ca and Mg contents and pH (Fig. 8), whereas the relative abundance of Acidobacteria was negatively correlated with soil Ca content and pH (Fig. 8).

Compared to correlations of soil properties with alpha-diversity and certain dominant microbial phyla at the OTU level, only a few soil factors (e.g., C/N ratio, P and Mg contents, and pH) showed significant correlations with relative abundance of dominant microbial functional groups (Fig. 9). For example, soil pH was positively correlated with relative abundance of aerobic ammonia oxidizers and general nitrifiers, but negatively correlated with cellulolytic bacteria, aerobic chemoheterotrophs, and general chemoheterotrophs (Fig. 9).

4. Discussion

In this research, we investigated individual and interactive effects of land-use intensification, grazing and fire disturbances on soil bacterial and fungal communities in subtropical seasonal wetlands, and assessed potential mechanisms of treatment effects via alterations in soil physiochemical properties. Overall, our results showed that both microbial taxonomic and functional composition responded to agricultural land use and wetland management.

4.1. Effects of wetland management on soil microbial taxonomic composition

Bacteria. Changes in bacterial community composition were reflected in the shifts of relative abundance among taxonomic groups (Fig. S3, S4, S7, and S8), but did not translate to effects on the alpha diversity (Table 2), suggesting a 'reshuffle' without simplification of bacteria communities due to land-use intensification. Indeed, at the

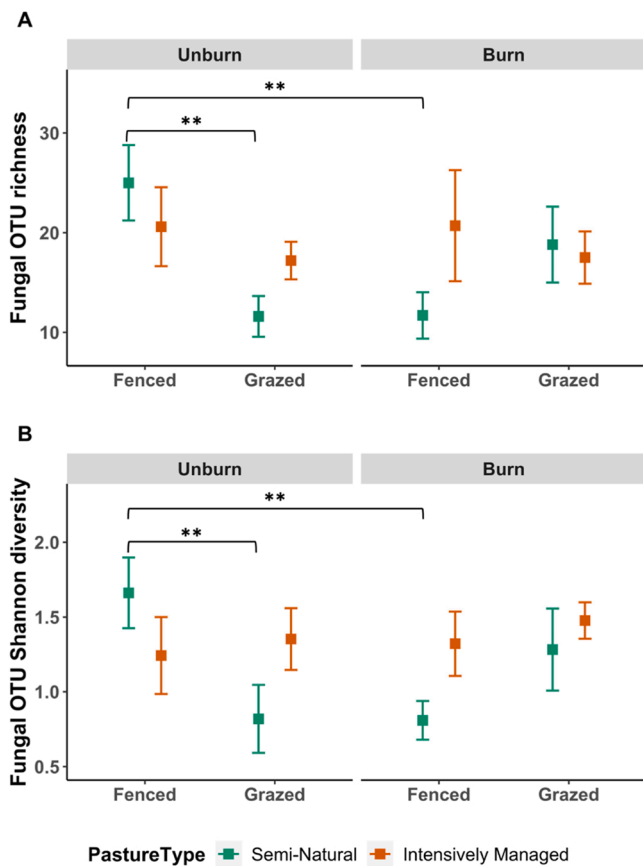


Fig. 3. Effects of land-use intensity, cattle grazing and prescribed fire on fungal OTU richness (A) and fungal OTU Shannon diversity (B) (mean ± SE; N = 10). Pairwise significant differences were determined by the Tukey post hoc test. * indicates $p \leq 0.05$, ** indicates $p \leq 0.01$.

phylum level, we detected that upland land-use intensification increased the relative abundance of Bacteroidetes but decreased the relative abundance of Acidobacteria in wetland soils (Fig. S7A). Since the upland land-use practices were not directly applied within the seasonal wetlands, but on their surrounding pastures, effects of land-use intensification on wetland microbes likely manifested indirectly through altered soil physiochemical properties, hydrology, and vegetation (Banerjee et al., 2019; Boughton et al., 2016; Ho et al., 2018; Yang et al., 2021, Fig. S1). Our analyses indeed showed strong positive correlations between relative abundance of Bacteroidetes with soil pH, and Ca and Mg

contents (all of which were affected by land-use intensification and cattle grazing; Table S3) (Fig. 8), and a strong negative correlation between relative abundance of Acidobacteria and soil pH (Fig. 8). Our results were also consistent with findings from Lauber et al. (2009) and Jeanbille et al. (2016) that soil pH and availability of secondary nutrients (i.e., Ca and Mg) are large drivers of the structure of bacterial community (Fig. S1).

Moreover, such shifts among dominant bacterial groups due to upland management intensification (i.e., nutrient enrichment and pH neutralization) could also be detected and manifested at lower taxonomic levels. For example, relative abundance of the class Bacteroidia was greater in IM wetlands, while the class Acidobacteriia, Ktedonobacteria, and Verrucomicrobiae were less abundant in IM wetlands (Fig. S8). Therefore, our results indicated that wetlands embedded in intensively managed agricultural landscapes, which were characterized by more neutral pH and higher nutrient contents resulting from receiving lime and fertilizer runoff, would favor phylum Bacteroidetes or in particular class Bacteroidia, and suppress Acidobacteria and class Ktedonobacteria and Verrucomicrobiae. These results aligned with the ecological classification of bacterial groups according to Fierer et al. (2007), in which Bacteroidetes are considered copiotrophs (i.e., tend to perform well in nutrient rich environment), whereas Acidobacteria, Ktedonobacteria, and Verrucomicrobiae are considered oligotrophs (i.e., thrive in environments with low nutrient levels).

Fungi. Effects of wetland management on overall fungal community composition were reflected in both altered alpha diversity (Fig. 3, Table 2) and shifts of taxonomic composition (Fig. S5, S6, and S9). The significant responses of fungal alpha diversity to interacted wetland treatments revealed that soil bacteria and fungi differentially responded to land management, because bacterial alpha diversity metric were not affected by management practices, even though wetland treatments significantly affected soil properties (Table S3), as well as water quality (Jansen et al., 2019) and plant communities (Boughton et al., 2016). Reduction in fungal diversity but unchanged bacterial diversity in response to intensive agricultural land management has been found consistently in other studies (Choudhary et al., 2018; Wagg et al., 2018). This has been attributed to greater versatility of metabolism in bacteria than fungi (Paul, 2014), and thus different parts of bacterial communities succeeding depending upon the environmental conditions. On the other hand, fungi are mainly obligatory osmotrophic aerobic heterotrophs, and thus not able to quickly shift in response to changing environmental conditions (e.g., nutrient loading and hydrological regime, oxygen availability) (Engelhardt et al., 2018; Leff et al., 2015). Indeed, correlations between microbial diversity and soil properties (Fig. 7) also supported this idea, where only fungal OTU Shannon diversity showed significant negative correlations with soil P and OM content, whereas bacterial OTU Shannon diversity did not (Fig. 7).

Table 2

Results of linear mixed-effects models of land-use intensity, cattle grazing, and prescribed fire effects on bacterial and fungal OTUs alpha diversity metrics (richness, Shannon diversity index, and Pielou's evenness). Bold values indicate significant coefficients. * indicates $p \leq 0.05$.

	Treatment			Richness		Shannon diversity		Evenness	
		numDF	denDF	F value	P	F value	P	F value	P
Bacteria	Land-use intensity (L)	1	71	0.45	0.51	0.08	0.78	0.48	0.49
	Grazing (G)	1	71	0.57	0.45	0.13	0.72	0.01	0.94
	Fire (F)	1	71	0.00	0.98	0.20	0.65	0.06	0.81
	L × G	1	71	1.56	0.22	1.00	0.32	0.05	0.82
	L × F	1	71	0.06	0.81	0.08	0.77	0.95	0.33
	G × F	1	71	1.31	0.26	1.95	0.17	0.51	0.48
	L × G × F	1	71	0.32	0.57	0.15	0.70	0.25	0.62
	Fungi	Land-use intensity (L)	1	72	0.93	0.34	1.93	0.17	1.30
	Grazing (G)	1	72	1.00	0.32	0.03	0.86	0.24	0.63
	Fire (F)	1	72	0.60	0.44	0.10	0.75	0.03	0.86
	L × G	1	72	0.17	0.68	1.15	0.29	0.86	0.36
	L × F	1	72	0.37	0.54	1.00	0.32	1.58	0.21
	G × F	1	72	4.76	0.03 *	5.29	0.02 *	1.96	0.17
	L × G × F	1	72	4.12	0.05 *	4.62	0.04 *	2.40	0.13

Table 3

Results of linear mixed-effects models of land-use intensity, cattle grazing, and prescribed fire effects on richness, Shannon diversity index, and Pielou's evenness of bacterial and fungal functional groups. Bold values indicate significant coefficients. * indicates $p \leq 0.05$, ** indicates $p \leq 0.01$.

Treatment	numDF	denDF	Richness		Shannon diversity		Evenness		
			F value	P	F value	P	F value	P	
Functional groups of bacteria	Land-use intensity (L)	1	71	8.55	0.005 **	12.13	0.001 **	0.43	0.51
	Grazing (G)	1	71	0.02	0.88	0.01	0.92	0.66	0.42
	Fire (F)	1	71	1.16	0.28	0.10	0.75	1.53	0.22
	L × G	1	71	2.40	0.13	0.81	0.37	0.01	0.94
	L × F	1	71	0.39	0.53	0.74	0.39	0.53	0.47
	G × F	1	71	7.21	0.009 **	2.20	0.14	0.39	0.53
	L × G × F	1	71	0.31	0.58	0.51	0.48	0.02	0.88
Functional groups of Fungi	Land-use intensity (L)	1	72	4.15	0.045 *	0.41	0.52	0.14	0.71
	Grazing (G)	1	72	2.38	0.13	1.57	0.21	1.05	0.31
	Fire (F)	1	72	0.35	0.56	3.55	0.06	3.97	0.05
	L × G	1	72	0.54	0.47	0.18	0.67	0.07	0.79
	L × F	1	72	0.04	0.84	0.00	0.97	0.00	0.97
	G × F	1	72	2.23	0.14	0.38	0.54	0.40	0.53
	L × G × F	1	72	1.51	0.22	1.96	0.17	1.68	0.20

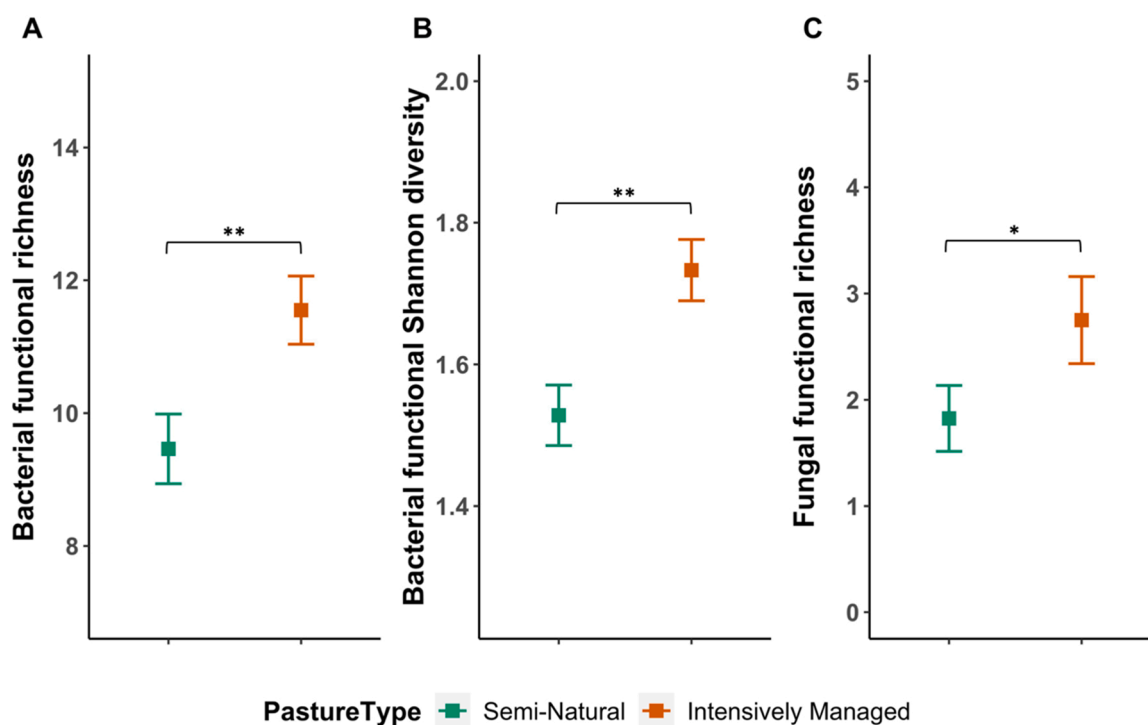


Fig. 4. Effects of land-use intensity on bacterial functional richness (A), bacterial functional Shannon diversity (B), and fungal functional richness (C) (mean \pm SE; N = 40). Pairwise significant differences were determined by the Tukey post hoc test. * indicates $p \leq 0.05$, ** indicates $p \leq 0.01$.

Responses of fungal OTU diversity to wetland treatments might also be due to the stronger coupling between plants and fungi compared to bacteria, as a significant proportion of fungi are symbionts and parasites (Bergelson et al., 2019; Paul, 2014, Fig. S1). Specifically, in the same experimental wetland systems, Boughton, et al., (2016) found that land-use intensification, grazing and fire interactively affected the composition and structure of plant communities. The overall pattern of management effects on plant community was similar to the patterns in our fungal communities. For example, we found cattle grazing and prescribed fire only exerted negative influences on fungal OTU alpha diversity individually in SN wetlands but not in IM wetlands (Fig. 3), while Boughton et al., (2016) observed grazing or fire disturbance only promoted plant alpha diversity in SN wetlands, but did not change plant diversity within IM wetlands. At first glance, grazing and fire disturbances appeared to show opposite effects with increased plant diversity but decreased fungal diversity in SN wetlands. Yet details in the vegetation data might explain this phenomenon. In SN and ungrazed

wetlands, fire lead to a higher diversity of non-native plants (Boughton, et al., 2016). Nevertheless, invasive plants have been found to decrease soil fungal diversity, simplify fungal co-occurrence networks (Zhang et al., 2021), and reduce abundance of fungal mutualists of native plants (Stinson et al., 2006). Although it is unclear whether soil fungal communities are primarily shaped by overall plant composition or by key plant species (Li et al., 2018), such congruence in plant and fungal responses to wetland treatments indicate a strong coupling between wetland plants and fungi, suggesting potential pathways of wetland treatments to affect fungal communities through altering plant communities (Peay et al., 2013; Zhang et al., 2021, Fig. S1).

4.2. Effects of wetland management on soil microbial functional composition

Bacteria and fungi perform diverse ecologically relevant functions, participating in almost every aspect of soil organic matter

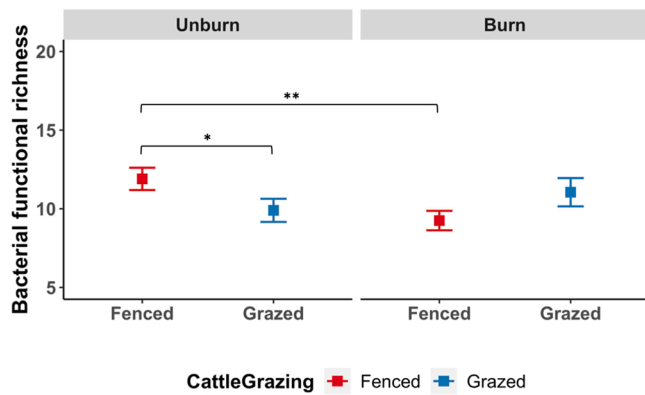


Fig. 5. Effects of cattle grazing and prescribed fire on bacterial functional richness (mean \pm SE; N = 20). Pairwise significant differences were determined by the Tukey post hoc test. * indicates $p \leq 0.05$, ** indicates $p \leq 0.01$.

decomposition, production, and sequestration (Paul, 2014). Although functional annotations were only possible for a relatively small fraction of the microbial communities, the identified functional annotations showed that land-use intensification significantly altered the composition of bacterial functional groups (Table 1, Fig. 2C) by increasing the relative abundance of nitrifiers, aerobic ammonia oxidizers, predatory/exoparasitic bacteria, methylotrophs, methanotrophs, hydrocarbon degraders, aromatic compound degraders, and fermentative bacteria (Fig. S10). These results suggest IM wetlands might have higher denitrification, organic matter decomposition, and methane consumption activities than SN wetlands, which is consistent with results of previous teabag decomposition experiments showing that IM wetlands had more complete organic matter decomposition than SN wetlands (Guo et al., 2021).

We initially expected that microbial functional composition would be more stable and resistant to external disturbance than taxonomic composition because of functional redundancy and metabolic plasticity within wetland soil microbial communities (Allison and Martiny, 2008; Louca et al., 2018). However, our results showed otherwise that wetland microbial functional composition more actively responded to land management and disturbance than taxonomic features. For example, we observed that wetland management did not affect bacterial OTU diversity, but we found upland land-use intensification increased bacterial functional diversity (Fig. 4). Therefore, our results suggest that there

could be a lack of functional redundancy in microbial communities in these subtropical seasonal wetlands. Similarly low functional redundancies have been found in permanently inundated freshwater ecosystems where were strongly influenced by anthropogenic activities (Berga et al., 2012; Liu et al., 2021). However, the mechanisms behind such weakened functional redundancies are not clear yet and need further investigation.

Moreover, we expect that intense disturbances in wetlands could destabilize the functional attributes of soil microbial communities, which possibly resulted in the functional attributes of microbial communities, instead of taxonomic attributes, being more responsive to disturbance and altered environmental factors (Gibbons, 2017). Indeed, we found that soil pH and a few indicators of soil cation exchange capacity (i.e., Ca and Mg) were the main factors shaping the functional structure of bacterial communities, rather than the taxonomic structure (Figs. 7 and 9), which is consistent with results from L. Liu et al. (2021); Y. Liu et al. (2021), Wang et al. (2019), and Xiao et al. (2021). In addition to yielding higher bacterial functional diversity, more neutral pH was also correlated with lower relative abundance of organic matter mineralization-related groups, such as cellulolytic bacteria and chemoheterotrophs. However, we suspect that these results did not necessarily indicate less cellulolysis or chemoheterotrophic activity, because the reduced relative abundance could be due to higher functional diversity and shifts to relatively minor microbial groups in IM wetlands. Nevertheless, studies have proposed that the relationships between microbial community structure, function, and environmental gradients are not static and have important temporal dynamics (Comte et al., 2013; Waldrop and Firestone, 2006). Further long-term time series observations of soil microbes in seasonal wetlands would be required to test and validate those dynamic relationships.

4.3. Methodological limitations

There are a few possible methodological limitations in our study that are worth discussion. First, effects of agricultural land management on wetland soil microbes might be also manifested through alterations in plant stoichiometry and shifts in the composition and structure of plant communities (Dassen et al., 2017; Valencia et al., 2018). However, this field study by design is not able to disentangle those potential mechanisms through vegetational responses and local plant-soil-microbe interactions due to insufficient plant data and logistic constraints, which would be necessary in future research to uncover such complex plant-microbe interactions (e.g., using controlled greenhouse pot

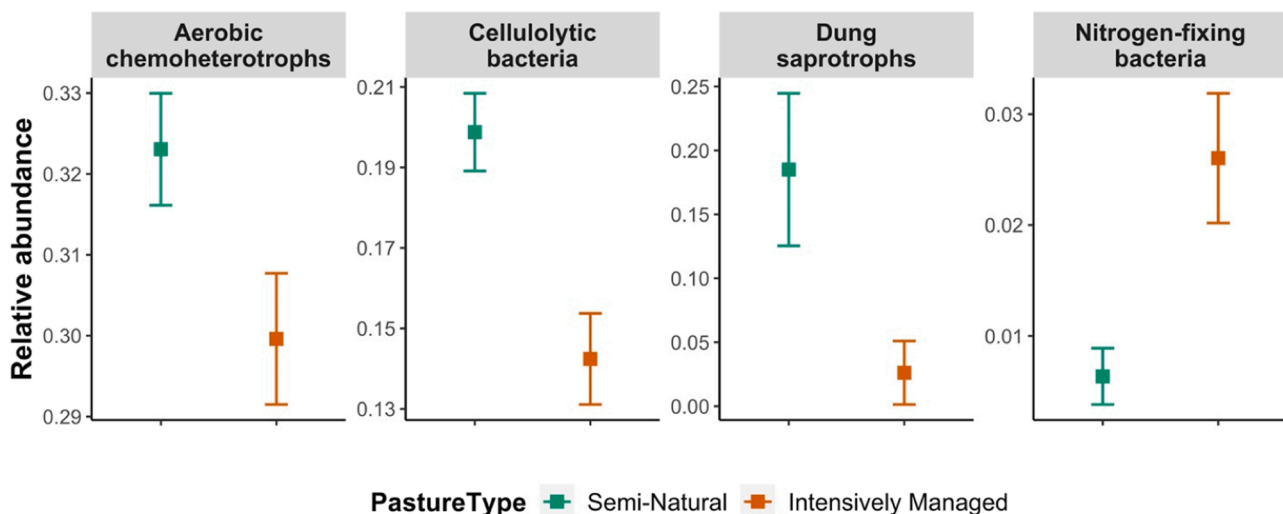


Fig. 6. Relative abundance of bacterial and fungal functional groups that showed a significant response (as resulted from ANCOM test with W-values higher than 0.7) to the land-use intensity treatment (mean \pm SE; N = 40).

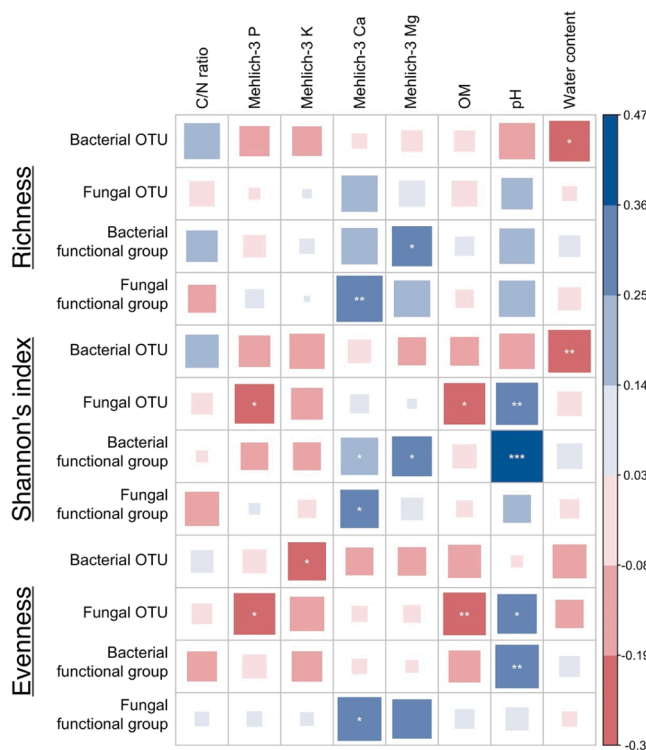


Fig. 7. Correlation coefficients (spearman's rank) of alpha-diversity metrics of bacterial and fungal OTU and functional groups (richness, Shannon's index, evenness) with soil physiochemical properties across all wetland soil samples. Blue color indicates positive correlations and red color indicates negative correlations. * indicates $p \leq 0.05$, ** indicates $p \leq 0.01$, *** indicates $p \leq 0.001$.

experiment). Second, the functional annotation databases used in this study are rather conservative databases that only provide ecologically relevant functions according to known properties of cultured taxa (Nguyen et al., 2016; Sansupa et al., 2021), and thus do not present predictions based on phenotypes of gene families or enzyme activities. Other functional prediction tools such as PICRUSt2 may assign functions to higher relative proportions of taxa; however, functional prediction tools inherently underestimate microbial functional diversity relative to shotgun metagenomic approaches (Toole et al., 2021). Therefore, to infer changes in microbial community functions more accurately, large-scale shotgun metagenomic and metatranscriptomic sequencing, albeit at a higher cost, will be required.

4.4. Management implications

Soils in agroecosystems have been increasingly under anthropogenic pressures from intensively land management and boost agricultural production, which challenge their capacity to maintain biotic heterogeneity and sustain essential ecosystem functions and services over time (Smith et al., 2016; Yang et al., 2021). Our results showed significant responses of soil bacterial and fungal communities to land-use intensification, cattle grazing, and prescribed fire, suggesting that microbes in wetland soils were susceptible to land management and disturbances. These findings highlight the importance of accounting for the 'invisible' microbial consequences of land management in agroecosystems and implications for soil health (Arbruster et al., 2021). In particular, inconsistency between taxonomic and functional attributes of microbial communities in response to anthropogenic environmental changes (Gibbons, 2017; Liu et al., 2021) points to the need for comprehensive evaluation to inform management decisions for sustainability of agricultural landscapes and conservation of wetlands.

The primary goal for agroecosystem management is to

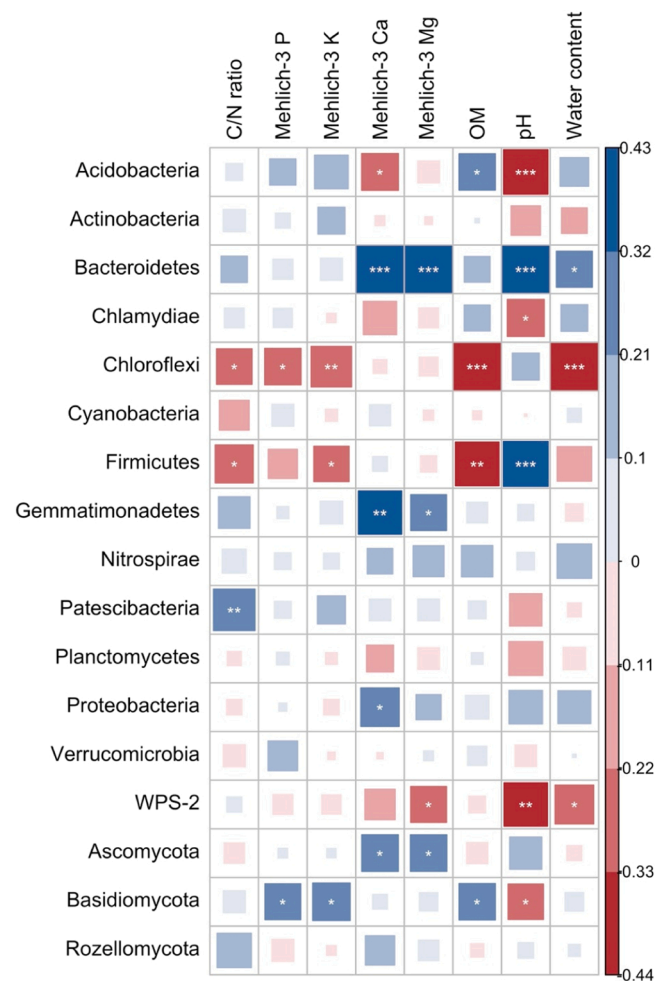


Fig. 8. Correlation coefficients (spearman's rank) of relative abundance of dominant bacterial and fungal phyla (relative abundance >1%) with soil physiochemical properties across all wetland soil samples. Blue color indicates positive correlations and red color indicates negative correlations. * indicates $p \leq 0.05$, ** indicates $p \leq 0.01$, *** indicates $p \leq 0.001$.

simultaneously balance agricultural production, biodiversity, and environmental sustainability. Improved understanding of wetland soil microbial responses to upland grassland and onsite management practices could provide valuable insights for conservation of wetlands and holistic provision of multiple ecosystem services in agriculture landscapes (Qiu and Turner, 2013). Our study showed that intensification of upland agriculture was not necessarily negative for bacterial taxonomic and functional diversity of embedded wetlands. However, this finding does not suggest that increasing land-use intensity should be used for wetland management and conservation. In fact, studies have shown that multiple agricultural practices associated with intensification, such as fertilization and tillage, could adversely affect soil microbial communities (Choudhary et al., 2018; Zhou et al., 2020). Yet our results did show that a neutral pH environment resulting from lime application associated with IM wetlands was key for promoting more diverse microbial groups in subtropical wetlands. As agricultural intensification normally encompasses and combines a suite of complex practices (Xu et al., 2016), our study suggests that it might be beneficial to integrate lime application with fertilization especially when there are N fertilizers that strongly acidify soils to maintain soil microbial diversity and their sustained functions.

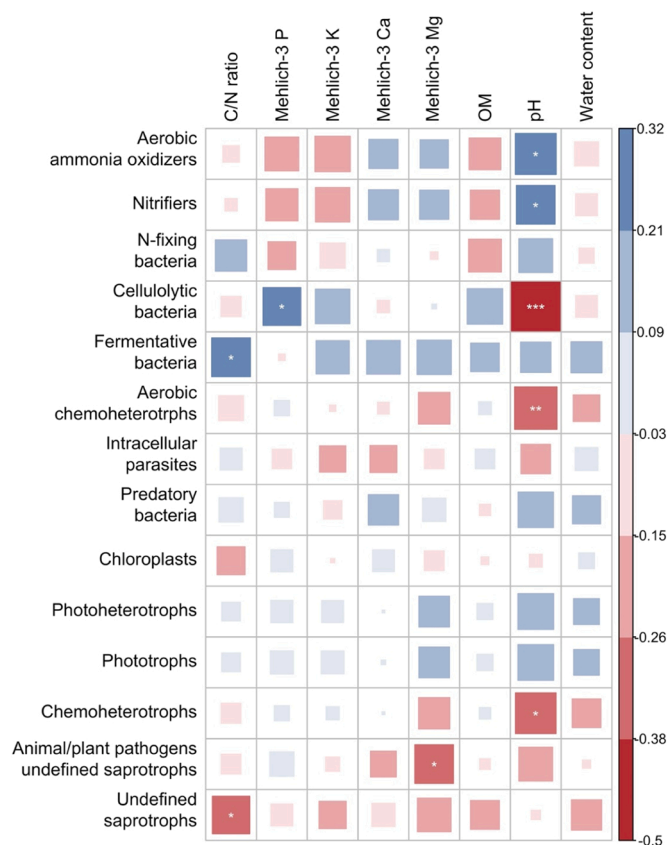


Fig. 9. Correlation coefficients (spearman's rank) of relative abundance of pervasive bacterial and fungal functional groups (present in at least 50% of samples) with soil physiochemical properties across all wetland soil samples. Blue color indicates positive correlations and red color indicates negative correlations. * indicates $p \leq 0.05$, ** indicates $p \leq 0.01$, *** indicates $p \leq 0.001$.

5. Conclusion

The results of our study demonstrate that upland land-use intensification and two common agricultural disturbances can substantially alter the composition and structure of bacterial and fungal communities in subtropical seasonal wetlands. However, responses of bacterial and fungal communities (e.g., composition, diversity) to anthropogenic land uses and disturbances are not consistent, and differed across taxonomic and functional level. Specifically, we found: (1) upland land-use intensification was the most consistent and strongest driver of bacterial and fungal community composition, as compared to grazing and fire disturbances; (2) at the OTU level, land-use intensity, grazing, and fire interacted to affect fungal diversity, but exerted no effects on bacterial diversity; (3) at the functional level, land-use intensification increased bacterial and fungal functional richness, whereas grazing and fire interactively affected bacterial functional richness; (4) for both taxonomic and functional levels, effects of wetland management on microbial communities could be manifested through its effects on certain soil physiochemical properties. This study highlights the importance of assessing microbial responses from both taxonomic and functional perspectives to better evaluate the impacts of agricultural land use and wetland management on belowground biological communities and associated ecosystem functions. Our results on microbial responses are relevant for earth system models for predicting global environmental change effects on wetland functions and services in the tropical/subtropical regions, and informative for sustainable landscape management to conserve seasonal wetlands.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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.agee.2022.108314](https://doi.org/10.1016/j.agee.2022.108314).

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