CONSEQUENCES OF LAND MANAGEMENT ON ECOSYSTEM FUNCTIONS AND SERVICES IN SUBTROPICAL AGROECOSYSTEMS

By

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A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

2022

To the giants whose shoulders I stand on

ACKNOWLEDGMENTS

I would not have been able to fulfill the commitment towards a PhD without the support of many people. I would like to express sincere gratitude to my advisor Dr. Jiangxiao Qiu, for his guidance, support, and encouragement. He has always been the first responder to my research questions, provided numerous insightful discussions, and helped me overcome many challenges. I am honored to have Jiangxiao as my committee chair. I would also like to sincerely appreciate my committee members, Drs. Elizabeth Boughton, Hui-Ling (Sunny) Liao, Willm Martens-Habbena, and Stephanie Bohlman, for their kindness, wisdom and perspectives in navigating the scientific research, and their valuable feedback and assistance to improve my work.

I would like to thank my Landscape Ecology and Sustainability Science lab colleagues: Chase O’Neil, Amy Wiedenfeld, Bei Huang, Hui Zhao, Marx Gedeon, Hayley Henderson, and Bibiana Lopez, who have offered extensive help on my research and shared laughs, caring, friendship, and precious memories with me. I wish to thank the chemist in Fort Lauderdale Research and Education Center, Hanzhuo (Andy) Fu, for his assistance on my laboratory measurements. I want to thank Buck Island Ranch research staff, particularly Steffan Pierre and Grégory Sonnier, for their assistance on my field infrastructure construction and sample collection. I am also thankful to researchers and staff in the labs of Drs. Sunny Liao and Willm Martens-Habbena, particularly Chih-Ming Hsu and Seemanti Chakrabarti, for their guidance on my microbiome analysis.

Finally, I am and will forever be grateful to my parents, Jie Bai and Cunde Guo, and my boyfriend, Ke Zhang, with whom I built and shared my life and work every step of the way.

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LIST OF ABBREVIATIONS

AIC	Akaike Information Criterion
ANCOM	Analysis of composition of microbiomes
ANPP	Annual net primary productivity
BIR	Buck island ranch
C	Carbon
Ca	Calcium
DNA	Deoxyribonucleic acid
FDR	False discovery rate
GHG	Greenhouse gas
ICP-OES	Inductively Coupled Plasma Optical Emission Spectrometry
IM	Intensively managed
ITS	Internal transcribed spacer
IVOMD	*In vitro* organic matter digestibility
K	Potassium
LiDAR	Light Detection and Ranging
LTAR	Long-Term Agroecosystem Research
MF	Multifunctionality index
Mg	Magnesium
N	Nitrogen
NCBI	National Center for Biotechnology Information
NMDS	Non-metric multidimensional scaling
OM	Organic matter
OTU	Operational taxonomic unit
P	Phosphorus

PCA	Principal component analysis
PCR	Polymerase chain reaction
PERMANOVA	Permutational multivariate analysis of variance
rRNA	Ribosomal ribonucleic acid
SEM	Structural equation modeling
SN	Semi-natural
SRA	Sequence Read Archive
SS	Sums of squares
TBI	Tea Bag Index
USDA	United States Department of Agriculture

Abstract of Dissertation Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Doctor of Philosophy

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August 2022

Chair: Jiangxiao Qiu
Major: Forest Resources and Conservation

Management intensification and human-induced disturbances in agroecosystems are crucial to boosting agricultural yields and attaining human livelihoods, but meanwhile are dominant global change drivers that may lead to adverse environmental consequences, such as water pollution, greenhouse gas emission, and biodiversity loss. Therefore, it is critical to understand how agricultural management and disturbances affect biological communities, ecological processes, and their underlying functions and services. Such knowledge is particularly scarce in subtropical landscapes that are unique in regard to their humid climate and numerous embedded natural wetlands in agroecosystems. Using a combination of data synthesis, field experiment, laboratory soil, plant, and microbiome analyses, I investigated effects of land management on multiple ecosystem functions and services in subtropical agroecosystems, and further focused on organic matter decomposition and soil microbial communities in embedded wetlands that fundamental to maintaining their functions and services. Specifically, in Chapter 2, I demonstrated that management intensification could alter magnitude and relationship of multiple ecosystem services, and their effects cascaded from managed grasslands to embeded natural wetland ecosystems. In Chapter 3 and 5, I found that decomposition of both standardized plant material and *in situ* plant litter in wetlands were either individually or interactively affected

by upland management intensification, grazing and fire disturbances, with effects manifested through changes in wetland biotic and abiotic conditions. Regarding to biotic conditions, I further identified the composition of wetland soil bacterial and fungal communities in Chapter 4, and revealed their significant responses to land management from both taxonomic and functional potential perspectives. These microbial responses, in turn, interacted with litter traits or soil properties to affect wetland litter decomposition. Collectively, my dissertation demonstrated that intensified management and human-induced disturbances promoted provisioning services at the expense of declining regulating and supporting services, such as by accelerating decomposition rates, promoting long-term decomposition completeness, and reducing carbon sequentration potentials. Findings from this work fill data gaps in Earth System Models for predicting global change effects on ecosystem functions and services in subtropical grasslands and natural wetlands. This research also provided scientific bases for sustainable management to conserve subtropical agroecosystems in an era of increasing anthropogenic environmental changes.

CHAPTER 1
INTRODUCTION

Agricultural landscapes, as the principal interface between people and the environment, represent areas of both pressure and promise (Cassman and Grassini, 2020; Garnett et al., 2013). In the era of an expanding global populations as well as wealth that leads to resource-intensive diets, demands for food are rising and aggravate competition for land, water, and other resource inputs into food production. While a key to attaining human livelihoods, intensive agriculture is the main driver of global change and may lead to critical environmental consequences such as water pollution, biodiversity loss, and increased greenhouse gas emission (Foley et al., 2011; Tilman et al., 2001). In addition to intensified management practices (e.g., through nutrient applications to boost yields), agroecosystems are subject to disturbances such as deliberate livestock grazing and prescribed fire, all of which are prominent drivers that alter the structure of biological communities and functions of ecosystems (Bengtsson et al., 2019). Hence, it is critical to understand how these agricultural management and disturbances affect ecological processes and their underlying functions and services in agroecosystems.

Subtropical agroecosystems are unique and complex, because they are experiencing great variabilities in precipitation and variations in soil moisture throughout the year (Beaumont et al., 2011). In the state of Florida, for example, notable rainfall fluctuations (e.g., distinct dry vs. wet seasons) along with the high groundwater table form numerous seasonal wetlands embedded and interspersed within managed grasslands (Kasischke et al., 2003). Such unique landscape mosaic makes subtropical grasslands substantially different from their temperate or arid/semi-arid counterparts in terms of their biophysical conditions of abiotic components, biological assemblages, biogeochemical cycles and energy fluxes, and therefore consequences of management practices. Given the inherent landscape linkages of wetlands and grasslands in the subtropical region, it is crucial to investigate them together as an integrated grassland-wetland landscape mosaic, which has been rarely done previously. In other words, it is important to consider and explicitly address the combined provision of ecosystem functions and services in integrated grassland-wetland systems and associated tradeoffs and synergies resulting from different land management practices.

Changes in land-use intensity and human-induced disturbances have potentials to interact and affect the provision and stability of multiple ecosystem functions and services simultaneously (Bennett et al., 2009; De Groot et al., 2010). To optimize decisions on agricultural land management, it is important to take a systematic and holistic approach to quantifying a suite of ecosystem functions and services as well as their relationships induced by dominant drivers of environmental changes. Such knowledge can help identify the directional and causal linkages between ecosystem management and ecosystem services across spatial scales (Raudsepp-Hearne and Peterson, 2016; Swain et al., 2013; Wilson, 2008). It is also vital to inform how to sustainably manage subtropical agroecosystems to balance food production with the maintenance of regulating and supporting services.

Therefore, in this dissertation, I firstly performed a comprehensive data synthesis in Chapter 2 by focusing on an extensively studied grassland-dominated agricultural landscape in central-south Florida, where experience two intensification levels that are exemplars of the local and other subtropical areas (Boughton et al., 2016): semi-natural (SN) and intensively managed (IM). In this Chapter 2, I aimed to address how agricultural intensification affect multiple ecosystem functions and services in managed grasslands and natural wetlands in subtropical agroecosystems, as well as any resulting tradeoffs and synergies.

Based on synthesized results from Chapter 2, I found strong cascading effects of agricultural intensification on the multifunctionality of embedded natural wetlands where management were not even directly applied. In particular, I identified an important data and knowledge gap in regard to subtropical wetland decomposition, which is a fundamental process that underlies many important ecosystem functions and services in wetlands but highly susceptible to surrounding land management. Specifically, in terrestrial ecosystems, more than 50% of net primary production is returned to soil through the decomposition of plant litter (Wardle et al., 2004). Decomposition also regulates the rates of nutrient cycling, maintains primary production, and affects the release of greenhouse gases (Swift et al., 1979; Weathers et al., 2012). In addition, decomposed mass could enable transport of nutrients from wetlands downstream water bodies, which further affects water quality and trophic structures at the regional scale (Taylor and Middleton, 2004; Weathers et al., 2012). Therefore, understanding how anthropogenic activities alter litter decomposition has major implications for predicting carbon budgets, and achieving sustainable land management and multifunctional agriculture (Zuazo et al., 2011). While there has been a long history of research on litter decomposition, direct and indirect effects of multiple interactive land management on wetland decomposition through altering biotic and abiotic factors yet remain less well understood. Such understanding is relatively scarce in subtropical freshwater seasonal wetlands – one of the most biologically productive and detritus-dominated systems where dead organic matter serves as the major nutrient source (Findlay and Sinsabaugh, 2004; Murkin and Wrubleski, 1988).

In tropical and subtropical regions, due to climate and hydrologic conditions, there are numerous isolated seasonal wetlands embedded across agricultural landscapes (Mitsch et al., 2010). These wetlands are an integral component of the local agriculture, deemed as hotspots of primary productivity and biogeochemical cycling (McClain et al., 2003), and are particularly important for regulating regional hydrology and supporting biodiversity (Whigham, 1999). Although these isolated seasonal wetlands do not occupy a large proportion of the land surface, they are numerous, are widely distributed across the landscapes, and function as indispensable environmental buffers between agricultural lands and residential or other natural areas by providing flood protection, nutrient retention, organic material accumulation, and water purification services (Janse et al., 2019; Whigham, 1999). These wetlands are also experiencing agricultural management practices (e.g., land-use intensification and livestock grazing) that are typically less common compared to natural or less human-disturbed wetlands. Hence, I decided to use multiple approaches (i.e., Tea Bag Index and the litterbag technique) to investigate the response of decomposition to global change drivers in wetlands embedded in subtropical agroecosystems, and explore key mechanisms for quantitative assessments of consequences for carbon and nutrient cycling and associated ecosystem services underpinned by decomposition. In Chapter 3, I used the Tea Bag Index (TBI) to measure decomposition in a whole-ecosystem wetland experiment in central-south Florida (Boughton et al., 2016; Ho et al., 2018) that manipulated presence/absence of grazing and fire in wetlands embedded in upland grasslands managed at two levels of land-use intensity (i.e., semi-natural vs. intensively managed systems). I chose TBI to quantify decomposition processes of a standardized tea material, because (1) TBI allows multiple short period measurements across wet/dry seasons, and (2) enables comparisons across ecosystems and geographic regions by standardizing litter quality (Petraglia et al., 2019). I specifically addressed how did upland management intensification and wetland management practices affect teabag decomposition through altering soil properties along with hydrological gradient and climatic conditions.

Results from Chapter 3 indicated that land management and associated abiotic conditions only explained 28% and 42% of the variations in decomposition rates and stabilization factors, respectively. It was because standard TBI approach cannot capture indirect effects through changing biotic factors, such as microbial decomposers and its interactions with soil properties or litter traits. Further, agricultural management could alter plant stoichiometry, the structure and composition of plant communities, and thus the quality of litter material (Boughton et al., 2010, 2016) which is one of the most crucial controlling factor for decompositon. Yet, the TBI approach could not capture such local plant-soil-microbe interactions on litter decomposition. Therefore, to further integrate and disentangle these biotic and abiotic interactions on organic matter decomposition, the first necessary step would be to uncover the composition and structure of soil microbial communities in subtropical wetlands, which is the main focus of Chapter 4.

Bacteria and fungi are primary components in wetland soil microbial communities and predominantly perform decomposition of organic materials (Paul, 2014). Different microbial taxonomic groups and their metabolic activities collectively determine the overall wetland mediation of biogeochemical cycling of carbon (C), nitrogen (N), phosphorus (P), and sulfur (S) in sediments and soils (Lamers et al., 2012). Although the 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 environmental changes (Leff et al., 2015; Liu et al., 2021; Wagg et al., 2018), yet there has been limited understanding of 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. 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 agricultural land management. Therefore, in Chapter 4, I examined individual and interactive effects of upland land-use intensification, livestock grazing, and prescribed fire on taxonomic composition and functional potential of wetland soil bacterial and fungal communities. Further, I explored whether and to what extent would these management and disturbance effects on wetland microbial communities manifest through alterations in soil properties.

Finally, in the Chapter 5, I came back to address the question on how subtropical wetland *in situ* plant litter decomposition respond to multiple interacting agricultural management practices that account for both biotic and abiotic pathways. Prior research has demonstrated that litter decomposition is primarily controlled by litter quality, environmental condition, and decomposing organisms (Chapin et al., 2002; Swift et al., 1979), but mostly focused on effects of individual factors (Ferreira et al., 2015; Krishna and Mohan, 2017; Yarwood, 2018). Effects of multiple land management practices (which are interactive and often operating in concert) on litter decomposition through interactions among litter traits, soil properties, and soil microbial communities yet remain less well understood (Krishna and Mohan, 2017; Yarwood, 2018). To address these knowledge gaps, in this Chapter 5, I used the litterbag technique (Hackney and De La Cruz, 1980) to investigate *in situ* plant litter decomposition processes in isolated seasonal wetlands embedded in managed grasslands. The Chapter 5 was also built on the same long-term whole-ecosystem wetland experiment (as in Chapters 2 and 3) (Boughton et al., 2016) that systematically manipulate effects of land-use intensification, livestock grazing, and prescribed fire. I measured litter traits of dominant plant species, wetland soil properties, and soil bacterial and fungal communities to elucidate the mechanisms of litter decomposition processes in response to agricultural land-use intensification and management practices. I specifically addressed how did upland land-use intensification and wetland on-site disturbances affect *in situ* plant litter decomposition through altering litter, soil, and microbial attributes and their interactions.

In this dissertation, I used multiple approaches that integrate data synthesis, field expeirment, laboratory soil, plant, and microbiome analyses to address the above interlinked research questions to improve the understanding of the consequences of land management on ecosystem functions and services in subtropical agroecosystems.

CHAPTER 2

EFFECTS OF LAND INTENSIFICATION ON ECOSYSTEM MULTIFUNCTIONALITY CASCADE ACROSS ECOSYSTEMS

Introduction

Securing food supplies while safeguarding natural capital remains one of the grand challenges in the 21st century and as a top priority on the global policy agenda (Cassman and Grassini, 2020; Garnett et al., 2013). In the era of an expanding populations as well as wealth that leads to resource-intensive diets, demands for food are rising and aggravate competitions for land, water and other resources into food production. While a key to attaining human livelihoods, intensive agriculture is the main driver of global change and the largest contributor to rising environmental risks (e.g., water pollution, biodiversity loss, and greenhouse gas emission) (Foley et al., 2011; Tilman et al., 2001). On the other hand, climate change also poses substantial threats to resilience of agriculture (Pretty, 2018; Wheeler and Braun, 2013) with disproportionate impacts on developing countries and marginal communities. Hence, it is vital to transition current agricultural systems towards a more ‘sustainable intensification’ paradigm (Pretty, 2018; Rockström et al., 2017) that encapsulates the aims of increasing productivity, bolstering ecosystem services, and improving multifunctionality and resilience of agricultural landscapes.

Grasslands, as a primarily component of rangelands, occupying ~25% of the earth’s land surface and 70% of global agricultural production area (Asner et al., 2004) and are critical components to global food security. At present, grazing lands are responsible for 40% of global agricultural output (e.g., milk, meat, and dairy products) and support livelihoods of 1.3 billion people worldwide (Phelps and Kaplan, 2017). Besides providing agricultural products, grasslands also deliver a wide array of essential ecosystem services (Isbell et al., 2011; Zavaleta et al., 2010). For example, grasslands contribute to >10% of terrestrial net primary productivity, store up to 30% of global soil organic carbon (Follett and Reed, 2010; Lal, 2004; Scurlock et al., 2002), and serve as key habitats for diverse fauna and flora. At local to regional scale, grasslands provide vital regulating services, such as soil health, flood abatement, nutrient retention, pollination, and pest control (Bengtsson et al., 2019; Qiu, et al., 2018; Werling et al., 2014).

Grassland intensification entails a portfolio of management practices that include fertilization, irrigation, soil disturbance, introduction of exotic productive forage species, and other hydrologic modification (Xu et al., 2016). Conceptually, grassland intensification is perceived as a continuum from extensively managed (i.e., no or low artificial input) to intermediate and fully cultivated (Beckmann et al., 2019; Paudel et al., 2021). Historical conversion of natural and semi-natural grasslands to intensively managed grasslands occurred predominantly in humid and mesic temperate and subtropical/tropical regions (White et al., 2000). Although intensification increases forage and livestock production and maintains rural economic prosperity, it could also lead to unwanted tradeoffs in other ecosystem services, such as carbon sequestration, nutrient retention, and biodiversity maintenance (Qiu and Turner, 2013), thus compromising grassland multifunctionality (Gamfeldt et al., 2008; Manning et al., 2018).

Prior research has investigated effects of land intensification on the capacity of grasslands to support specific taxa and biotic communities (Gossner et al., 2016; Manning et al., 2015) or provide individual ecosystem service. However, few studies have taken a multifunctional and systems approach that considers consequences for a broad suite of organisms and ecosystem services and their interactions (i.e., tradeoffs and synergies) from intensified management (Neyret et al., 2021; Paudel et al., 2021). In addition, most research thus far has focused on effects *within* grasslands (e.g., either local empirical studies or global synthesis) (Humbert et al., 2021; Simons and Weisser, 2017). Research that explicitly addresses cascading effects of intensification via spatial flows of energy, materials, and organisms *across* boundaries of grasslands in the landscape are scarce (Jeanneret et al., 2021; Loreau et al., 2003; Tscharntke et al., 2005). Such a holistic and cross-scale understanding is crucial for designing well-functioning agricultural landscapes and informing management decisions that often occur at larger spatial scales (e.g., watersheds) and can exert far-reaching impacts beyond production areas. Further, much research on grassland intensification has focused on temperate or semiarid regions. Subtropical/tropical grasslands, unique due to their humid climate, biophysical characteristics, and management practices but at the forefront of intensification, have received less attention. Such knowledge is especially relevant, given that ~50% of global population will reside in the tropics by 2050 (Edelman et al., 2014), potentially leading to more intensified land uses and anthropogenic pressures in this biogeographic region.

In this research, we aim to address these knowledge gaps by focusing on an extensively studied grassland-dominated agricultural landscape in central Florida, USA (Figure A-1) that experience two intensification levels that are exemplars of the regional and other (sub)tropical areas (Boughton et al., 2016): semi-natural (SN) and intensively managed (IM) grazing lands (Table A-1). Subtropical/tropical grasslands are distinguished from their northwestern counterparts in North America with dominant C_4 grass/forb species and humid climate, creating a landscape mosaic with numerous embedded seasonal wetlands (Buisson et al., 2019; Swain et al., 2013). These geographically isolated wetlands (Cohen et al., 2016) are keystone landscape elements providing many irreplaceable ecosystem services, particularly flood mitigation and nutrients retention (Creed et al., 2017), and are vital refugia for invertebrates, amphibians, and migratory birds. Yet these wetlands are highly sensitive to surrounding grassland management and have been substantially altered and lost due to anthropogenic pressures (Creed et al., 2017). We ask four specific research questions: (1) How does management intensification affect a suite of grassland ecosystem services and biodiversity of multiple taxa? (2) Do intensification effects cascade to natural wetlands embedded within grasslands? (3) What are consequences of intensification for grassland and wetland multifunctionality? (4) How does intensification alter interactions among multiple ecosystem services and biodiversity in grassland-wetland mosaics? To address our questions, we synthesized long-term datasets (2003-2019) of 31 indicators of biodiversity and ecosystem services (Table A-2) and calculated multifunctionality index. We used SN as baseline to estimate land intensification effects using linear mixed-effects models, which were compared across indicators and systems using standardized effect size. Based on our synthesized results, we further alluded to possible solutions to sustainable intensification in these grasslands.

Materials and Methods

Study Region and Data Source

Our study area is Archbold Biological Station’s Buck Island Ranch (BIR) in south-central Florida (27°09’N, 81°11W) (Figure A-1) that has been historically managed under two intensities (Table A-1). As a part of the Long-term Agroecosystem Research (LTAR) Network, BIR represents managed grasslands in humid tropical/subtropical biomes. Over the past two decades, there has been comprehensive long-term monitoring and assessment (e.g., soil nutrients, water quality, greenhouse gas (GHG), and plant and animal communities) on grasslands and wetlands. Details of the study region and data sources can be found in the Supplementary Materials (SM).

Multifunctionality Index Calculation

Prior to data analysis, we first standardized each indicator of ecosystem service and biodiversity (*total* = 31) to 0-1 range (Equation 2-1) to remove effects of measurement scale differences among indicators. We then transformed certain indicators (e.g., water chemistry and GHG fluxes) so that higher indicator values correspond to greater service provision.

$$z_i = \frac{x_i - \min(x)}{\max(x) - \min(x)} \tag{2-1}$$

Major ecosystem service and biodiversity indicators measured at the BIR were grouped into six broad categories: soil nutrient maintenance, water quality regulation, carbon storage and greenhouse gas mitigation, biodiversity maintenance, non-native species invasion resistance, and agricultural production. Based on scaled values of individual indicators (Tables A-2, A-3), we calculated ecosystem multifunctionality using multiple common multifunctionality index (MF) (Manning et al., 2018). The multifunctionality index presented in main context was calculated using a service-based weighted averaging approach (Manning et al., 2018), which averaged all scaled indicator values within a grassland or wetland by assigning equal weight to the indicators that represent same category of ecosystem service (Equation 2-2).

$$MF_{weighted} = \frac{1}{m} \times \sum_{i=1}^{n} c_i \times s_i \tag{2-2}$$

where m was the number of ecosystem services being measured; n represented the number of all indicators; c_i represented the weight coefficient that was 1 divided by the number of indicators in the corresponding ecosystem service category; and s_i was the scaled values of indicator i.

A plethora of methods exists for calculating MF. Here we chose service-based weighted averaging approach because it provides a comprehensive quantification of all available indicators and meanwhile avoids overweighting of certain category of services with more indicators. However, this approach assumes that all services share equal weight, which could be subject to debate in certain circumstances. Hence, we also calculated unweighted averaging MF (Byrnes et al., 2014; Maestre et al., 2012), cluster-based weighted MF (Manning et al., 2018), and top 50% quantile-based threshold MF (Pasari et al., 2013) to assess robustness of our results (Table A-4).

Statistical Analysis

To address our first and second question, we tested effects of land intensification on each ecosystem service indicator using linear mixed-effects models, in which land-use intensity was treated as a fixed factor, and sampling location and time were treated as random factors. To eliminate influence of confounding factors such as geographic gradient in physiochemical properties, we also included elevation of sampling location as covariates. Transformations (i.e., log, square root, or cubic root) of response variables were performed when residuals of raw data failed to satisfy assumptions of linear regressions. Detailed model specification for each ecosystem service and biodiversity indicator can be found in Table A-5. To compare effects of land intensification across indicators, we calculated Hedge's *D* as the standardized effect size and its 95% confidence intervals following the approach in Werling et al., (2014). We averaged the standardized effect size of indicators within each category of ecosystem services, and used the average value to indicate the overall intensification effect on that ecosystem service (Figure 2-5).

To address our third question, we performed linear regression models to analyze effects of land intensification on MF index of grasslands and wetlands. To address our last question on whether there were management-driven or intrinsic relationships (i.e., tradeoffs or synergies) among ecosystem service and biodiversity indicators, we examined selected pairs of indicators using scattered plots and quantified differences in their relationships using Spearman's rank correlations that are more robust to outliers and linear assumptions. Datasets of different indicators contained a mixture of single-time and repeated measurements. For those paired indicators with repeated measurements within same time periods, indicator values were averaged and matched by measuring time and locations (either in grassland or wetland). For other indicators with single-time measurement, values were only averaged and matched by measuring locations. All statistical analyses were performed in R version 4.0.4 (Team, 2016). Linear mixed-effects models were fitted using the “lme4” package (Bates et al., 2007).

Results

Management intensification substantially altered provisioning of multiple ecosystem services and biodiversity in subtropical grasslands, and such effects cascaded consistently to embedded wetlands. For upland areas, management intensification altered at least one indicator in every ecosystem service category, and for 4 of the 6 ecosystem service categories, at least half of the indicators differed between IM and SN (Figure 2-1). As expected, in grasslands, intensified management resulted in higher soil Mehlich-3 phosphorus (i.e., plant-available P), but effects on other soil nutrients (e.g., total N, inorganic N) were negligible (Figure 2-1). In addition, management intensification decreased adjacent ditch water quality with more released P (i.e., higher water orthophosphate (PO_4^{3-}) and total P concentrations; Figure 2-1). Intensification in grasslands tended to favor potential carbon storage with greater above- and below-ground biomass production, but on the other hand produced greater greenhouse gas emissions including CO_2 and CH_4 (Figure 2-1). Our results also revealed compromised biodiversity due to intensification, as reflected in lower plant and vertebrate diversities (Figure 2-1). Intensification had mixed effects on invasion resistance with increased non-native plant richness yet decreased non-native vertebrate diversity (Figure 2-1). As for agricultural production, intensified management supported greater high-quality forage and livestock production by improving forage quantity and quality (i.e., indicated by higher P content and *in vitro* organic matter digestibility (IVOMD)), and sustaining higher cattle stocking density (Figure 2-1).

Wetlands embedded in grasslands were not being directly managed but influenced by intensified practices from surrounding uplands. For 4 of the 5 ecosystem service categories, at least half of the indicators showed a significant difference between SN and IM wetlands. Specifically, wetlands embedded in IM grasslands showed significantly lower water quality, as evidenced by higher total N, total P, and PO_4^{3-} concentrations in the waterbodies (Figure 2-2). Like the upland areas, wetlands also showed mixed effects on carbon cycling. Compared to SN wetlands, IM wetlands showed lower root biomass, but higher aboveground primary productivity and more methane emissions (Figure 2-2). Further, upland grassland intensification also reduced wetland plant diversity but increased vegetation structure complexity (i.e., indicated by higher coefficient of variation in vegetation height) (Figure 2-2); and resulted in more invasive plant species (Figure 2-2), as compared to wetlands in SN grasslands. Interestingly, soil nutrient indicators showed no difference between IM and SN wetlands (Figure 2-2).

Overall performance across all measured indicators of ecosystem services and biodiversity was assessed using the service-based weighted averaging multifunctionality (MF) index. Our results demonstrated that management intensification slightly decreased grassland MF by 7.3% (Figure 2-3), but caused a stronger negative cascading effect on embedded wetlands, with 23.8% reduced wetland MF (Figure 2-3). Our results were overall robust and qualitatively consistent with using other comparable multifunctionality indexes (Figure A-4). The differences in individual ecosystem services that contribute to the multifunctionality index differences can be visualized looking at differences in scaled indicators in each service cateogy (Figure 2-5). The small difference in multifunctionality between SN and IM grasslands is due to SN systems having greater biodiversity and invasion resistance, which is offset by the greater food production of the IM systems (Figure 2-5). Wetlands showed similar patterns with greater biodiversity and invasion resistance in SN systems compared to IM systems, but this was not offset by greater agricultural production because this was not measured for the wetlands.

Management intensification altered interactions among certain ecosystem service and biodiversity indicators. Specifically, we found that intensification removed positive correlations between soil nutrients (e.g., organic matter) and potential carbon storage (e.g., root biomass) in grasslands (that were not affected by management intensity, such as positive correlation between soil nutrients Figure 2-4). Moreover, intensification changed correlations between plant β-diversity and ANPP from non-significant to negative in grasslands (Figure 2-4). In embedded wetlands, upland intensification also reversed positive associations between wetland nutrients (e.g., soil TN, water NH_4^+) and primary productivity (i.e., ANPP) (Figure 2-4). On the other hand, there were also intrinsic relationships between ecosystem services (e.g., organic matter) and forage quality (e.g., forage N) in grasslands (Figure 2-4) and negative correlation between water quality (e.g., TP) and plant diversity (e.g., richness) in wetlands (Figure 2-3).

Discussion

In this research, we performed a holistic synthesis of management intensification effects on a broad suite of grassland and wetland ecosystem services in a subtropical agroecosystem using two-decade of >11,000 measurements of soil, water, GHG fluxes, and biotic communities. Collectively, our results suggest that management intensification strongly altered the magnitude and relationship of multiple ecosystem service provisions and biodiversity maintenance in grasslands, which in consequence cascaded to exert even stronger impacts on embedded natural wetlands where management applications were not even directly implemented. Specifically, we found that, compared to semi-natural systems: (1) intensified management promoted provisioning services including forage and livestock production, but at the expense of regulating and supporting services, including water quality regulation, greenhouse gas mitigation and biodiversity maintenance (Figure 2-5); (2) consistent cascading effects from upland intensification were observed in wetlands situated within IM grasslands (Figure 2-5); (3) intensified management caused >3 times reduction in multifunctionality in wetlands than their upland grasslands; and (3) intensification altered the interactions among certain ecosystem services and biodiversity in both grasslands and embedded wetlands. Our results highlight that none of two land management intensities (i.e., SN and IM) performs absolutely better than the other, but instead are complementary in their supported ecosystem services. Hence, these two management intensities should be implemented together and spatially optimized (e.g., in their relative proportion and optimal locations) at the landscape scale to achieve sustainable intensification that effectively balance agricultural production, conservation, multifunctionality, and rural prosperity.

Shifts in some ecosystem services due to management intensification, such as increased soil nutrients (Figure 2-1), reduced ditch water quality (Figure 2-1), boosted forage yields (Figure 2-1) and improved forage quality (Figure 2-1), can be attributed in large part to its embraced fertilization practices. Fertilization is key to maintaining bahiagrass-dominated (*Paspalum notatum* Flugge) grasslands, which occupies >2 million acres in Florida, serves as the foundation to cow-calf production in the state (Wallau et al., 2019), and dominates in other managed (sub)tropical grasslands. As the most widely used C_4 grass species in low-latitudes, bahiagrass adapts well to infertile sandy soils and is highly resistant to diseases and pests (Chambliss, 1999). Yet bahiagrass, due to its lower nutritional values than C_3 grasses, is deemed as inadequate for livestock (Myer et al., 2011), which makes fertilization vital to improve its nutritional quality and ensure success of livestock operations. Other practices associated with intensification such as extensive drainage ditch and lacking water retention infrastructure

(Boughton et al., 2019) could also contribute to reduced water quality. In addition, fertilization likely counteracted adverse effects of grazing on belowground biomass. Because intensive grazing had been found suppressed root and rhizome growth of bahiagrass (Chambliss, 1999), thus negatively affected its drought resistance and C accumulation. However, our results showed higher root biomass in IM than in SN grasslands (Figure 2-1), likely due to higher soil plant-available P and constantly supplemental N that efficiently stimulates the below-ground primary productivity (Santos et al., 2018).

On the other hand, fertilization along with species conversion in intensified management account for compromised biodiversity in IM grasslands (Figure 2-1) and embedded natural wetlands (Figure 2-2). Human-induced species conversion to bahiagrass is perhaps the largest factor for declined plant diversity. Such decline is reflected not only in lowered richness, but also decreased ß-diversity and vegetative structure (indicated by plant height coefficient of variation). Fertilization also contributes to reduced plant diversity because of its homogenization effects (Gossner et al., 2016), which could further cascade to other taxa and trophic groups (Manning et al., 2015). This is evidenced in our results of decreased vertebrates diversity in IM grasslands (Figure 2-1), likely due to uniform bahiagrass-dominant vegetation cover and associated homogenous habitats (Demarais et al., 2017). Plant diversity in embedded wetlands was negatively affected as well, which probably resulted from nutrient runoff from upland intensification that eutrophicated wetlands (Figure 2-2). Excessive nutrients can alter competition between fast-growing and slow-growing plant species and favor some species over others (Soons et al., 2017), thus wetlands surrounded by fertilized upland areas would be unfavorable to the coexistence of different plant species. Moreover, fertilization could lead to eutrophication in wetlands that foster algae growth, decrease light availability thus intensifying light competition (Hautier et al., 2009), thereby only favoring tall macrophytes and simplifying plant community and vegetation structure.

Management intensification declined climate mitigation service from both grasslands and wetlands, as revealed by GHG emission results (Figures 2-1, 2-2). A mixture of multiple practices embodied in intensification, including livestock grazing, fertilization, and hydrology modification, could collectively contribute to such negative impacts. For example, beef cattle is one of the largest sources of C release through its enteric fermentation and manure decomposition (Z. Chen et al., 2020), contributing one-third of anthropogenic CH_4 production and 18% of total global GHG (Steinfeld et al., 2006). Fertilization increases litter quality (Figure 2-1), which could accelerate the rates of plant litter decomposition and soil C turnover (Grandy et al., 2013; Y. Guo et al., 2021). Extensive drainage ditches and dry-season irrigation from intensified management (Kohmann et al., 2021), could increase soil drying-wetting cycles that breakdown soil aggregates, stimulate deep soil respiration and finally deplete the soil C pool (Qi et al., 2018; Xu et al., 2017).

Our findings have three important management implications: (1) *holistic and landscape perspective in sustainable intensification*; (2) *consideration of spatially cascading effects*; and (3) *altered tradeoffs and synergies from intensification*. First, agricultural intensification often fails to achieve simultaneous positive ecosystem service and well-being outcomes (Rasmussen et al., 2018), urging for pathways towards sustainable intensification. Our results demonstrated that the two focused management intensities produce complementary outcomes in ecosystem service provisions and biodiversity maintenance (Figures 2-3, 2-5), where intensified management leads to improved agricultural productivity at the expense of GHG emission, biodiversity loss, water contamination, and overall multifunctionality. However, if only SN systems were allowed, production areas may have to be expanded to meet the basic food demands and support the local economy, or otherwise will likely be converted to development if not self-sustainable and profitable. Either of these options would cause even greater adverse ecological impacts, in particular on carbon sequestration, natural communities, water quality, and native biodiversity (Edenhofer, 2015; Foley et al., 2011; Newbold et al., 2015). Hence, it alludes that neither of these management itself would individually qualify as sustainable intensification. Rather, sustainable intensification is likely more achievable at the landscape scale where both management approaches are implemented and spatially distributed (Qiu and Turner, 2013). Such landscape-level ensemble strategies could be further optimized by spatially targeting locale across the landscape that are more desirable for one or the other management intensities and balancing their relative proportion in area, thus finding a good compromise among agricultural production, conservation, and rural livelihoods. The findings about specific changes in ecosystem services found in this study may be useful in guiding where SN and IM are best implemented, including considering both upland and wetland areas that have different outcomes from SN and IM approaches.

Second, an evaluation or comparison of agricultural intensification should go beyond the local production area and consider their cascading effects across ecosystems within the agricultural landscape (Qiu et al., 2018; Seppelt et al., 2011; Tscharntke et al., 2005). Our results showed that compared to grasslands, IM caused 3x more reduction in ecosystem multifunctionality in embedded wetlands where management practices are not even directly imposed (Figure 2-3). Hence, it is crucial to holistically account for these potentially more pronounced cascading, distant (or off-site) impacts from intensification. It also implies opportunities for interventions that aim to optimize intensification and mitigate its negative impacts. For example, we can adopt inspirational management practices that help improving nutrient-use efficiency (Spiegal et al., 2018), such as patch-burn grazing (McGranahan et al., 2014; Ricketts and Sandercock, 2016), to reduce reliance on high fertilizer inputs and ameliorate the burden of nutrient retention of embedded wetlands. Another example is that payment for ecosystem services from wetland nutrient retention and water retention (Boughton et al., 2019; Canning et al., 2021) could potentially contribute to establish buffer strips or improved nutrient management to reduce local and regional effects of upland intensification. Our data suggested that wetlands were more sensitive and less resilient to external modifications than their upland grasslands. Hence, interventions (e.g., restoration, incentives, policies) could have a priority on these fragile and more degraded natural systems.

Finally, our results revealed that land-use intensification could alter certain tradeoffs or synergies among ecosystem services, consistent with other studies (Beckmann et al., 2019; Lindborg et al., 2017; Qiu et al., 2021). For example, synergies between soil nutrients and carbon storage could disappear, or emerging tradeoffs between plant diversity and primary productivity could occur in both grasslands and wetlands due to intensified management. Hence, it is crucial to consider such shifts and dynamic nature in ecosystem service interactions in sustainable intensification that aim to take advantages of synergies and mitigate unwanted tradeoffs.

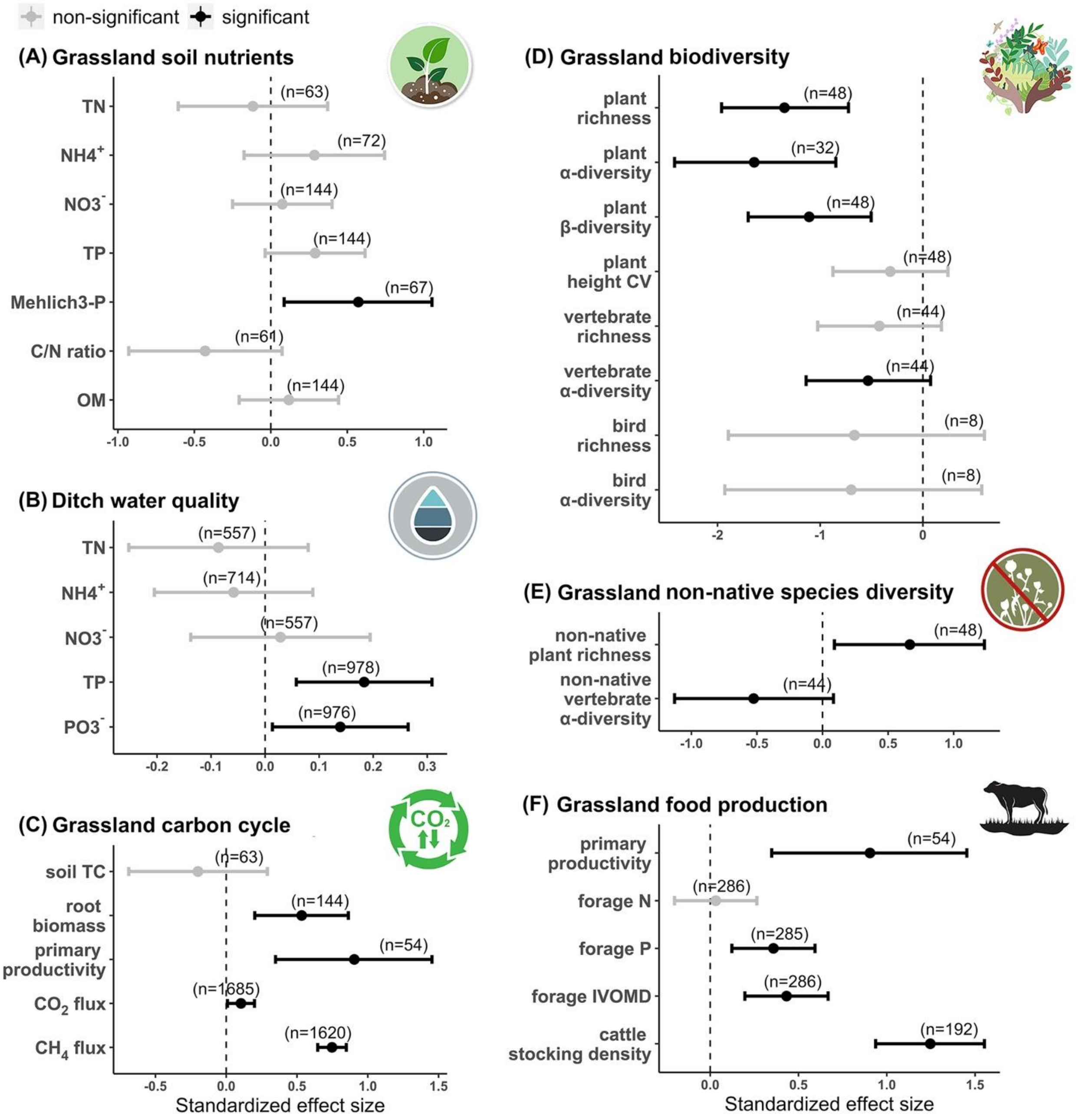

Figure 2-1. Standardized effect sizes (Hedge's *d*) of land intensification on ecosystem services and biodiversity indicators in grasslands: (*A*) Soil nutrients; (*B*) Adjacent ditch water quality; (*C*) Carbon sequestration; (*D*) Biodiversity; (*E*) Invasion resistance; and (*F*) Food production. Effect sizes of intensification were estimated by comparing intensively managed (IM) vs. semi-natural (SN) grasslands, with error bars representing 95% confidence intervals. Black bars indicate significant differences (α= 0.1) between IM and SN grasslands, whereas grey bars indicate non-significant differences. Numbers in parentheses indicate the total independent sample size for estimating effect size of each indicator.

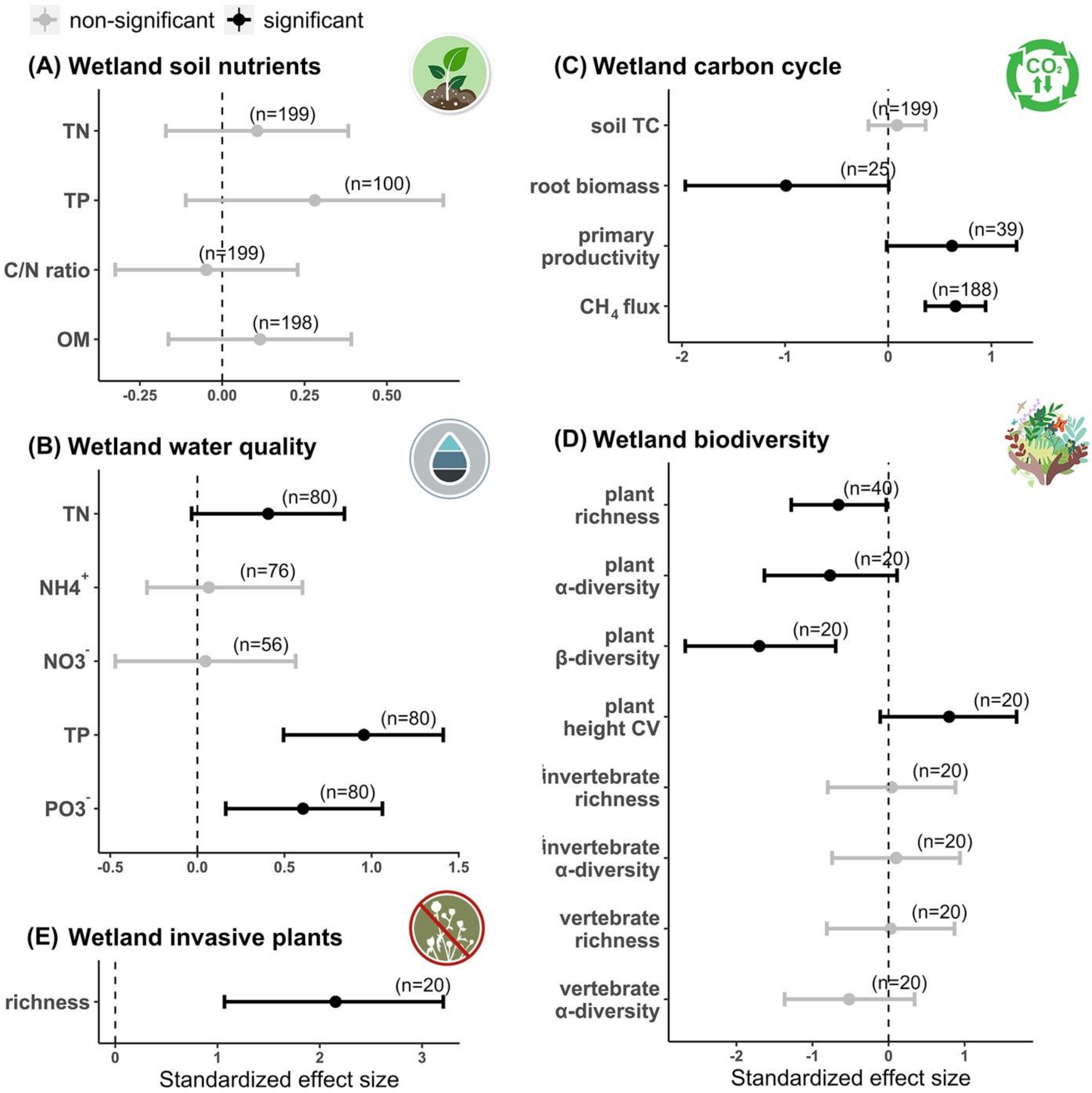

Figure 2-2. Standardized effect sizes (Hedge's *d*) of land intensification on ecosystem services and biodiversity indicators in adjoining wetlands: (*A*) Soil nutrients; (*B*) Water quality; (*C*) Carbon sequestration; (*D*) Biodiversity; and (*E*) Invasion resistance. Effect sizes were estimated by comparing wetlands embedded in intensively managed (IM) vs. semi-natural (SN) grasslands, with error bars representing 95% confidence intervals. Black bars indicate significant differences ($\alpha = 0.1$) between wetlands embedded in IM and SN grasslands, whereas grey bars indicate non-significant effects. Numbers in parentheses indicate the total independent sample size for estimating effect size of each indicator.

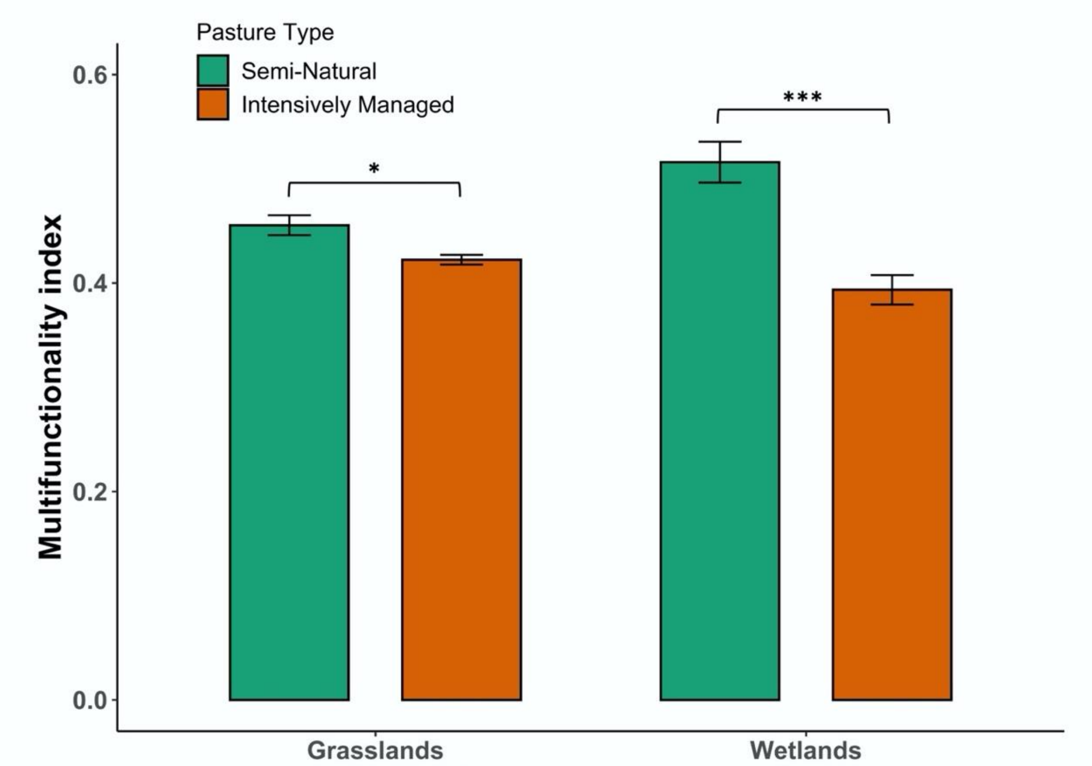

Figure 2-3. Effects of agricultural land intensification on ecosystem multifunctionality of grasslands and embedded wetlands. Multifunctionality were quantified using the approach of weighted average by categories of ecosystem services, following Manning et al. (2018). Level of significance: * p<0.5, ***p<0.01from linear regression models.

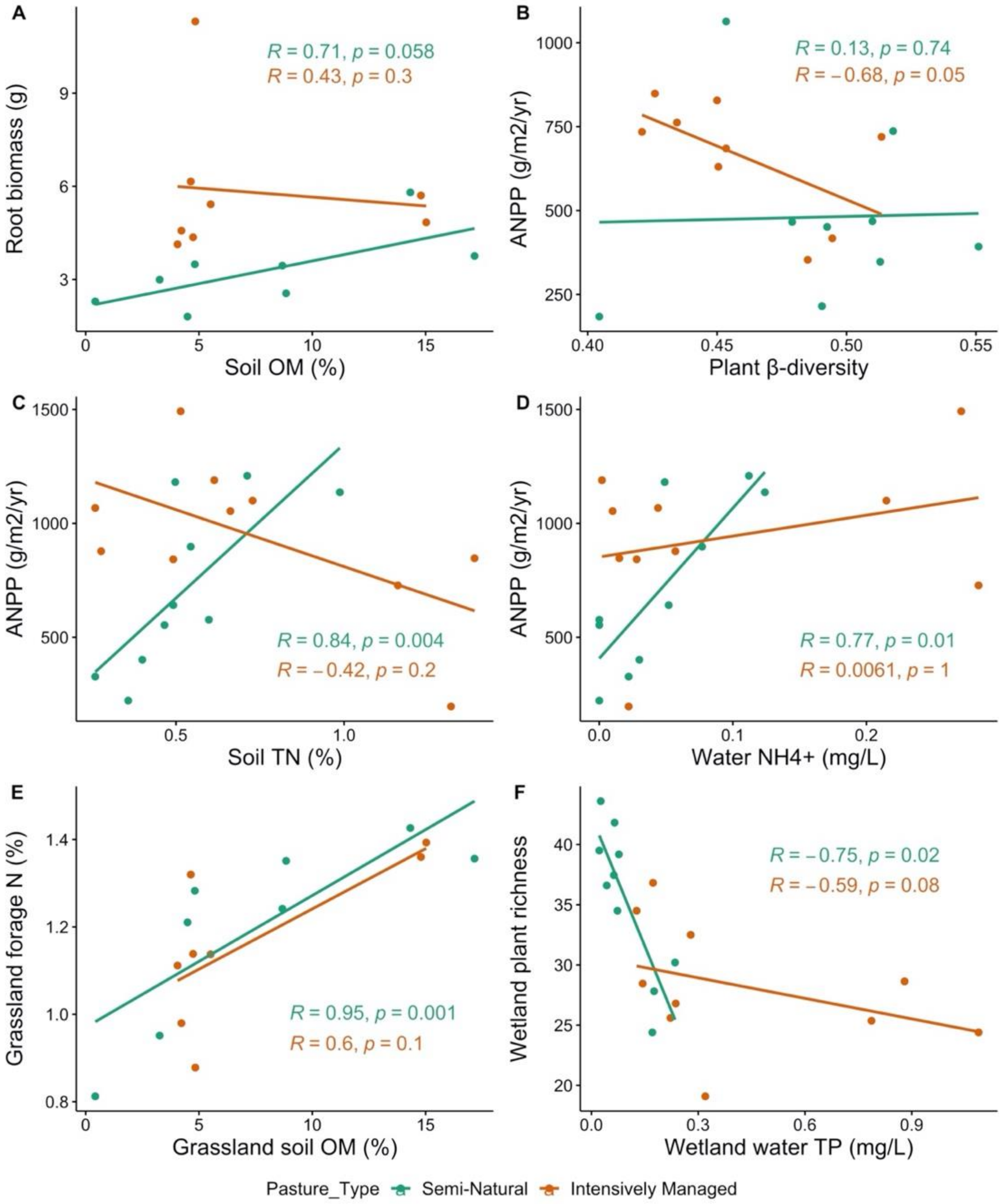

Figure 2-4. Spearman correlations between ecosystem service and biodiversity indicators in intensively managed (orange) and semi-natural (green) systems: (A) Soil organic matter content (OM) vs. Root biomass in grasslands; (B) Plant **β** diversity vs. ANPP in grasslands; (C) Soil total nitrogen (TN) vs. ANPP in wetlands; (D) Water ammonia nitrogen (NH_4^+) vs. ANPP in wetlands; (E) Soil OM vs. Forage N in grasslands; (F) Water total phosphorus (TP) vs. plant richness in wetlands.

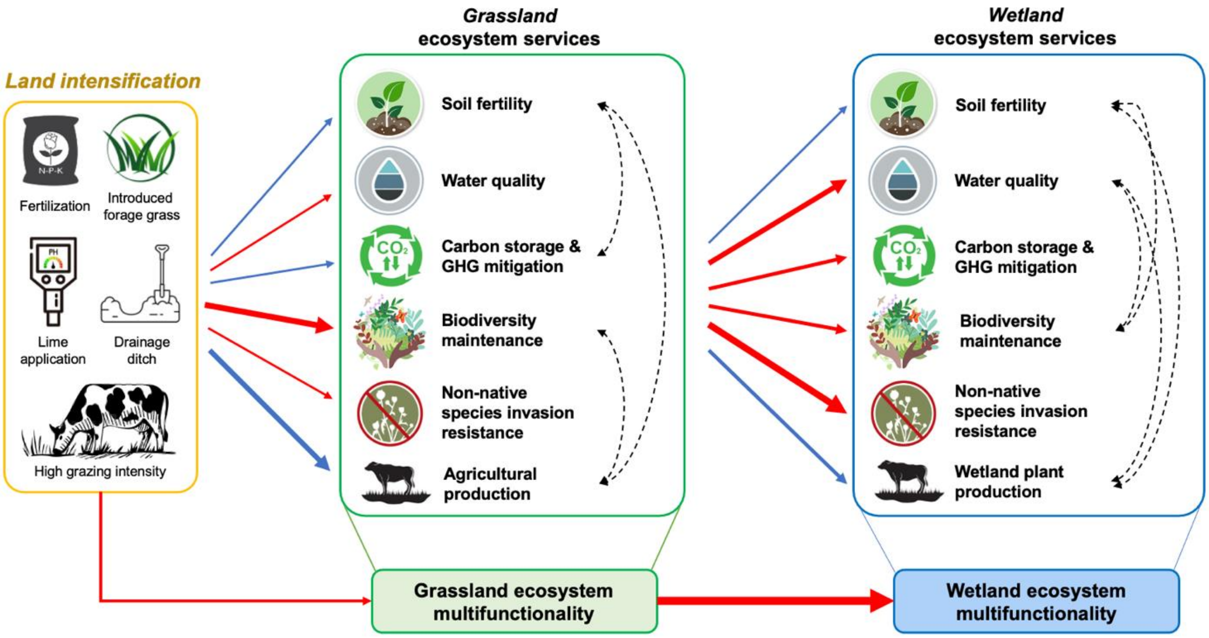

Figure 2-5. Conceptual diagram illustrating how ecosystem service and biodiversity were affected by land intensification. Blue arrows indicate positive effects and red arrows indicate negative effects. Width of the arrow corresponds to the magnitude of effect size, calculated by averaging the standardized effect size of indicators within each ecosystem service category. Dotted arrows indicate interactions between two ecosystem service categories existed.

CHAPTER 3[*]

INTERACTIVE ROLES OF LAND-USE INTENSITY, GRAZING AND FIRE IN DECOMPOSITION OF SUBTROPICAL SEASONAL WETLANDS

Introduction

Organic matter decomposition is one of the most fundamental processes underlying a range of ecosystem functions and services. For example, decomposition regulates biogeochemical cycling, maintains primary production, contributes to soil formation, and affects greenhouse gas (GHG) emissions (Chapin et al., 2002; Swift et al., 1979; D. Zhang et al., 2008). However, global change drivers (e.g., land-use intensification and altered disturbance regimes) (Steffen et al., 2006) have substantially modified the rates and controlling factors of decomposition, including physicochemical properties of the environment, plant functional traits, and decomposer communities (Solly et al., 2014; Y. Wang et al., 2015). Hence, it is critical to investigate the response of decomposition to global change drivers, and explore key mechanisms for quantitative assessments of consequences for carbon and nutrient cycling and associated ecosystem services underpinned by decomposition.

It has been acknowledged that the relative controls on decomposition and its responses to environmental changes are likely ecosystem- or context-dependent (Swift et al., 1979). Prior research has thoroughly studied litter decomposition across an array of terrestrial and aquatic ecosystems (Chapin et al., 2002; D. Zhang et al., 2008). However, less is known about decomposition in transitional systems, such as seasonal freshwater wetlands. Inland seasonal or intermittently inundated wetlands have often been lumped into surrounding uplands, presumably due to their small size, embedment in landscape mosaics, and inundation dynamics (Kollmann et

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al., 2016; Reis et al., 2017). Yet these ecosystems are disproportionately important, given their: (1) large global presence (i.e., 10.6×10^6 km^2, or 62% of total global wetland extents) (Davidson et al., 2018; Fluet-Chouinard et al., 2015); and (2) social-ecological value because they support high biodiversity and productivity (Brinson et al., 1981), (3) regulation of GHG and global carbon budget (Raymond et al., 2013), and (4) provision of diverse ecosystem services, such as water purification and erosion control (Qiu, et al., 2018; Qiu and Turner, 2013).

Compared to permanently inundated wetlands or aquatic ecosystems, hydrologic pulses in seasonal wetlands could enhance primary productivity (Gomez-Casanovas et al., 2020; Odum et al., 1995), particularly in tropical/subtropical biomes (Aselmann and Crutzen, 1989), leading to greater litter inputs and therefore a larger carbon sink. On the other hand, sediment exposure during dry periods in seasonal wetlands could accelerate decomposition and may then switch seasonal wetlands to a carbon source (Moura et al., 2008), although the net carbon effect remains inconclusive due to large uncertainties (Raymond et al., 2013). In addition, during wet seasons, compared to permanently inundated wetlands, seasonal wetlands tend to have shallower water levels and thus less vertical stratification, which makes their physicochemical conditions more sensitive to external environmental changes that further affect wetland litter decomposition (McLatchey and Reddy, 1998). In central Florida, for example, many shallow seasonal wetlands are embedded in managed grasslands and thus highly susceptible to agricultural management, including fertilization, lime application, water pumping and ditch construction (Boughton et al., 2010, 2016). Furthermore, livestock grazing and prescribed fire are two additional typical yet essential management factors and disturbances that may also exert significant impacts on litter decomposition in seasonal wetlands (Steffen et al., 2006).

Prior research has demonstrated that land-use intensification, livestock grazing and prescribed fire have exerted individual and sometimes interactive effects on soil properties, water quantities and qualities, methane fluxes, and plant communities in seasonal wetlands (Bohlen and Gathumbi, 2007; Boughton et al., 2016; DeLucia et al., 2019; Ho et al., 2018; Jansen et al., 2019). Yet whether and how these drivers affect wetland decomposition, either directly, indirectly, or interactively, remains largely unclear. For instance, decomposition could be directly affected by cattle trampling, or indirectly affected via higher available soil nutrients from cattle dung and fertilization inputs from surrounding grasslands. Fire-grazing interactions have also been demonstrated to affect wetland plant productivity, leaf nutrient content and water nutrients (Jansen et al., 2019; Sonnier et al., 2020), which could also further affect organic matter decomposition in wetlands. Hence, a key step is to unravel the effects of these land management practices and their interactions, as well as the relative contributions of direct or indirect pathways through which decomposition is shaped in seasonal wetlands. Such knowledge is vital to informing management of seasonal wetlands embedded in agricultural landscapes to sustain their essential functions and services given accelerating anthropogenic environmental changes.

To address these knowledge gaps, we focus on decomposition in subtropical wetlands – a biome less well understood in the literature but largely represented in global seasonal wetlands (Davidson et al., 2018; Prigent et al., 2007). We used the Tea Bag Index (TBI) to quantify decomposition processes that include decay rate *k* and stabilization factor *S*, because (1) TBI allows multiple measurements across time, and (2) enables comparisons across ecosystems and geographic regions by standardizing litter quality (Petraglia et al., 2019). We ask: (1) How do land-use intensity and management practices (i.e., cattle grazing and prescribed fire) interact to affect seasonal wetland decomposition? (2) How does wetland decomposition vary with hydrological gradient and seasonal climatic condition? (3) What are the dominant direct and indirect pathways through which land-use intensity and management practices affect wetland decomposition? To answer these questions, we measured decomposition in a whole wetland field experiment in central-south Florida, USA (Boughton et al., 2016; Ho et al., 2018) that manipulated presence/absence of grazing and fire in wetlands embedded in upland grasslands managed at two levels of land-use intensity (i.e., high vs. low) (Figure 3-1). We hypothesized that land-use intensity would exert greater effects than grazing and prescribed fire, due to strong modifications of hydrologic regimes and soil properties. However, the direction of the land-use intensity effect is uncertain, because under intensified land-use, neutral pH resulting from upland lime application may stimulate microbial activities and accelerate decomposition, but N-enrichment from upland fertilization may inhibit labile organic matter decomposition (Y. Li et al., 2016). We also hypothesized that grazing and fire were more likely to show interactive effects on organic matter decomposition in wetlands embedded within high-intensity upland grasslands, considering wetland soil nutrients were only interactively modified by grazing and fire under high-intensity land-uses (Ho et al., 2018).

Materials and Methods

Study Area

Our study took place at Archbold Biological Station’s Buck Island Ranch (BIR) in central-south Florida, USA (Figure 3-1). BIR is a working ranch (4,336 ha) with commercial-scale cow-calf operations (~3,000 cows) for research purposes. BIR is part of the Archbold Biological Station-University of Florida USDA Long-term Agroecosystem Research (LTAR) site. This is one of 18 sites forming a nationwide network designed to assess strategies for sustainable intensification of agriculture at the national scale (Kleinman et al., 2018; Spiegal et al., 2018). BIR contains two pasture (or grassland) types representing typical land-use intensities for this region (Figure 3-1): intensively managed (IM) vs. semi-natural (SN). IM pastures are heavily drained, planted with non-native bahiagrass (*Paspalum notatum*), and are intensively grazed by cattle during wet seasons (June-October). IM pastures were also regularly limed, N-fertilized every 1-2 years, and were historically fertilized with P and K until 1987. SN pastures were never fertilized (Swain et al., 2007), composed of mixed non-native bahiagrass and native grasses, and are grazed primarily during dry seasons (November-May). Average animal use days per hectare for IM and SN pastures are 391.2 and 177.7, respectively (data from 2014-2019).

Isolated seasonal wetlands are an important feature of tropical/subtropical landscapes that are embedded within and thus influenced by surrounding upland pastures (Figure 3-1). In BIR, there are more than 600 wetlands (~ comprising of 12% of the area) (Boughton et al., 2010) with hydroperiods ranging 2-10 months and size between 0.007-41.9 ha. Plant communities of wetlands within SN pastures are diverse and dominated by native and perennial species, such as *Panicum hemitomon, Rhynchospora inundata, Diodia virginiana, Sagitarria lancifolia, Pontedaria cordata*. However, in wetlands within IM pastures, plant communities have lower richness, more exotic and annual species, and are often dominated by *Juncus effusus* var. *solutus, Persicaria punctata, and Pontedaria cordata* (Boughton et al., 2011). Cattle grazing and prescribed fires are the two most common grassland management actions in this region and elsewhere, which also have shown significant effects on wetland vegetation. For example, grazed wetlands tended to have greater abundance of short graminoids and forbs, such as *Luziola fluitans*, *Eleocharis vivipara* and *Bacopa caroliniana*, than ungrazed wetlands, which were dominated by taller species such as *P. hemitomon* and *Sacciolepis striata* (Boughton et al., 2011, 2016). Moreover, fire interacted with grazing that increased shrubs in ungrazed wetlands but had no effect on shrubs in grazed wetlands (Boughton et al., 2016). In addition, wetland edges typically have greater diversity and more variable plant composition compared to wetland centers which are lower diversity and primarily dominated by broadleaf emergent vegetation (*P. cordata and Sagitarria lanc*ifolia) (Boughton et al., 2019, 2021).

Experimental Design

Our research was conducted in a long-term whole-ecosystem wetland experiment initialized in 2008 with a randomized complete block design. Details in experimental design can be found in Boughton et al. (2016) and Ho et al. (2018). In brief, forty similar-size wetlands (0.5-1.5 ha, Figure 3-1) were selected across the BIR and organized into five blocks. In each block, the crossed presence/absence of grazing and fire treatments were implemented in wetlands within IM and SN pastures (i.e., 2×2×2 factorials). For ungrazed treatment, cattle exclosures were installed in 2007; for grazed treatment, cattle use (stocking rate typical of local region) activities were tracked for pastures in which wetlands were embedded (number of cattle and days in and out). Prescribed fire treatment was imposed to 5 random wetlands in each combination of pasture type × grazing treatment during dry seasons of 2008, 2011, 2013-2014, and 2020 mimicking local return intervals. Standard drip torch and overlapping strip lines were used to ignite fires and ensure that >80% of the wetland was burned. Such a whole-ecosystem and long-term manipulative experiment allows us to tease apart effects of different factors of environmental changes in real-word settings.

Given our design, three sites (i.e., center, middle, and edge) were selected per wetland that vary along the hydrological gradient to measure aboveground decomposition (Figure 3-1). The center site is the lowest point of the wetland; the edge site is determined by the longest distance between the center and wetland margin where transitions in vegetation normally occur along the hydrological gradient; the middle site is the midpoint with equal distances to the center and edge sites. Our selection of three sites per wetland not only captured local variation in wetland decomposition, but also can be aggregated to obtain the representative wetland-level estimates (Table B-1).

Tea Bag Index Approach

TBI uses two standardized materials – labile (Lipton green tea) and recalcitrant (Lipton rooibos tea) litter (Figure 3-1) – to assess decay rate k and stabilization factor S within a single incubation (Keuskamp et al., 2013). K quantifies mass loss rate that reflects short-term litter decomposition dynamics (Equations 3-1, 3-2), with higher values indicating faster decomposition of labile organic components. S measures the amount of hydrolysable compounds turning recalcitrant (Equation 3-3) and reflects completeness of decomposition. Thus, high S values suggest inhibition of litter decomposition, likely due to factors such as anoxia or low pH, leading to high carbon sequestration potential (Keuskamp et al., 2013). As an effective and standardized method, TBI has been widely tested to study decomposition across diverse ecosystems globally, including montane and lowland forests (Elumeeva et al., 2018; Fujii et al., 2017), temperate grasslands and lakes (Petraglia et al., 2019; Seelen et al., 2019), peatlands (MacDonald et al., 2018), and subarctic wetlands (Whigham et al., 2017).

Before deployment, all tea bags were weighed (±0.001-g) and labelled. A pair of tea bags (one green-EAN:8714100770542 and one rooibos-EAN:8722700188438) were deployed at each sampling site in January and March 2019, then retrieved in March and June 2019, respectively. These two deployments were intended to capture effects of intra-annual climatic variations during dry season (January to March) and dry-wet season transitions (March to June). We did not measure decomposition during wet seasons because of high water level, logistic constraints, and high risks of losing tea bags. Hence, a total of 240 pairs of tea bags were deployed in all wetlands (i.e., 40 wetlands×3 sites×2 incubations). To capture aboveground litter decomposition (i.e., where most decomposition occurs in wetlands) (Brinson et al., 1981), we placed tea bags on the sediment surface and secured them using meshed nylon net and landscape pins (Figure 3-1). The retrieval times of tea bags (i.e., incubation period) were within 60-90 days after deployments, according to Keuskamp et al. (2013) for the tropical/subtropical biome. At the end of each incubation, we retrieved tea bags and transported them to the lab, removed adhering sediments, and oven-dried tea bags at 60°C for at least 48 hours until constant weight. Since the weights of bag, cord, and label of each teabag are initially consistent and provided by Keuskamp et al. (2013), after the incubation, we only weighed dried tea materials that were carefully taken out of each bag to avoid the confounding effect of weight changes in bag, cord, and label.

TBI Calculation

Based on our measurements, decomposition rate *k* was calculated by the rooibos tea mass loss *W* after incubation time *t*, following a negative double exponential regression describing the breakdown of decomposable fraction a_r at the beginning, and then gradually slowing down, with the recalcitrant fraction (1-a_r) left over time (Equation 3-1):

$$\mathrm{W(t)} = a_r e^{-kt} + (1 - a_r) \tag{3-1}$$

The decomposable fraction of rooibos tea (a_r) was determined by its hydrolysable fraction H_r and stabilization factor *S* (Equation 3-2):

$$a_r = H_r(1 - S) \tag{3-2}$$

The stabilization factor *S* was calculated based on decomposable fraction a_g and hydrolysable fraction H_g of green tea (Equation 3-3):

$$S = 1 - \frac{a_g}{H_g} \tag{3-3}$$

Hydrolysable fractions of rooibos tea (H_r) and green tea (H_g) were provided in Keuskamp et al., (2013).

Environmental Variables Collection

At each site per wetland, three soil cores (5-cm depth×10-cm diameter) were taken in March and June 2019 (in synchrony with tea bag retrieval times) to measure soil properties. Top 5-cm soils were sampled because they exert most influences on aboveground decomposition. All soil samples were sieved (with 2-mm mesh) and then used to measure water content, pH, organic matter, total C and N, and plant-available P, K, Ca, Mg, all of which could affect decomposition (Chapin et al., 2002; W. Zhang et al., 2008). Gravimetric soil water content was measured by weight loss after oven drying in 105°C for 72-hr, and soil pH was measured by 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 a 450°C muffle furnace (Schulte & Hopkins, 2015). Soil C and N content were measured using LECO CN628 C/N Determinator (LECO Corporation, MI) (Bremner, 2018). Plant-available P, K, Ca and Mg were extracted with Mehlich-3 solution (Mehlich, 1984), and analyzed using inductively coupled plasma atomic emission spectrometry technique (Soltanpour et al., 2018) with the Perkin Elmer Avio 200 (Perkin-Elmer, CT).

Meteorological data over the two incubations were collected by the main BIR weather station, including daily temperature (°C) and precipitation (mm). Daily average temperature and precipitation were calculated based on the timing and duration over which the tea bags were incubated in each experimental wetland. Elevations of wetlands (i.e., a variable that could affect wetland hydrology) were extracted from their geographic locations and the Light Detection and

Ranging (LiDAR) by Optech 1233 Airborne Laser Terrain Mapper system in 2006, and averaged by their extents.

Statistical Analyses

To determine individual and interactive effects of land-use intensity and management factors on wetland decomposition (*Q1*), and how it varies across hydrological gradient (i.e., sampling sites) and climatic conditions (i.e., incubation period) (*Q2*), we performed linear mixed-effects models separately for k and S. In the model specification, wetland management (i.e., land-use intensity, grazing, and prescribed fire), sampling site (center, middle, and edge), and incubation period (January through March, and March through June) were treated as fixed factors, and block as a random factor. Residual plots were assessed to check assumptions of normality and homogeneity of variance; no violations were detected. *P*-values were estimated using Type III Sums of Squares, due to multiple specified non-sequential variables and their interactions. We conducted our analyses at both site and aggregated whole-wetland levels (Table B-1) and found that the significant factors in determining k and S were consistent.

To disentangle the direct and indirect pathways through which management practices affected decomposition (i.e., *Q3*), we performed structural equation modeling (SEM) (Hooper et al., 2008). First, we reduced dimensions of soil properties using principal component analysis (PCA), because of high levels of collinearities among soil variables (Figure B-1). We used the derived first three components in our subsequent analyses, which together accounted for 82% of total variance. In addition, we performed multiple linear regressions as exploratory analyses to determine which principal components affected the decay rate k and stabilization factor S (Table 3-2). Based on these analyses and system knowledge, we constructed hypothesized path diagrams (Figure B-5) before fitting empirical data, along with other covariates that were hypothesized to affect decomposition (Figures 3-5, 3-6). Given that land-use intensity, grazing, and fire treatments were categorical variables, we evaluated their effects using x^2 likelihood ratio tests. We also performed post-hoc Tukey tests to determine significance of differences in categorical variables within each grouping (Bowen et al., 2017). Linear mixed-effect models, PCA, and SEM were performed in R 3.6.1 using “lme4”, “pls”, and “piecewiseSEM” packages, respectively (Bates et al., 2014; Lefcheck, 2016; Wehrens and Mevik, 2007).

Results

Land-use intensity, cattle grazing, and prescribed fire showed significant individual and interactive effects on decomposition rate *k* and stabilization factor *S*. For *k*, cattle grazing overall reduced *k* (Table 3-1); in addition, under fire treatment, wetlands embedded in IM pastures also showed marginally lower *k* than those in SN pastures (Table 3-1). For *S*, a significant three-way interaction among land-use intensity, grazing, and fire was detected (Table 3-1). Specifically, wetlands embedded in IM pastures tended to have lower *S* than those in SN pastures across most combinations of grazing and fire treatments, except for grazed and unburned wetlands (Figure 3-2). In addition, grazing and fire only showed significant interactive effects in IM wetlands, where grazing increased *S* in unburned wetland but did not affect *S* in burned wetlands (Figure 3-2).

Substantial temporal and spatial variation in wetland decomposition were also revealed, presumably associated with climatic conditions and hydrological gradient, respectively. The teabags incubated from January-March 2019 had significantly higher *k* than those from March-June 2019 (Table 1, Figure 3-3). In addition, spatial variation was more pronounced for *S* along the hydrologic gradient, where wetland edge sites had higher *S* than centers (Table 1, Figure 3-3).

Direct and indirect pathways on how land-use intensity and management affected decomposition were also revealed. Our SEM of *k* (Fisher's C=4.74, *p*=0.58) showed that *k* was directly affected by land-use intensity and grazing (Figure 3-5), yet stronger effects of land-use intensity were indirectly manifested through PC2 of soil attributes (indicated by soil C/N, C/P and pH), which negatively associated with *k* ($p=0.017$). In addition, effects of sampling site and wetland elevation were also manifested via PC2 (Figure 3-5). Daily precipitation directly affected *k* ($p<0.001$), but also indirectly through altering soil conditions (i.e., PC2).

SEM of *S* (Fisher's C=6.24, $p=0.40$) showed that *S* was directly affected by land-use intensity, sampling site, and daily average temperature ($p<0.05$, effect size=-0.01) (Figure 3-6). Effects of land-use intensity and daily temperature were also manifested through PC3 (indicated by soil C/P and pH), which negatively associated with *S* ($p<0.05$, effect size=-0.02). Effects of geographic location and wetland elevation on *S* were also manifested through PC1 (defined by soil nutrients and water content), which positively associated with *S* ($p<0.001$, effect size=0.01).

Discussion

Our research demonstrated that wetland decomposition was affected by land-use intensity, management, geographical features, and seasonal climate, either directly or indirectly through altering soil properties. Among all factors considered, land-use intensity was the most influential driver of wetland decomposition (Table 3-1), with complex interactions among grazing and fire disturbances. Specifically, our results highlighted that: (1) grazing slowed down decomposition (Figures 3-2, 3-5), and intensified land-use also reduced decomposition rate, but only when wetlands were burned; (2) intensified land-use reduced plant litter stabilization and thus carbon sequestration potentials (Figure 3-6); fire increased litter stabilization when wetlands were ungrazed but showed no effects in grazed wetlands, and such fire-grazing interactions only occurred in intensively-managed pastures (Figure 3-3); (3) substantial spatial variation in *S* from wetland edge to center were revealed, likely reflecting its sensitivity to hydrological gradient (Figures 3-3, 3-5); and (4) significant temporal variation in *k* were detected, indicating its sensitivity to seasonal climate conditions (Figures 3-3, 3-6). Findings from this research have important implications for managing tropical/subtropical seasonal wetlands to sustain their crucial ecosystem functions and services in the context of global environmental changes.

Interactive Effects of Land-Use Intensity and Management Practices on Wetland Decomposition

Intensified land-uses could slow down decomposition of labile components in plant litter (Figure 3-5), consistent with previous studies (e.g., Solly et al., 2014; Violita et al., 2016). The primary mechanism could be that when mineral N nutrients are adequate through runoff from surrounding upland fertilized pastures, soil microbes are provided sufficient amounts of easily digested nutrient, hence, lowering the microbial nutrient demands and delaying the associated N mineralization (Fog, 1988). Such inhibitory effects of N enrichment on decomposition rate have also been observed in other studies (Entry, 2000; Hobbie, 2008; Y. Li et al., 2016; Song et al., 2017; Tao et al., 2013). However, another seemingly 'contradictory' theory posits that nutrient addition may stimulate decomposers' activities, thereby facilitating decomposition (Swift et al., 1979). Yet as a matter of fact, this facilitation refers to decomposition completeness (i.e., reducing S), rather than the rate of initial decomposition (i.e., increasing k), because nutrient enrichment tends to enhance fungal growth that are the major decomposers of recalcitrant organic compounds (Ferreira and Chauvet, 2011). A recent molecular study confirmed this explanation by showing that N-induced inhibition only existed on labile litter components (e.g., cellulose) but not on complex macromolecules (e.g. lignin) (Z. Chen et al., 2019). Therefore, in tandem, both mechanisms explain why wetlands in IM pastures had lower k and meanwhile lower S than those embedded in SN pastures (Figure 3-2). Such results also highlighted the importance of investigating decomposition processes of different litter components and their dominant actors and mechanisms.

Moreover, in IM wetlands, increased soil pH was also found to decrease *S* (Figure 3-6), which could be another mechanism related to land-use intensity, which concurs with findings from Elumeeva et al., (2018). The lime applied in IM pastures contributed to a more pH-neutral environment in wetlands (Figure B-2), which may favor microbial diversity and activities (Paul, 2014) and then promote decomposition completeness. However, the specific effects of microbial composition and diversity on organic matter stabilization need to be further explored.

Grazing decreased *k* and *S*, especially in SN wetlands (Figures 3-2, 3-5, 3-6). Similar to effects of intensified land-uses on *k*, cattle grazing could return nutrients to wetlands and lead to N inhibitory effect on labile material decomposition, because much of animal-excreted N was in readily available urea or dung forms to decomposers (Y. Wang et al., 2015). In addition to animal waste nutrient inputs, grazing reduced vegetation canopy and promoted the abundance of short graminoids and forbs from foraging activities (Boughton et al., 2016; Ho et al., 2018), which could increase exposure of underlying plant litter to the solar radiation, increase the surface temperature and enable photodegradation that consequently enhances decomposition completeness (i.e., *S*) (Austin et al., 2016). Grazing effects on *k* and *S* were more profound in SN wetlands, probably because intensified land-uses had the same directional effects as grazing, and the magnitude of land-use intensity effects through nutrient enrichment might already saturate for wetland decomposition in IM wetlands (Chapin et al., 2002).

Individual effects of prescribed fire on decomposition were much weaker as compared to land-use intensity and grazing, perhaps due to its infrequent occurrence and pulse dynamics that resulted in no stable or persistent impacts over time, coupled with weak lag effects. Overall, in IM wetlands, fire tended to increase *S* where wetlands were released from grazing but did not affect *S* in grazed wetlands (Figure 3-2), while fire had no effect on *S* in SN wetlands. The interactive fire-grazing effect on litter decomposition might result from complex plant-soil-microbe interactions that vary with grazing regimes (Churchland and Grayston, 2014). Fire had been found to reduce overall N stocks in wetland soils (Ho et al., 2018), which might have positive effects on litter stabilization (Figure 3-6). However, cattle grazing could add soil nutrients through excrement and reduce nutrient loss by favoring growth of unpalatable and slow-growing wetland plants and associated decomposers (Boughton et al., 2016), which counteracted fire effects on *S*. Moreover, burned and ungrazed wetlands had more non-native plants (Boughton et al., 2016). Invasive plants might release phenolic acids that suppress microbial respiration (Jones et al., 2019) and thereby increase litter stabilization.

Spatial and Temporal Variations in Wetland Decomposition

Substantial spatial variation in wetland litter stabilization *S* (Table 3-1, Figure 3-3) suggested that in the lowest and wettest point of a wetland there was lower litter stabilization. This trend is consistent with MacDonald et al. (2018) that found wetter sites in peatlands were associated with lower *S*. Indeed, our SEM results (Figure 3-6) corroborated this finding by showing that sampling site (i.e., center, middle, edge) affected *S* through changes in soil water content and nutrient levels. Given the relationships among soil properties where higher water contents were associated with higher soil nutrients (Figure B-1), we argue that the completeness of litter decomposition could be enhanced by higher soil moisture and nutrient concentrations and associated decomposers activities.

Our results also showed significant temporal variation in decomposition rate *k* (Table 3-1, Figure 3-3), likely resulting from influences of seasonal climatic conditions (Zhang et al., 2008). Two teabag incubation periods had significantly different meteorological characteristics (Figure B-4). The second incubation from March through June 2019 had higher temperature and less precipitation than the first incubation, which led to lower soil water content during the second incubation (Figure B-3). Based on the SEM results (Figure 3-5), we infer that increased precipitation during dry seasons could accelerate decomposition rate by increasing soil moisture and pH, as well as associated decreased soil C/N and C/P ratio.

Management Implications

Our findings have important implications for wetland management in landscapes dominated by agriculture. Our observation of decomposition variability along the hydrological gradient within wetlands is relevant to understanding effects of ongoing water retention and hydrologic restoration measures (i.e., for providing flooding control and nutrient retention services). As water retention in the headwaters of the Florida Everglades – i.e., one of the world's largest and most diverse wetlands – garners more attention for controlling downstream eutrophication, the consequences of water retention need to be fully evaluated (Boughton et al., 2019; Sonnier et al., 2018). For example, water retention could increase wetland water table and depth, and extend hydroperiods, making future wetland edge/middle sites more similar to current center sites. Hence, hydrological restoration in this region might lead to lower litter stabilization (Figure 3-3), and thus less carbon sequestration potential (Chamberlain et al., 2017). This possible water-carbon tradeoff in seasonal wetlands due to water retention measures requires further testing in future studies.

Land-use intensification, grazing introduction and fire suppression are all typical anthropogenic management and disturbances in subtropical/tropical wetlands and marshes. Taking ungrazed, burned wetlands embedded in SN as the historical benchmark for a "natural" reference, we found that all types of human disturbances tend to lower litter stabilization and therefore carbon sequestration potentials of seasonal wetlands (Figure 3-2). Across all factors examined, intensified land-use of pastures had the strongest adverse impact on wetland decomposition, highlighting the importance of landscape context in wetland management, as well as the need of avoiding land-use intensification surrounding subtropical/tropical seasonal wetlands. Moreover, if pastures must be managed under intensified land-use (e.g., for achieving sufficient forage productivity for livestock), it is critical to avoid the combined practices of cattle exclusion and fire suppression. Further, in agricultural areas with focus on cattle production, management under low-intensities and with periodically prescribed fires could help counteract the potential negative grazing effects on long-term carbon sequestration and nutrients retention in seasonal wetlands.

Methodological Considerations

Several methodological limitations in our study are worth discussion. Human activities and external environmental changes could alter plant stoichiometry, and the structure and composition of plant communities (Boughton et al., 2010, 2016). Our TBI approach cannot capture those indirect effects through vegetational changes, litter quality and local plant-soil-microbe interactions, if any. One example of such plant-microbe interactions is that if certain wetlands are dominated by plants with inherently high lignin content, their local microbial communities are likely to be better adapted and “primed” to process lignin-rich organic matter in the tea bags. Hence, our results primarily reflect how human activities and other induced abiotic factors influence decomposition processes and potentials. Uncovering these complex plant-microbial interactions in wetland decomposition would be a fruitful avenue for future research (e.g., using controlled laboratory experiments). Another potential limitation is that we did not measure wetland decomposition before the initial implementation of management treatments that occurred over a decade ago. Nevertheless, our randomized block design can to some extent control the effects of differences in initial conditions that may confound treatment effects.

Table 3-1. Linear mixed-effects model results on wetland teabag decomposition in response to management factors and environmental covariates

Explanatory variable	Estimate	SE	*P*-value
	Decomposition rate *k*		
Pasture (IM)	0.0004	0.0017	0.128
Grazing (Grazed)	–0.0014	0.0017	0.022*
Fire (Burned)	0.0032	0.0016	0.437
Site (M)	–0.0101	0.0008	0.140
Site (C)	0.0018	0.0010	0.140
Period (March-June)	0.0002	0.0010	<0.001***
IM×Grazed	–0.0003	0.0024	0.391
IM×Burned	–0.0046	0.0024	0.083.
Grazed×Burned	–0.0022	0.0023	0.724
IM×Grazed×Burned	0.0034	0.0033	0.304
	Stabilization factor *S*		
Pasture (IM)	–0.159	0.026	<0.001***
Grazing (Grazed)	–0.032	0.025	0.897
Fire (Burned)	0.013	0.025	0.393
Site (M)	–0.017	0.013	<0.001***
Site (C)	–0.077	0.015	<0.001***
Period (March-June)	–0.133	0.015	0.169
IM×Grazed	0.146	0.036	0.003**
IM×Burned	0.080	0.036	0.717
Grazed×Burned	–0.011	0.035	0.002**
IM×Grazed×Burned	–0.139	0.050	0.005**

Notes. Units of teabag decomposition parameters are decomposition rate *k* ($mg \cdot g^{-1} \cdot day^{-1}$) and stabilization factor *S* (%). * $p<0.05$, ** $p<0.01$, *** $p<0.001$, • $p<0.10$.

Table 3-2. Multiple linear regression estimates of wetland teabag decomposition using the first three principal components (PCs)

PCs	Estimate	SE	*P*-value
	Decomposition rate *k*		
PC1	0	0.0002	0.993
PC2	–0.0015	0.0005	0.002**
PC3	0.0002	0.0005	0.597
	Stabilization factor *S*		
PC1	0.023	0.003	<0.001***
PC2	0.006	0.006	0.335
PC3	–0.019	0.006	0.003**

Notes. First three PCs were generated from the principal component analysis (PCA)

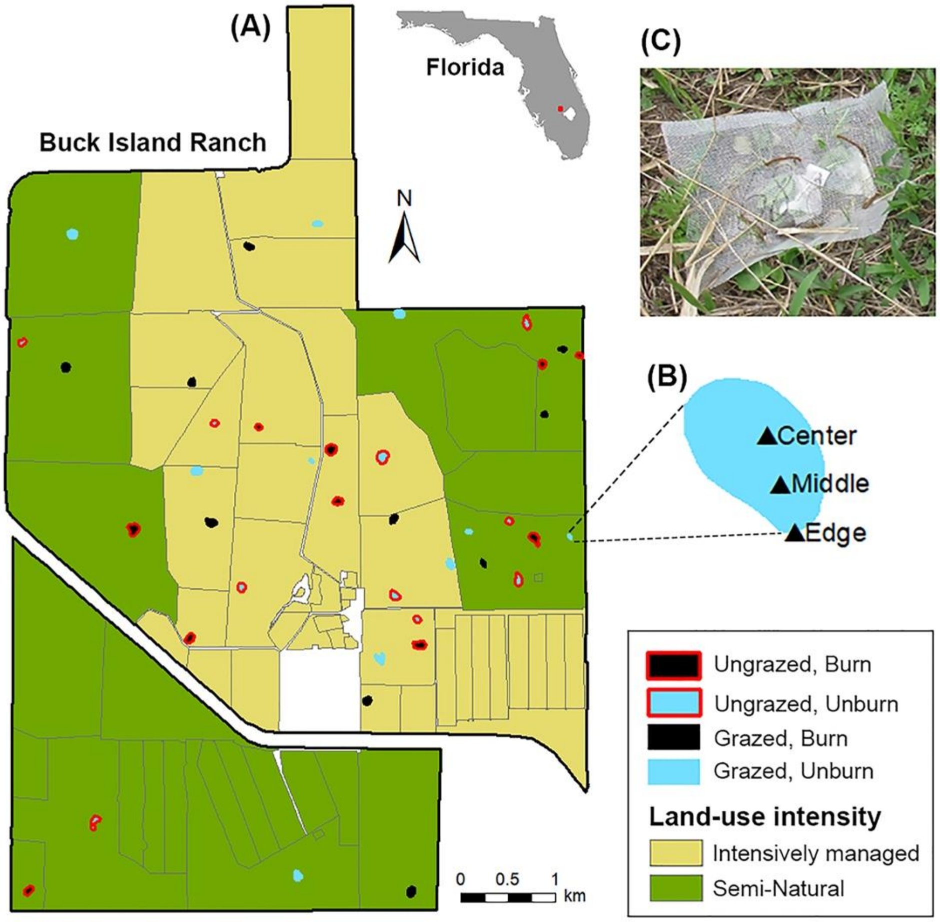

Figure 3-1. (A) Map of the 40 wetlands in a whole-ecosystem experiment at Archbold Biological Station's Buck Island Ranch. Intensively managed (IM) pastures are shown in yellow, and semi-natural (SN) pastures are shown in green. Fenced (ungrazed) wetlands are illustrated with red outlines, and grazed wetlands with no outlines. Burned wetlands are shown in black color, and unburned wetlands are filled with light blue color. (B) Each wetland has three sampling sites – center, middle, and edge – to capture the spatial variation along the hydrological gradient. (C) Photo is shown as an example of tea bag deployment in the field.

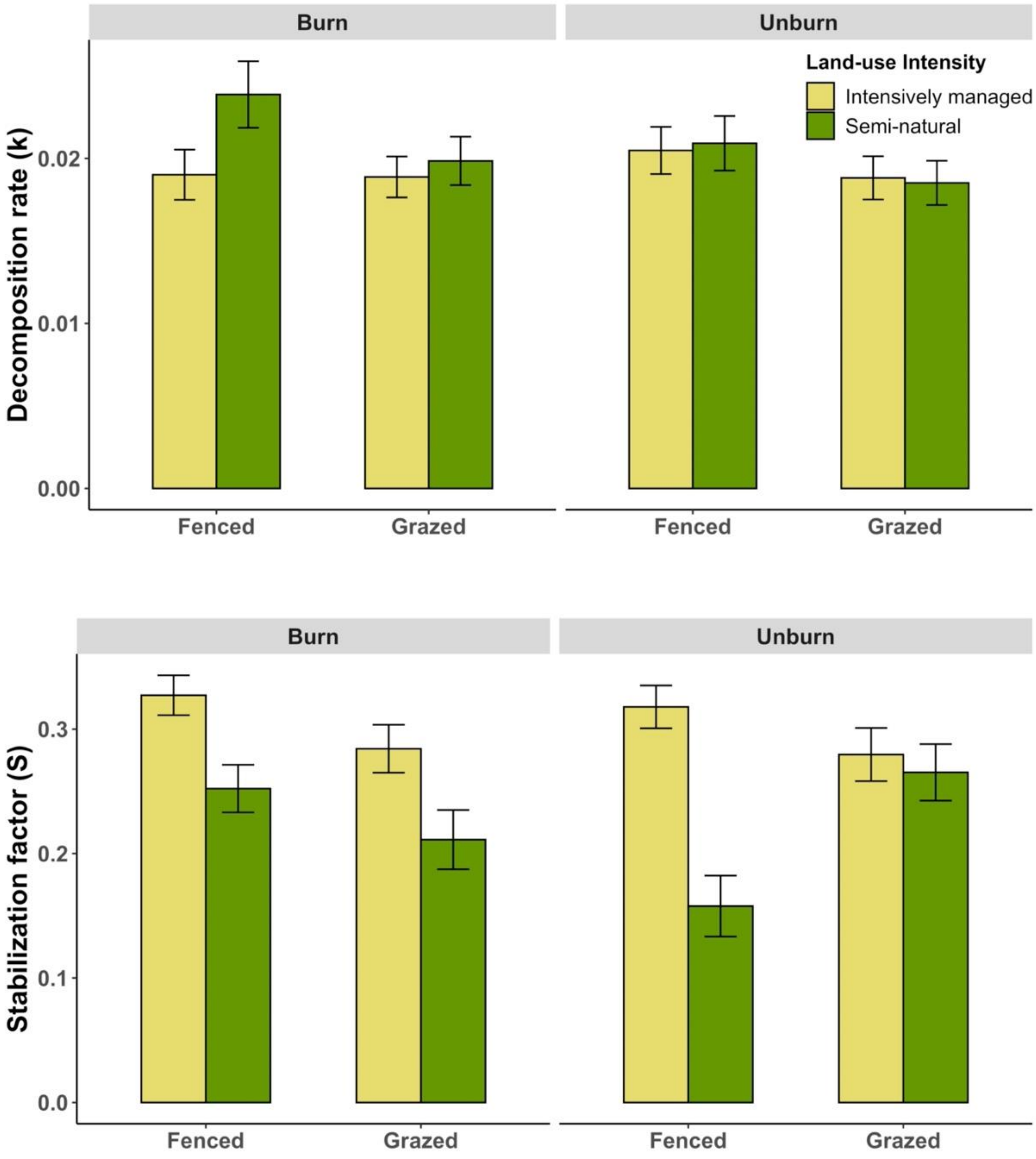

Figure 3-2. Differences in teabag decomposition rate *k* (A) and stabilization factor *S* (B) across wetland treatments (from embedded in intensively managed and semi-natural pastures, grazed and fenced (ungrazed) wetlands, burned and unburned wetlands). Error bars represent SEs. Full results of model fitting are shown in Table 2-1.

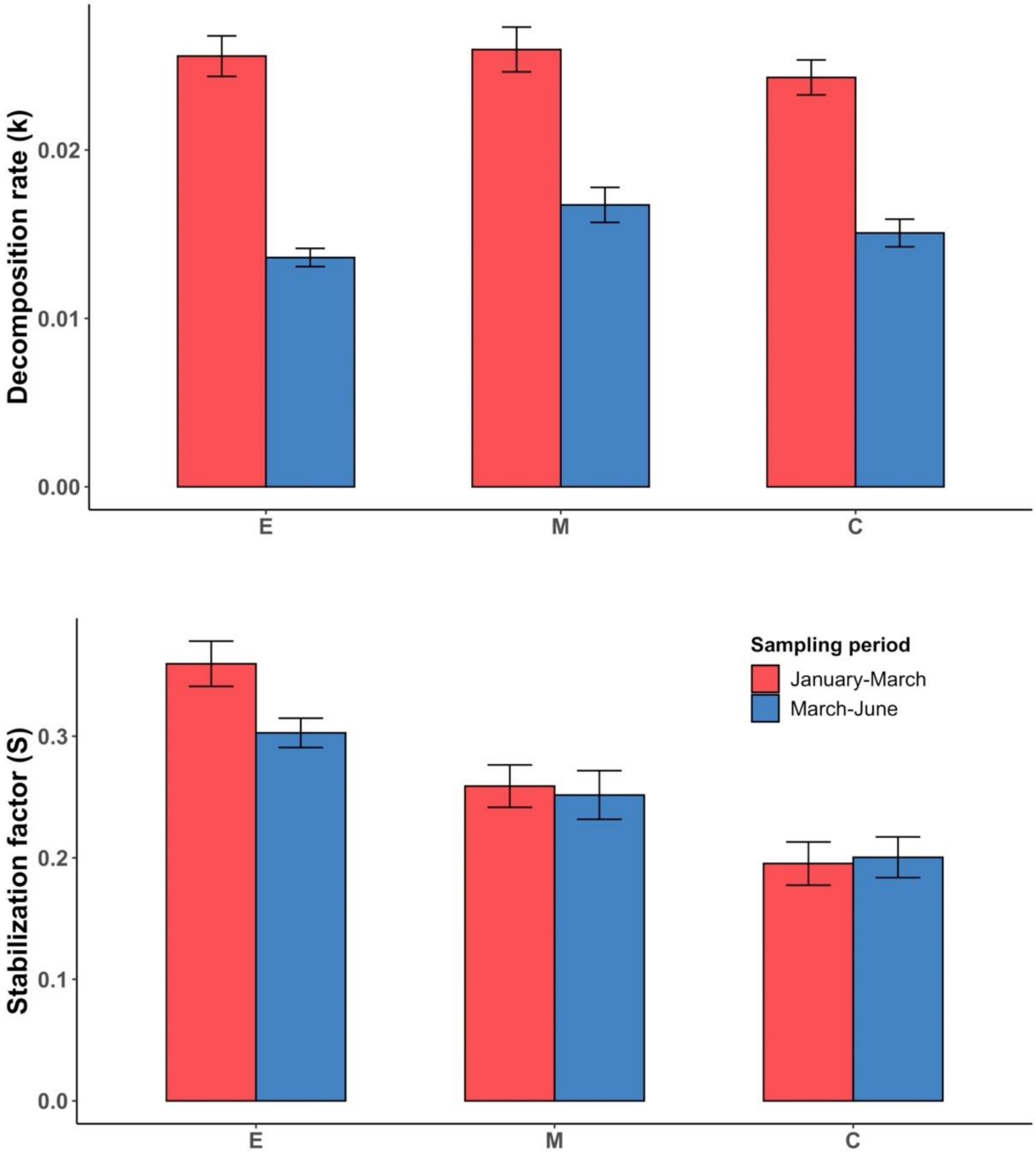

Figure 3-3. Differences in teabag decomposition rate *k* (A) and stabilization factor *S* (B) across three wetland sampling sites (edge, middle and center) and incubation periods (January to March, and March to June). Error bars represent SEs.

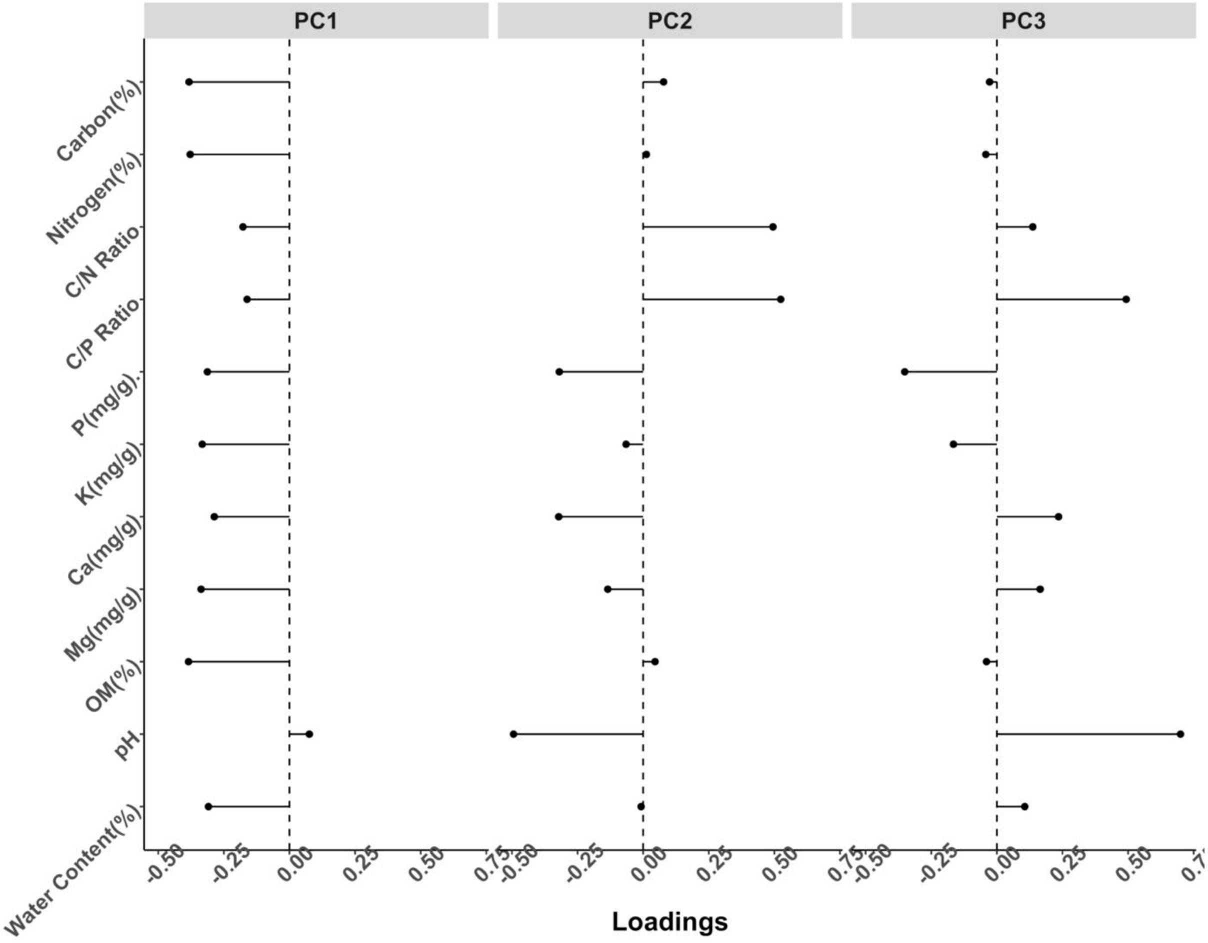

Figure 3-4. Loadings of predictor variables from the soil physical-chemistry properties principal component analysis (PCA). Resulting principal component (PC) 1, PC2 and PC3 altogether account for 81.5% of the total variation.

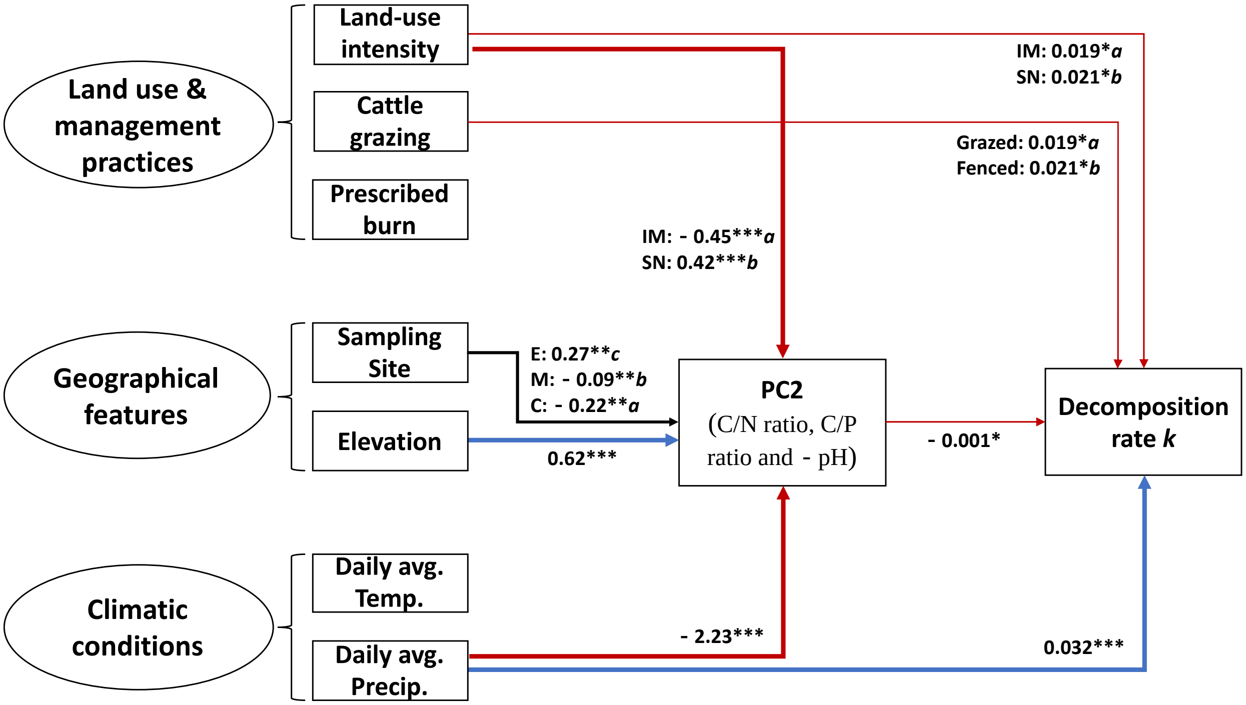

Figure 3-5. Structural equation modeling results for decomposition rate *k*. PC2 represents the second principal component of PCA on soil properties that is primarily defined by soil C/N ratio, C/P ratio and pH. Blue and red arrows represented each significant path (* indicates $p<0.05$; ** indicates $p<0.01$; *** indicates $p<0.001$), with blue and red colors indicate positive and negative treatment effects/relationships, respectively. Nonsignificant treatment effects or relationships ($p>0.05$) are omitted in the figure for clarity. The thickness of the arrow is proportional to the magnitude of effect size. Post-hoc tests (denoted as letters a, b and c) were performed to denote the level of significance within each group of categorical variables.

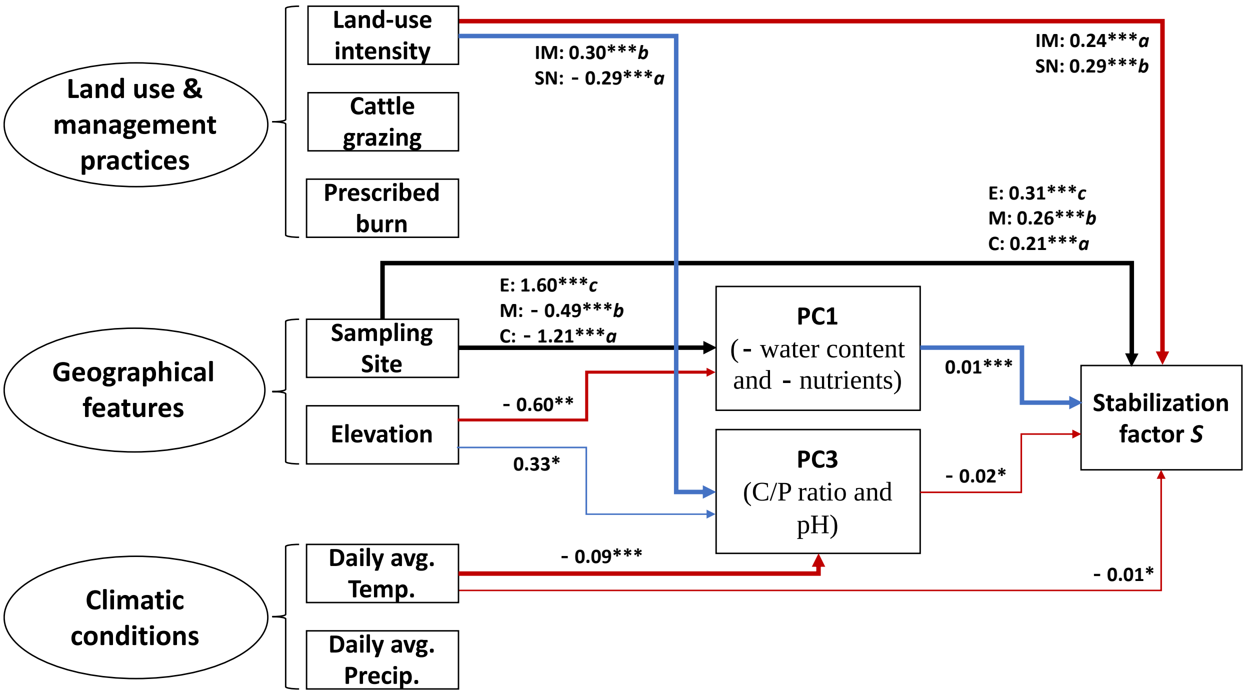

Figure 3-6. Structural equation models for stabilization factor *S*. PC1 represents the first principal component of PCA on soil properties that is primarily defined by soil nutrients and water content, while PC3 is primarily defined by soil C/P ratio and pH. Blue and red arrows indicate positive and negative treatment effects/relationships, respectively (* indicates p<0.05; ** indicates p<0.01; *** indicates p<0.001). Nonsignificant relationships (p>0.05) have been omitted for clarity. The thickness of the arrow is proportional to the magnitude of effect size. Post-hoc tests (denoted as letters a, b and c) were performed to denote the level of significance within each group of categorical variables.

CHAPTER 4[*]

INTERACTIVE EFFECTS OF LAND-USE INTENSITY, GRAZING AND FIRE DISTURBANCES ON SOIL BACTERIAL AND FUNGAL COMMUNITIES IN SUBTROPICAL WETLANDS

Introduction

Geographically isolated wetlands are disproportionately valuable landscape elements that provide critical ecosystem functions and services such as biodiversity support, flood mitigation, carbon sequestration, sediment and nutrient retention (Cohen et al., 2016; Marton et al., 2015; Rains et al., 2016). 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). These wetlands thus serve as important social-ecological linkages between agricultural production, natural communities, and rural livelihoods (Swain et al., 2013). However, these wetlands are facing constant and emerging 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., fertilization, extensive drainage) often results in increased nutrient runoff and eutrophication, greater hydrologic variability, and biodiversity loss in wetlands (Shukla et al., 2019). Grazing can also alter wetland plant community composition via dietary preferences and trampling effects from livestock (Boughton et al., 2010; Jones et al., 2011), which further modify soil properties by increasing soil compaction and incorporating surface litter into mineral soils (Ho et al., 2018). Furthermore, wetlands embedded in managed grasslands are often

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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 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; P. Upadhyay et al., 2019). 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; P. Upadhyay et al., 2019). 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 responses of wetland soil microorganisms to different agricultural and wetland management is critical to inform sustainable landscape 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), 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; 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, 2006a). 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 use and wetland 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 16S ribosomal RNA (16S 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 (Louca et al., 2016; Nguyen et al., 2016) to examine potential microbial functional responses. 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.

Material and Methods

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 ºC and precipitation is 1,360 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., 2011, 2016; 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 (Figure 4-

1). IM 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).

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 (2×2×2 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 (Figure 4-1). Half of the wetlands were located within IM pastures and the remaining half in SN pastures. For the ungrazed treatment, fences were installed in 2007 in 10 wetlands in IM and SN pastures, respectively, to exclude cattle grazing. In grazed wetlands, grazing was imposed in cattle foraging activities at typical local stocking rates. For prescribed fire, burn treatment was imposed to 5 random wetlands in each combination of land-use intensity × 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.

Soil samples were collected in March and June 2019 to capture distinctive seasonal variations. At each sampling effort, three soil cores (5-cm depth×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.

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 hours. 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 CN628 C/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).

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 16S 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 341F (5´-CCTACGGGNGGCWGCAG-3´) / 806R (5´-GGACTACHVGGGTWTCTAAT-3´) and ITS1F (5´-CTTGGTCATTTAGAGGAAGTAA-3´) / ITS4 (5´-TCCTCCGCTTATTGATATGC-3´), respectively. Quality and quantity of DNA, and size of PCR products were verified using a UV/VIS spectrophotometer (NanoDrop^{TM}, 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 × 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 1,735,406 high-quality 16S 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. Specifically, the tables of frequency of bacterial and fungal taxa at the 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 6,000 and 600 reads per sample for bacterial 16S rRNA and fungal ITS genes, respectively, according to the rarefaction curves (Figure C-1).

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 C-4), 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, relationships of microbial alpha diversity and relative abundances of dominant microbial phyla and functional groups with soil physiochemical properties were 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.

Results

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%) (Figure C-2). 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%) (Figure C-3). 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 C-1.

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%) (Figure C-4). At the class level, Tremellomycetes (20.7%), Dothideomycetes (11.2%), and Sordariomycetes (9.5%) were most frequent (Figure C-5). 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 C-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 4-1). Specifically, land-use intensification exerted individual effects on bacterial OTU composition (Figure 4-2, $p<0.05$), bacterial functional group composition (Figure 4-2, $p<0.001$), and fungal OTU composition (Figure 4-2, $p<0.05$). In addition, land-use intensification also showed interactive effects with prescribed fire to alter fungal functional group composition (Figure 4-2; $p<0.005$). Finally, cattle grazing and prescribed fire interactively affected bacterial OTU composition (Figure 4-2, $p<0.05$).

Treatment Effects on Wetland Microbial Alpha Diversity

Bacterial OTU alpha diversity was not affected by any of the treatments (Table 4-2). Fungal OTU richness and Shannon's diversity were interactively affected by land-use intensity, cattle grazing, and prescribed fire (Table 4-2). Specifically, in SN and unburned wetlands, grazing treatment reduced fungal richness by 53.6% (Figure 4-2; $p<0.01$) and reduced Shannon diversity by 50.7% (Figure 4-2; $p<0.01$). In SN and ungrazed wetlands, fire treatment resulted in a 53.2% lower fungal richness (Figure 4-2; $p<0.01$) and a 51.3% lower fungal Shannon diversity (Figure 4-2; $p<0.01$).

Although bacterial OTU alpha diversity did not respond to wetland treatments (Table 4-2), bacterial functional diversity metrics were affected individually by land-use intensity, and interactively by grazing and fire (Table 4-3). Specifically, land-use intensification increased bacterial functional richness by 22.1% (Figure 4-4; $p<0.01$) and bacterial functional Shannon diversity by 13.4% (Figure 4-4; $p<0.01$). In addition, in unburned wetlands, grazing decreased bacterial functional richness by 16.8% (Figure 4-5; $p<0.05$). In ungrazed wetlands, fire led to a 22.3% lower bacterial functional richness (Figure 4-5; $p<0.01$). 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 (Figure 4-4; $p<0.05$).

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 (Figure C-6), while the relative abundance of Acidobacteria was lower in IM wetlands (Figure C-6). Two major bacterial phyla responded to livestock grazing, with relative abundance of Actinobacteria being higher under grazing (Figure C-6) and that of Proteobacteria relatively higher without grazing (Figure C-6). 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 (Figure C-7). In addition, cattle grazing reduced the relative abundances of Alphaproteobacteria and Gammaproteobacteria (Figure C-7), and prescribed fire tended to reduce the relative abundance of Clostridia (Figure C-7).

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 Arnium were greater in SN wetlands than in IM wetlands (Figure C-8).

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 (Figure 4-6), while nitrogen-fixing bacteria had higher relative abundance in IM wetlands (Figure 4-6).

Relationship of Wetland Microbial Community 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 C-3), we further analyzed relationships of wetland microbial community with selected soil variables. Our results showed that soil pH 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 (Figure 4-7). Soil Ca and Mg content showed positive correlations with microbial functional diversity, while soil P, K, OM, and water contents showed negative correlations with microbial OTU diversity (Figure 4-7).

Soil properties were also significantly correlated with the relative abundance of certain dominant bacterial and fungal phyla (Figure 4-8). For example, the relative abundance of Bacteroidetes was highly positively correlated with soil Ca and Mg contents and pH (Figure 4-8), whereas the relative abundance of Acidobacteria was negatively correlated with soil Ca content and pH (Figure 4-8).

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 (Figure 4-9). 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 (Figure 4-9).

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 indirect pathways of these 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. 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.

Effects of Land Management on Soil Microbial Taxonomic Composition

Our results indicated that the overall OTU compositions of bacterial and fungal communities were significantly affected by agricultural land use and wetland management, particularly by upland land-use intensification (Figure 4-2, Table 4-1).

Bacteria

Changes in bacterial community composition were reflected in the shifts of relative abundance among taxonomic groups (Figures C-2, C-3, C-6, C-7), but did not translate to effects on the alpha diversity (Table 4-2), suggesting a ‘reshuffle’ without simplification of bacteria communities due to land-use intensification. Indeed, at the 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 (Figure C-6). 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. 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 C-3) (Figure 4-8), and a strong negative correlation between relative abundance of Acidobacteria and soil pH (Figure 4-8). 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 the major drivers for the structure of bacterial community.

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 (Figure 4-7). 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 also 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 (Figure 4-3, Table 4-2) and shifts of taxonomic composition (Figures. C-4, C-5, C-8). Our results revealed that either grazing or fire disturbance significantly reduced fungal OTU diversity in SN wetlands (Figure 4-3), indicating that bacterial and fungal taxonomic alpha diversity were differentially affected by wetland management. At the OTU level, only fungal richness and Shannon index responded to interactive treatments; bacterial OTU diversity was not affected by management practices, even though wetland treatments significantly affected soil properties (Table C-3), 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 (Figure 4-7) 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 (Figure 4-7).

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). 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 (Figure 4-3), 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).

Effects of Land Management on Soil Microbial Potential Functions

Bacteria and fungi perform diverse ecologically relevant functions, participating in almost every aspect of soil organic matter 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 4-1, Figure 4-2). For example, multiple bacterial functional groups were more frequently detected in IM wetlands than in SN wetlands, including nitrifiers, aerobic ammonia oxidizers, predatory/exoparasitic bacteria, methylotrophs, methanotrophs, hydrocarbon degraders, aromatic compound degraders, and fermentative bacteria (Figure C-9). These results suggest that IM wetlands might have higher denitrification, organic matter decomposition, and methane consumption activities than SN wetlands, which is consistent with our 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 (Figure 4-4). Furthermore, grazing and fire decreased bacterial functional richness, respectively, when the other treatments were statistically controlled (Figure 4-5). Therefore, these 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; L. 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 (Figures 4-7, 4-9), which is consistent with results from 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, functions, and environmental gradients are not static and have important temporal dynamics (Comte et al., 2013; Waldrop and Firestone, 2006b). Further long-term time series observations of soil microbes in seasonal wetlands would be required to test and validate those dynamic relationships.

Finally, 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 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 more accurately infer changes microbial community functions large-scale shotgun metagenomic and metatranscriptomic sequencing studies will be required.

Management Implications

Soils in agroecosystems have been increasingly under anthropogenic pressures from land management and agricultural productivity, 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 (Armbruster et al., 2021). In particular, inconsistency between taxonomic and functional attributes of microbial communities in response to anthropogenic environmental changes (Gibbons, 2017; L. 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 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 the agriculture landscape (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, 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.

Table 4-1. Results of PERMANOVA on Bray-Curtis distance of bacterial OTU, fungal OTU, bacterial functional, and fungal functional communities

			OTUs composition			Functional groups composition		
	Treatment	DF	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

Notes. Bold values indicate significant coefficients. * indicates $p \leqslant 0.05$, ** indicates $p \leqslant 0.01$, *** indicates $p \leqslant 0.001$.

Table 4-2. Results of linear mixed-effects models of wetland treatment effects on bacterial and fungal OTUs alpha diversity metrics

				Richness		Shannon diversity		Evenness	
	Treatment	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	0.26
	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

Notes. Alpha diversity metrics refer to richness, Shannon diversity index, and Pielou's evenness. Bold values indicate significant coefficients. * indicates $p \leqslant 0.05$.

Table 4-3. Results of linear mixed-effects models of wetland treatment effects on bacterial and fungal functional groups alpha diversity metrics

				Richness		Shannon diversity		Evenness	
	Treatment	numDF	denDF	*F* value	*P*	*F* value	*P*	*F* value	*P*
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
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

Notes. Alpha diversity metrics refer to richness, Shannon diversity index, and Pielou's evenness. Bold values indicate significant coefficients. * indicates $p \leqslant 0.05$, ** indicates $p \leq 0.01$.

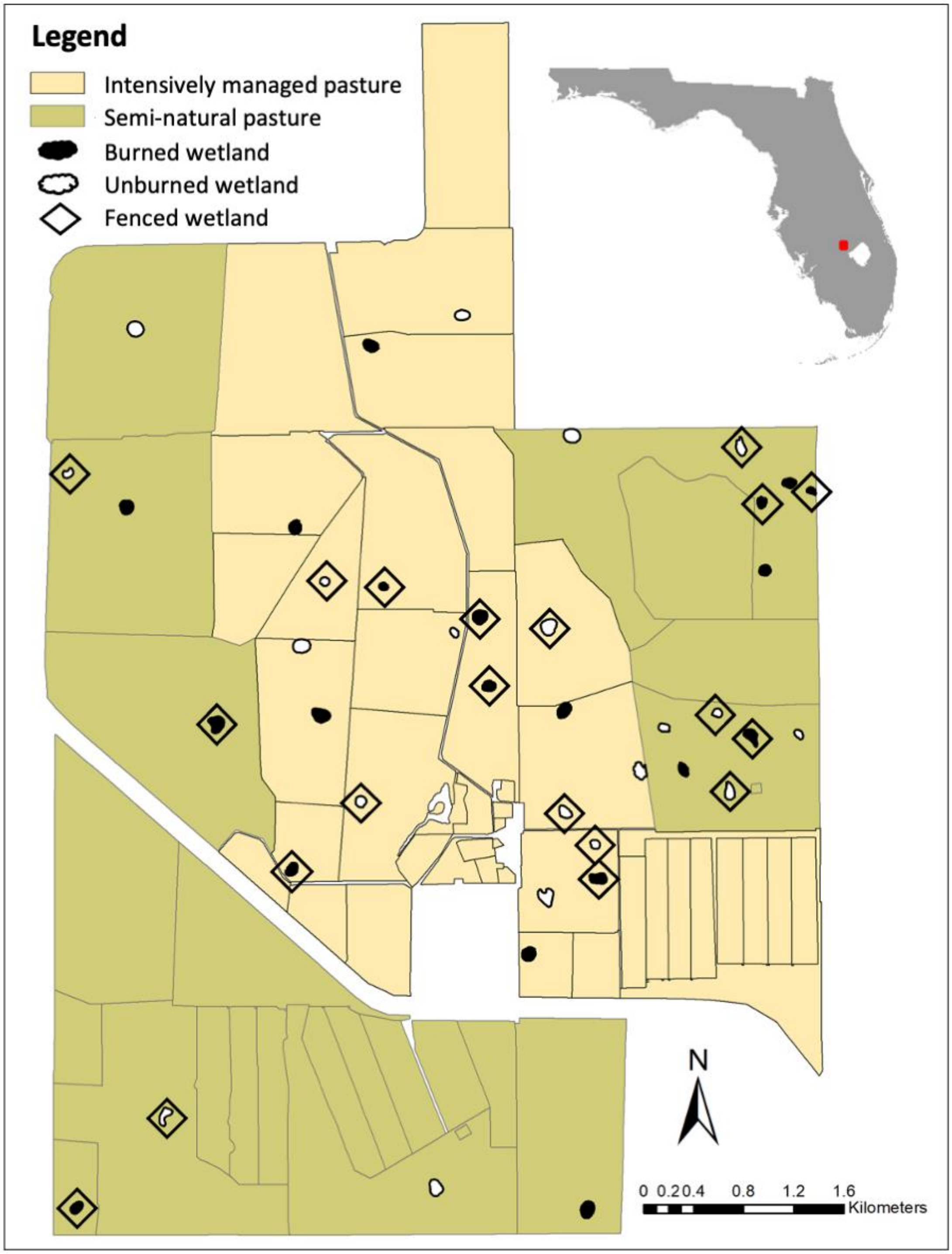

Figure 4-1. Map of the 40 experimental wetlands at Archbold Biological Station’s Buck Island Ranch. Intensively managed (IM) pastures are shaded light yellow, and semi-natural (SN) pastures are shown in green. Fenced (ungrazed) wetlands are surrounded by black diamond-shaped exclosure whereas grazed wetlands are show without dimond-shaped exclosure. Burned wetlands are filled with black color and unburned wetlands are filled with white color.

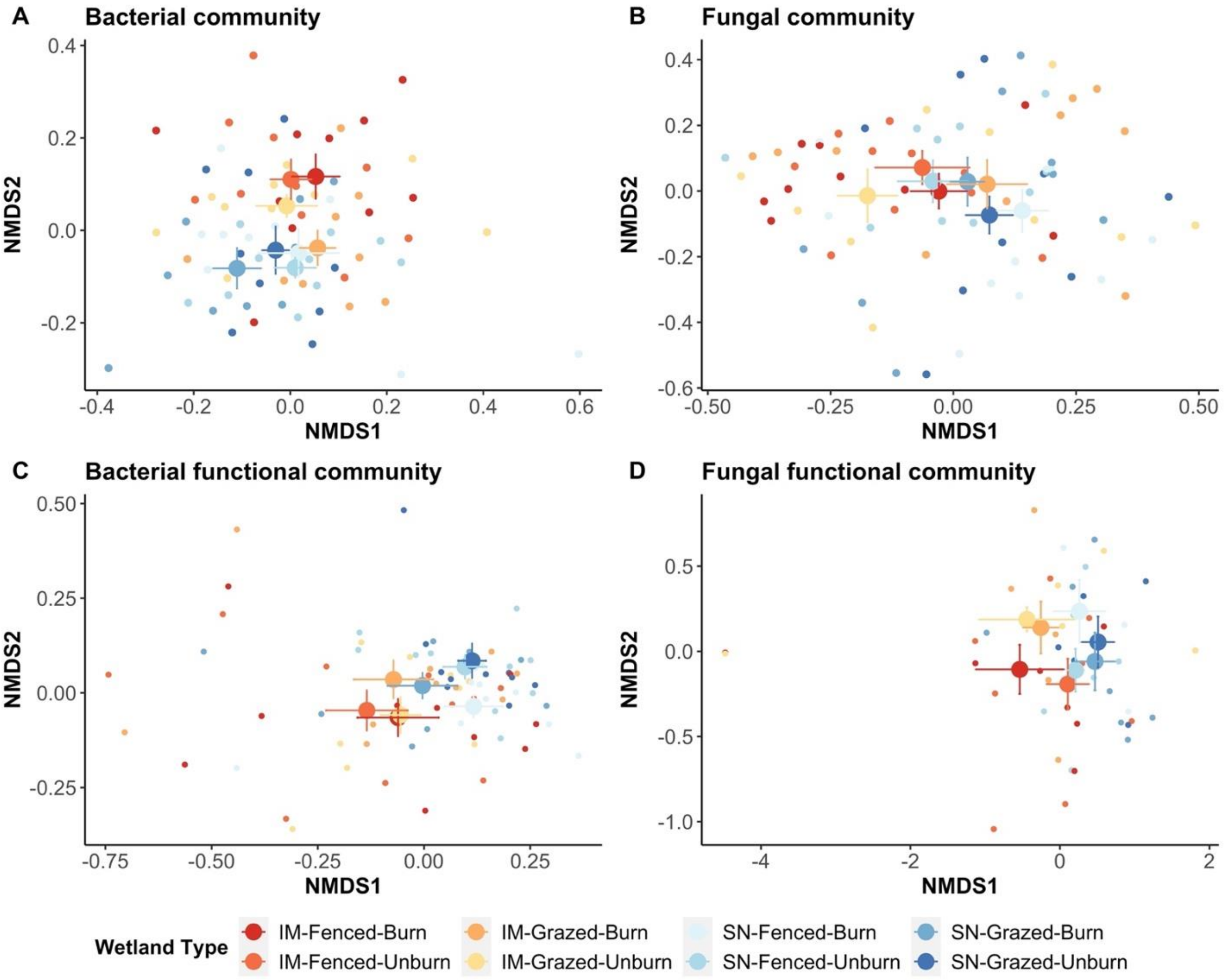

Figure 4-2. Nonmetric multidimensional scaling ordinations of bacterial (A), fungal (B), bacterial functional (C), and fungal functional (D) community dissimilarities using Bray-Curtis distance matrix (A: stress = 0.188, B: stress < 0.05, C: stress = 0.142, and D: stress = 0.135) among land-use intensity, grazing and fire treatments. Each small point indicates one wetland soil sample. Larger circle points with error bars indicate the mean ± SE for each combination of treatment (N=10; i.e., 5 replicates for each wetland type × 2 sampling times).

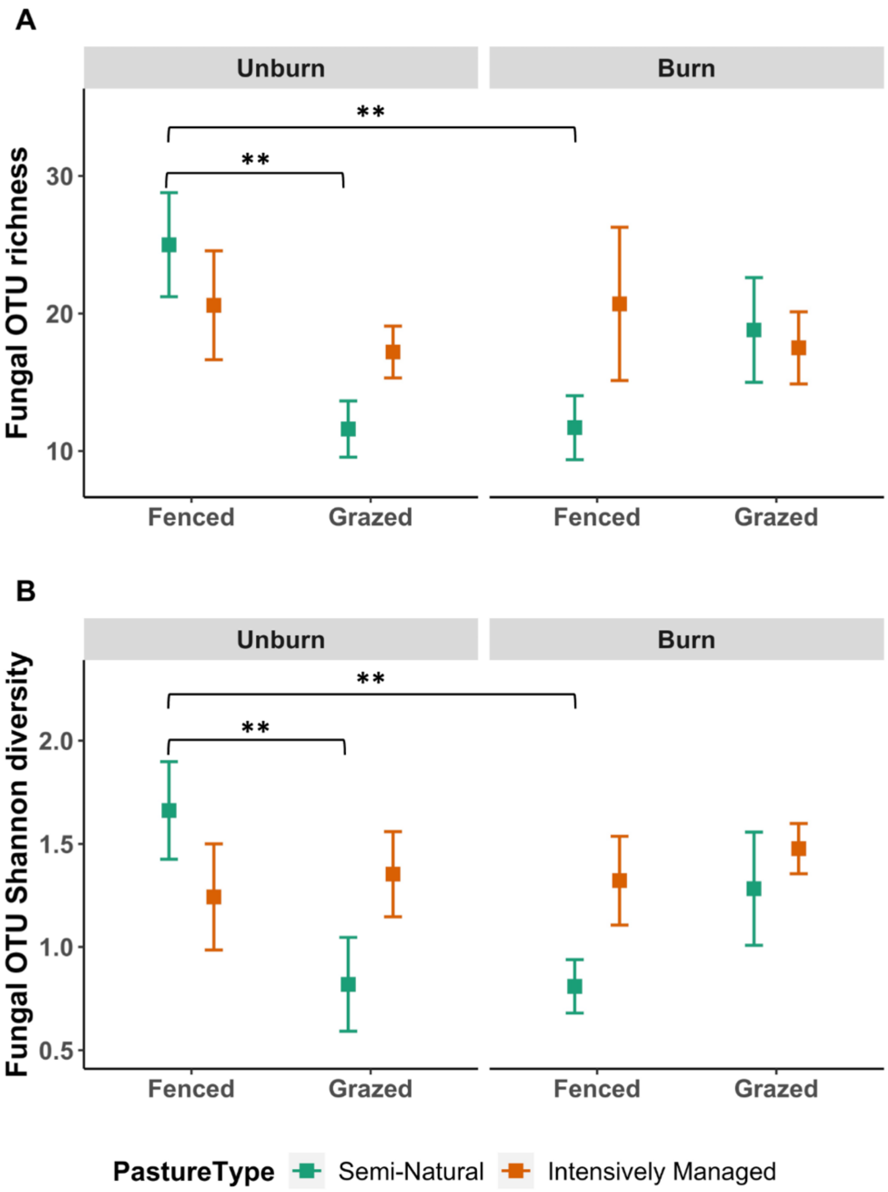

Figure 4-3. Effects of land-use intensity, cattle grazing and prescribed fire on fungal OTU richness (A) and fungal OTU Shannon diversity (B) (mean ± SE; N = 10). Pairwise significant differences were determined by the Tukey post hoc test. * indicates $p \leq 0.05$, ** indicates $p \leq 0.01$

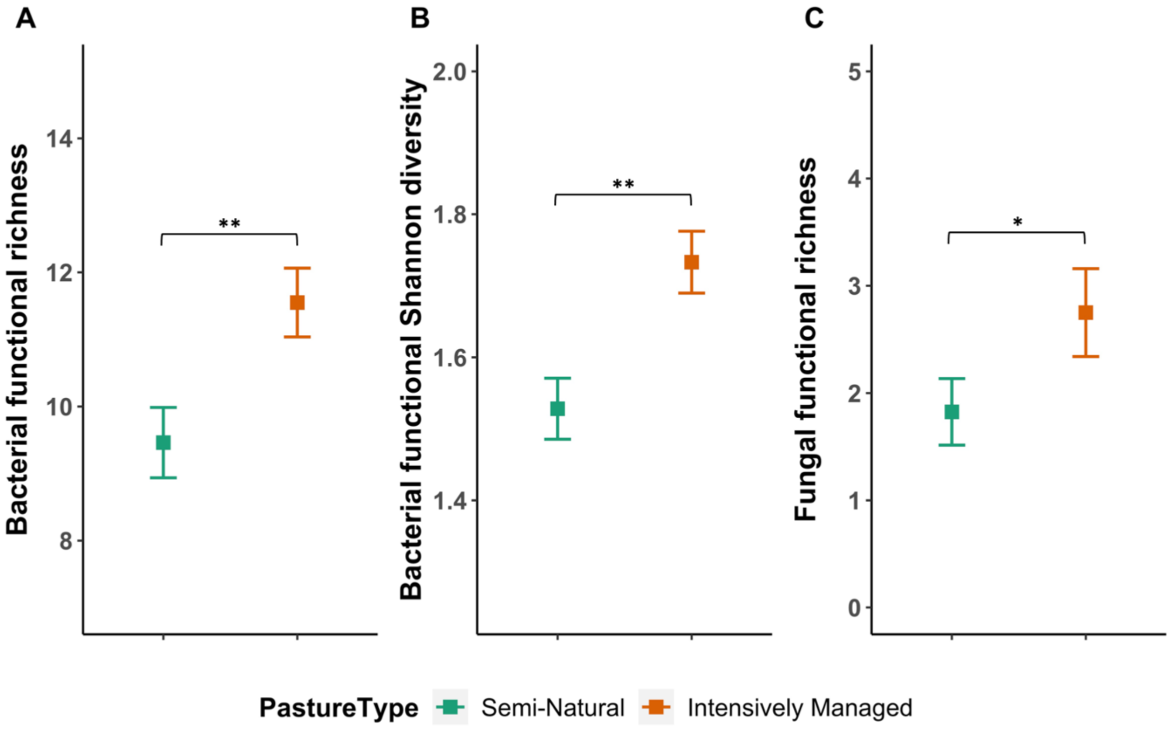

Figure 4-4. Effects of land-use intensity on bacterial functional richness (A), bacterial functional Shannon diversity (B), and fungal functional richness (C) (mean ± SE; N = 40). Pairwise significant differences were determined by the Tukey post hoc test. * indicates $p \leq 0.05$, ** indicates $p \leq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

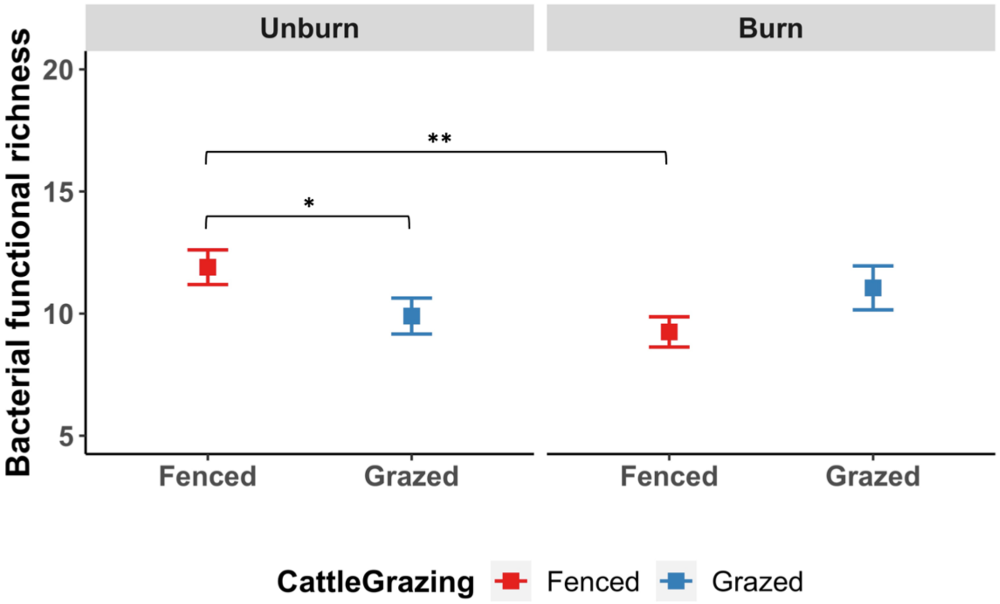

Figure 4-5. Effects of cattle grazing and prescribed fire on bacterial functional richness (mean ± SE; N = 20). Pairwise significant differences were determined by the Tukey post hoc test. * indicates $p \leq 0.05$, ** indicates $p \leq 0.01$

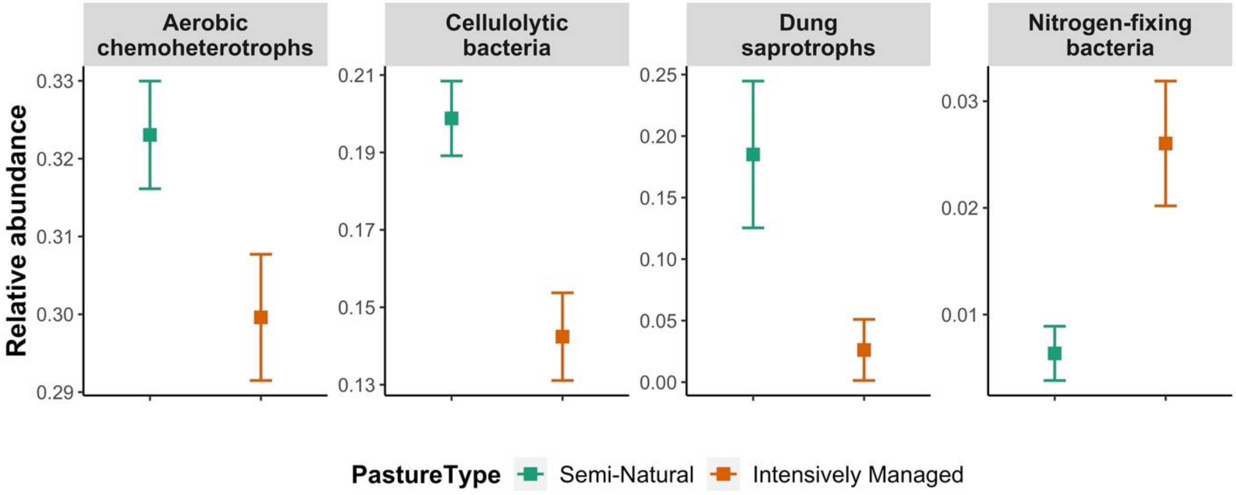

Figure 4-6. Relative abundance of bacterial and fungal functional groups that showed a significant response (as resulted from ANCOM test with W-values higher than 0.7) to the land-use intensity treatment (mean ± SE; N=40).

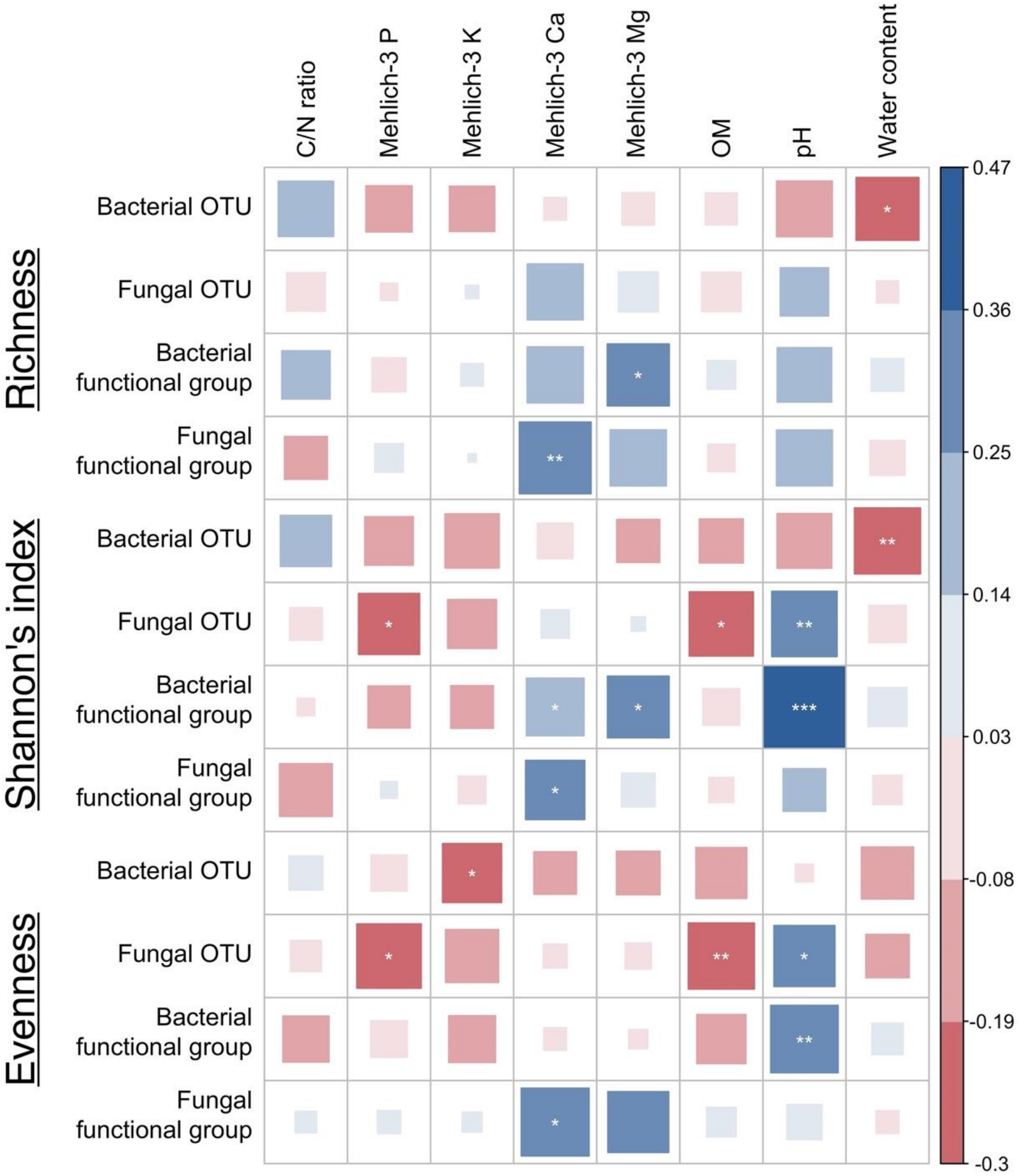

Figure 4-7. Correlation coefficients (spearman's rank) of alpha-diversity metrics of bacterial and fungal OTU and functional groups (richness, Shannon's index, evenness) with soil physipchemical properties across all wetland soil samples. Blue color indicates positive correlations and red color indicates negative correlations. * indicates $p \leq 0.05$, ** indicates $p \leq 0.01$, *** indicates $p \leq 0.001$.

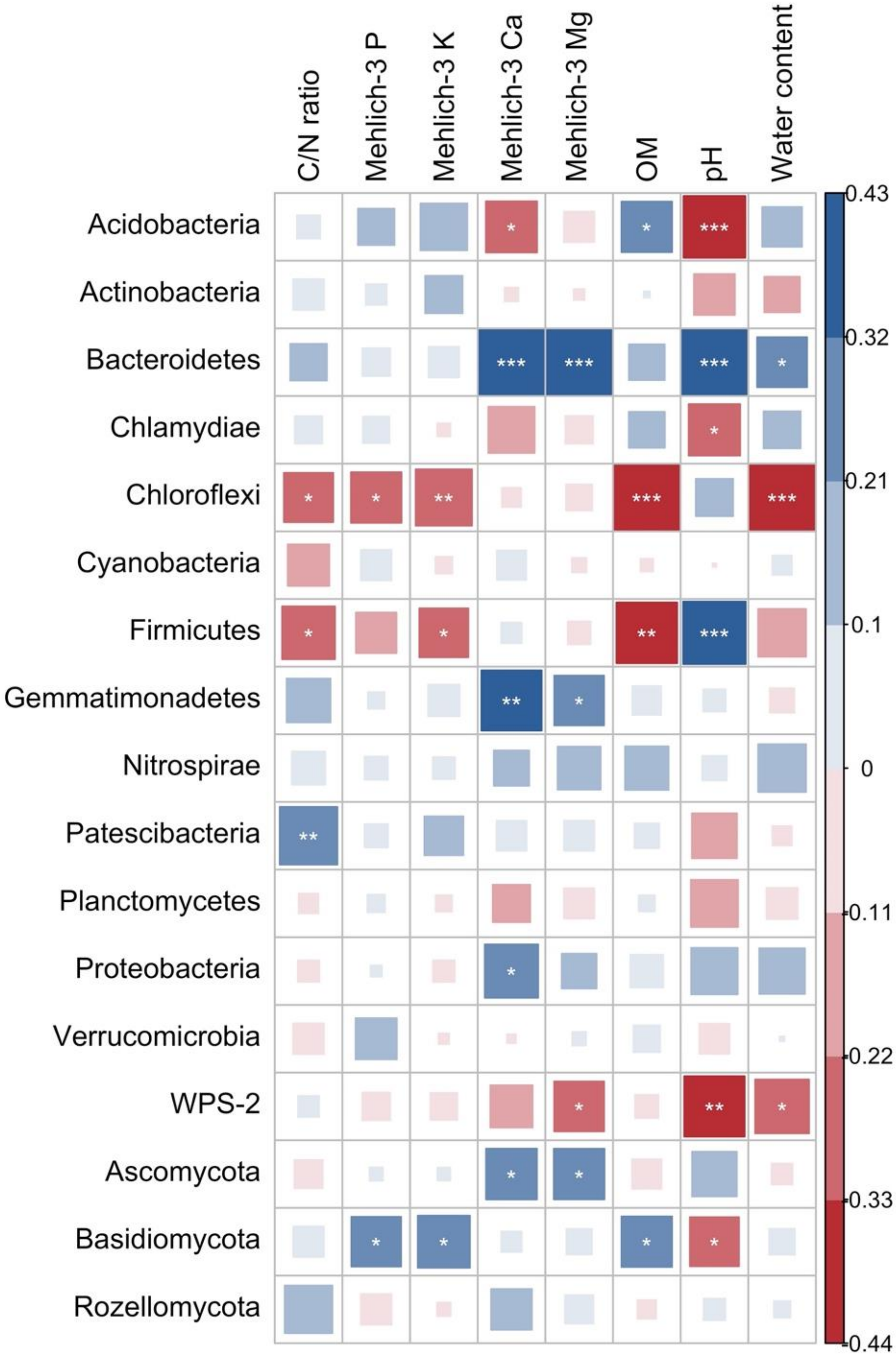

Figure 4-8. Correlation coefficients (spearman's rank) of relative abundance of dominant bacterial and fungal phyla (relative abundance >1%) with soil physipchemical properties across all wetland soil samples. Blue color indicates positive correlations and red color indicates negative correlations. * indicates $p \leq 0.05$, ** indicates $p \leq 0.01$, *** indicates $p \leq 0.001$.

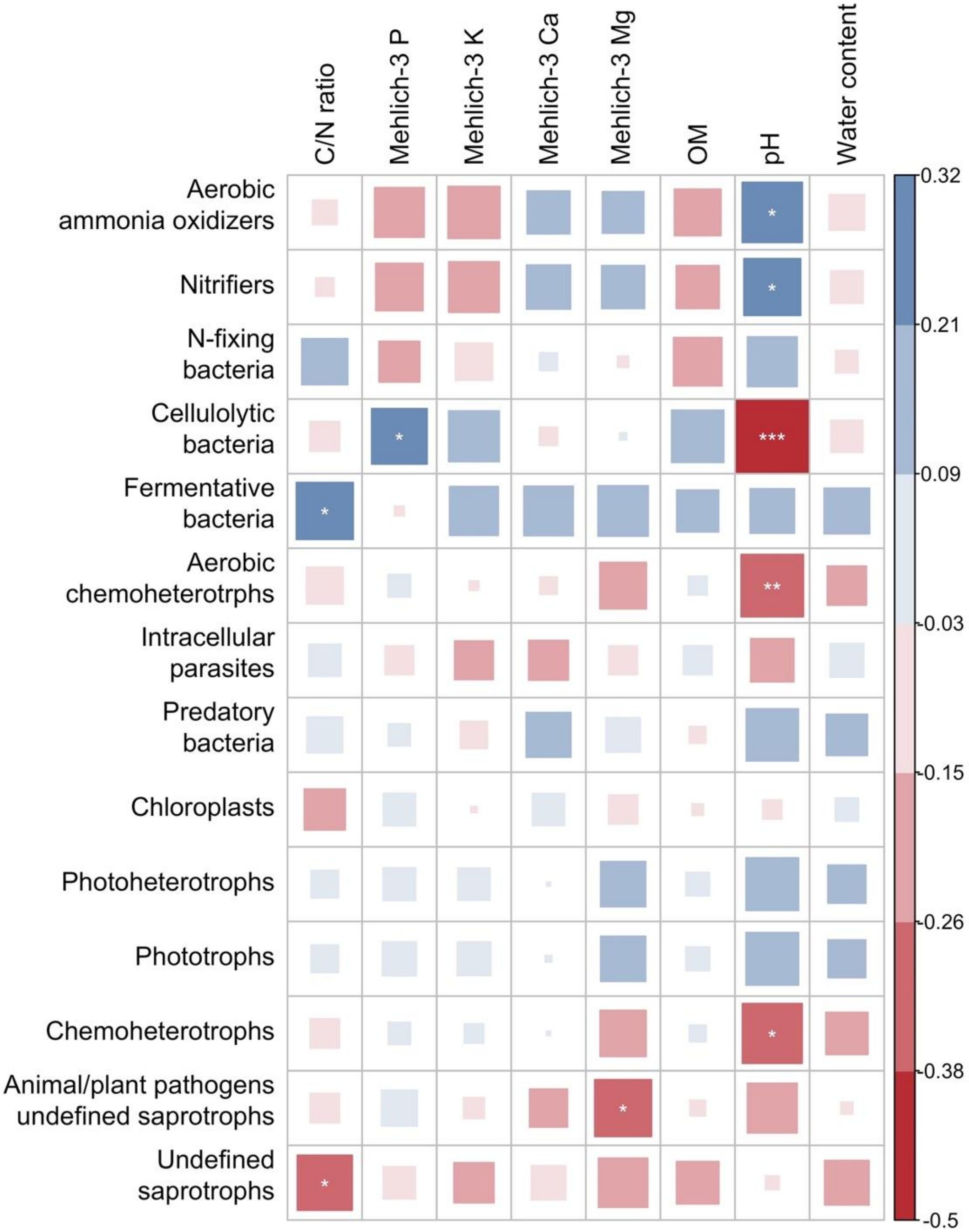

Figure 4-9. Correlation coefficients (spearman's rank) of relative abundance of pervasive bacterial and fungal functional groups (present in at least 50% of samples) with soil physipchemical properties across all wetland soil samples. Blue color indicates positive correlations and red color indicates negative correlations. * indicates $p \leq 0.05$, ** indicates $p \leq 0.01$, *** indicates $p \leq 0.001$.

CHAPTER 5
DIRECT AND INDIRECT PATHWAYS OF LAND MANAGEMENT EFFECTS ON WETLAND PLANT LITTER DECOMPOSITION

Introduction

As human population grows and demand for food and agricultural products increases, intensified agricultural land uses, expansion of livestock grazing, and associated agroecosystem management practices are occurring globally (Alexandratos and Bruinsma, 2012; Asner et al., 2004). These agricultural and land management activities, while fulfilling basic food demands, have inevitably transformed terrestrial landscapes in multiple ways, such as increased nutrient loading and soil disturbances, altered hydrologic regimes, species shifts in biological communities, and reduced habitat heterogeneity and biodiversity (Reid et al., 2005; Tilman, 1999). All these biotic and abiotic changes induced by land management are likely to affect fundamental ecological processes, such as organic matter decomposition, and consequently biogeochemical cycling and energy flows.

The processes of organic matter decomposition determine various essential ecosystem functions and services, including carbon sequestration, nutrients retention, greenhouse gas mitigation, soil formation, and soil health maintenance (Richardson and Hanna, 2021). In terrestrial ecosystems, more than 50% of net primary production is returned to soil through the decomposition of plant litter (Wardle et al., 2004). Therefore, understanding how anthropogenic activities alter litter decomposition has major implications for predicting carbon budgets, and achieving sustainable land management and multifunctional agriculture (Zuazo et al., 2011). While there has been a long history of research on litter decomposition, effects of agricultural land management on decomposition have been shown inconsistent with complex responses to different and sometimes interacting management practices. For example, nitrogen (N) and phosphorous (P) additions, as a common agricultural intensification practice, were reported to stimulate leaf decomposition of trees and shrubs in a Brazilian savanna (Jacobson et al., 2011) and subtropical/tropical forests (Hobbie, 2000; C. Wang et al., 2011), inhibit litter decomposition in forests under high levels of N deposition (Tu et al., 2014; Zheng et al., 2017), and show no effect on litter decomposition in corn production systems (Grandy et al., 2013). Similarly, livestock grazing exhibited varied effects on litter decomposition in different systems. For instance, cattle and sheep grazing reduced decomposition of tree leaf litter in an Australian temperate woodland (Lindsay and Cunningham, 2009), but enhanced grasses and forbs litter decomposition in a semi-arid grassland (Naeem et al., 2021). Thus, it is critical to investigate how and pathways in which multiple agriculture land use and management practices affect litter decomposition.

Prior research has well demonstrated that litter decomposition is primarily controlled by litter quality, environmental condition, and decomposing organisms (Chapin et al., 2002; Swift et al., 1979). Litter quality is commonly indicated by concentrations of N, P, and lignin, and ratios of C:N and N:lignin (Wardle et al., 2002). In addition, other plant functional traits, such as concentrations of essential macronutrients (e.g., potassium (K), calcium (Ca), magnesium (Mg)), have been increasingly recognized as important for predicting decomposition processes (Makkonen et al., 2012; Yue et al., 2021). However, the direction and magnitude of these litter traits effects on decomposition processes vary across study systems (Krishna and Mohan, 2017; Wang et al., 2013; Wardle et al., 2002), probably due to complex interactions with decomposers and soil properties that are responsive to management practices (García-Palacios et al., 2013; Ge et al., 2013). For example, litter with high quality (e.g., high N but low lignin content) usually decomposes faster or more completely in a certain amount of time than those with low quality

(Chapin et al., 2002; Swift et al., 1979), because microbial mining on complex polymer (i.e., lignin) requires synthesis of multiple extracellular enzymes that is metabolically costly (Ferreira et al., 2015). However, increasing availability of dissolved inorganic nutrients (e.g., fertilization) might stimulate activity of microbial decomposers, such as promoting fungal biomass accrual and sporulation (Gulis et al., 2006) and thus offsetting negative effects of high lignin content on litter decomposition. Moreover, litter quality could affect the composition of *in situ* soil microbial communities, which in turn drives its decomposition processes (Yan et al., 2018). Nevertheless, the specific role of soil microbial composition and structure in response to land management and consequences for litter decomposition have not been thoroughly investigated (Ge et al., 2013; Matulich and Martiny, 2015). In particular, compared to previous studies that focused on effects of individual factors (Ferreira et al., 2015; Krishna and Mohan, 2017; Yarwood, 2018), effects of multiple land management practices (which are interactive and often operating in concert) on litter decomposition through interactions among litter traits, soil properties, and soil microbial communities yet remain less well understood (Krishna and Mohan, 2017; Yarwood, 2018).

In tropical and subtropical regions, due to climate and hydrologic conditions, there are numerous isolated seasonal wetlands embedded across agricultural landscapes (Mitsch et al., 2010). These wetlands are an integral component of the local agriculture, deemed as hotspots of primary productivity and biogeochemical cycling (McClain et al., 2003), and are particularly important for regulating regional hydrology and supporting biodiversity (Whigham, 1999). Although these isolated seasonal wetlands do not occupy a large proportion of the land surface, they appear in a large number of quantity, are widely distributed across the landscapes, and function as indispensable environmental buffers between agricultural lands and residential or other natural areas by providing flood protection, nutrient retention, organic material accumulation, and water purification services (Janse et al., 2019; Whigham, 1999). These wetlands are also experiencing agricultural management practices (e.g., land-use intensification and livestock grazing) that are typically less common compared to natural or less human-disturbed wetlands. Therefore, we need a comprehensive understanding of how fundamental ecological processes (e.g., litter decomposition) in these wetlands respond to individual and interactive agricultural management practices, so that to inform effective and sustainable management for agroecosystems and ensure the provision of vital regulating and supporting ecosystem services.

To address these knowledge gaps, we used a field experiment to investigate plant litter decomposition in isolated seasonal wetlands embedded in managed grasslands in central-south Florida, USA (Figure 5-1). Our research is built upon a long-term whole-ecosystem and factorial wetland experiment (Boughton et al., 2016) that systematically manipulate effects of land-use intensification, livestock grazing, and prescribed fire, all of which are widely adopted agricultural management and important global change drivers for agroecosystems (Steffen et al., 2006). Specifically, we ask: (1) How do land-use intensification, cattle grazing, and prescribed fire interact to affect *in situ* plant litter decomposition processes in seasonal wetlands? (2) Among litter, soil, and microbial attributes, what are the most important predictors of wetland plant litter decomposition? (3) What are direct and indirect pathways through which land-use intensification and management practices affect wetland decomposition? To answer these questions, we used litterbag technique (Hackney and De La Cruz, 1980) with in-situ dominant plant species to measure decomposition processes that include quantification of decomposition rate k and recalcitrant fraction A. We also measured litter traits of dominant plant species, wetland soil properties, and soil bacterial and fungal communities to elucidate the mechanisms of litter decomposition processes in response to agricultural land-use intensification and management practices. Overall, we hypothesize that agricultural land management would alter wetland decomposition via modifying plant litter traits, soil properties, and composition and structure of soil microbial communities. We also expect that soil microbial attributes would exert interactive effects with litter or soil properties to affect wetland decomposition.

Materials and Methods

Study Area

Our research was conducted at the Archbold Biological Station's Buck Island Ranch (BIR) in Lake Placid, Florida, USA (27º09´ N, 81º11´ W). BIR is a 4,336-ha commercial cow-calf ranch and meanwhile serves as working grasslands as part of the Archbold-University of Florida Long-Term Agroecosystem Research (LTAR) site (Figure 5-1). The region has a humid subtropical climate consisting of a warm dry season (November-May) and a hot wet season (June-October), with annual average rainfall of 136-cm, 75% of which typically falls in the wet season. Average minimum and maximum daily temperatures are 15.9ºC and 29.0ºC, respectively. BIR has been managed under two land-use intensities that are exemplary of local ranching systems and the large subtropical South America regions (Swain et al., 2007): intensively managed (IM), and semi-natural (SN) pastures, along with seasonal wetlands embedded in these pastures (Figure 5-1). IM pastures have been heavily drained through constructed ditches, abundantly planted with forage grasses (mainly *Paspalum notatum*), and intensively cattle grazed during wet seasons, with an average animal use days (AUD) of 391 per hectare. Moreover, IM pastures are regularly limed, N-fertilized every 1-2 years, and until 1987 fertilized with P and K. In contrast, SN pastures are lightly drained, sparsely planted with forage grasses, moderately cattle grazed during dry seasons (with an average AUD of 178 per hectare), and never limed or fertilized.

In BIR, there are more than 600 isolated seasonal wetlands, comprising of 12% of the total area (Swain et al., 2013), which are important landscape elements and influenced by upland agricultural land management. These wetlands are sized between 0.007-41.9 ha with hydroperiods ranging from 2-10 months. Wetlands embedded in IM pastures had higher soil nutrients (Ho et al., 2018) but lower diversity in plant, macroinvertebrate, and ectothermic vertebrates communities (Boughton et al., 2016; Medley et al., 2015) than their counterparts in SN pastures. For convenience, in subsequent sections, we will term wetlands embedded in IM or SN pastures as IM or SN wetlands. In addition to upland intensification, grazing and prescribed fire are another two most common management and disturbances in these tropical/subtropical agroecosystems, which also have shown significant effects on wetland soil nutrients, water quality, and biodiversity from past studies (Boughton et al., 2016; Ho et al., 2018; Jansen et al., 2019). For example, research from the same study region found that cattle grazing and prescribed fire tended to promote P sequestration (Ho et al., 2018). However, for plant communities, grazing has been found to increase the abundance of short graminoids and forbs, such as *Bacopa caroliniana*, whereas fire reduced the abundance of graminoids (Boughton et al., 2016).

Experimental Design

To investigate effects of land management on organic matter decomposition, we conducted a litterbag decomposition experiment within an existing whole-ecosystem, long-term wetland experiment that was initialized in 2008. Details in the experimental design can be found in Boughton et al., (2016) and Guo et al., (2021). In brief, a 2 × 2 × 2 complete factorial design with a randomized complete block was setup using forty similar-size wetlands distributed across the BIR (0.5-1.5 ha) (Figure 5-1). The eight treatment combinations are the crossed presence/absence of cattle grazing and prescribed fire in wetlands embedded in IM and SN pastures, respectively, resulting in five replicates for each unique treatment combination. For grazed treatment, cattle activities were tracked in wetlands via GPS collars (Pandey et al., 2009); while for ungrazed (fenced) treatment, cattle exclosures were installed in 2007. For burned treatment, prescribed fire was implemented during dry seasons in 2008, 2011, 2013-2014, and 2017-2018 with return intervals mimicking naturel fire regimes. For each wetland, we selected three sites (i.e., center, middle, and edge) along the hydrological gradient to capture the variation in decomposition within a wetland (Figure 5-1). The center site was the lowest elevation point of the wetland; the edge site was estimated as one of the highest and driest point of the wetland and determined by the longest distance between center site and wetland margin; and the middle site was the midpoint with equal distances to the center and edge sites. The line formed by these three sites shows clear transitions in vegetation well aligning with the hydrological gradient (Boughton et al., 2010).

Litterbag Technique to Quantify Decomposition

We employed the litterbag technique (Hackney and De La Cruz, 1980) to measure decomposition processes of aboveground *in situ* plant litter in wetlands. Specifically, large 30 × 20 cm nylon bags with 2-mm mesh size were used as litterbags because we wanted to avoid manual fragmentation of plant litter and allow access of macroinvertebrate decomposers. Each litterbag was filled with 10-g dried plant materials representative of *in situ* litter. Composition of plant materials used in litterbag for each wetland type is shown in Table 5-1, as determined based on most recent plant surveys in these forty wetlands in 2018 (unpublished data from E.H. Boughton). Plant species with >10% frequency were selected, and their relative abundance in litterbags matched with those in the field (Table 5-1). Plant materials were collected in each type of wetlands at the end of the growing season in October 2018. Collected plants were carefully rinsed to remove sediment then oven-dried at 40°C for 72 hours.

Four litterbags were placed for each sampling site on the sediment surface, and secured using landscape pins (Figure 5-1) in December 2018. Hence, a total of 480 litterbags (40 wetlands × 3 sites × 4 litterbags per site) were deployed. In grazed wetlands, we constructed a 1-m diameter circular fence with chicken wire and T-posts surrounding deployed litterbags per sampling site to exclude direct cattle trampling. We then retrieved one random litterbag from each site at 4 intervals in January, March, June, and December 2019 (i.e., 1, 3, 6, and 12 months after deployment). Retrieved litterbags were sealed in plastic ziplock bags while transportation, then stored at 4°C in the laboratory until litter were gently rinsed to remove adhering sediments and oven-dried at 60°C for 72 hours. Remained litter was weighed, and the proportion of mass remaining (MR) was calculated by dividing the initial weight. The decomposition rate *k* was estimated using the remaining litter mass proportion over four times and the single negative exponential regression (Equation 5-1):

$$\mathrm{MR(t)} = e^{-kt} \tag{5-1}$$

where MR(t) is proportion of mass remaining after t days, and *k* is the decomposition constant. In addition, we estimated recalcitrant fraction *A* using MR and negative asymptotic regressions (Equation 5-2):

$$\mathrm{MR(t)} = A + (1 - A)e^{-k_a t} \tag{5-2}$$

where *A* is the asymptote, or the fraction of recalcitrant plant litter. Two different regressions were used for estimating *k* and *A*, respectively, because studies have shown that the single negative exponential regression provided a more accurate estimation of actual decomposition rate (Stagg et al., 2018).

Predictive Variables Collection

A subsample of initial dried plant litter was ground and oven-dried at 85°C for measuring initial litter traits, including concentrations of carbon (C), N, P, K, Ca, Mg, and lignin, as well as the ratio of C to N (C/N), C to P (C/P), and N to lignin (N/lignin). The litter C and N concentrations were determined by dry combustion (Bremner, 2018) using a LECO CN-628 analyzer (LECO Inc., St. Joseph, MI, USA). Separate litter subsamples were digested using 1 mol/L hydrochloric acid to measure P, K, Ca, and Mg concentrations by the Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES, Soltanpour et al., 2018) technique using a Perkin Elmer Avio 200 instrument (PerkinElmer, Inc., MA, USA). Litter lignin concentration was measured using AOAC Official Method 973.18-Fiber acid detergent and lignin in animal feed (AOAC, 1996) by the University of Florida Forage Evaluation Support Laboratory. Based on the measurement of litter traits of each plant species (Table D-1), the overall litter traits for the litterbags deployed to each corresponding wetland type were calculated using abundance-weighted averages (Equation 5-3), which are shown in Table D-2.

$$\mathrm{W} = \sum_{i=1}^{n} w_i T_i \tag{5-1}$$

where W is the weighted average for a given trait, n is the total number of dominant plant species in litterbags for each type of wetland, w_i is the relative abundance of *i* species in the litterbag (Table 5-1), and T_i refers to the average measured trait value of *i* species.

Soil subsamples for analyses of physiochemical properties were collected during the litterbag field incubation in March and June 2019 at each sampling site that capture distinctive seasonal variations. Physiochemical analyses include measuring water content, pH, organic matter content, total C and N contents, and plant-available (i.e., Mehlich-3) P, K, Ca, Mg contents. Detailed sampling and measurement protocols can be found in Guo et al., (2021). Briefly, three top 5-cm soil cores were collected and mixed at each site, then sieved through 2-mm mesh and stored within sealed plastic bags in 4 °C until further analyses. Gravimetric water content was measured using weight loss after oven drying at 105°C for 72 hours. Soil pH was measured in a 1:1 soil/distilled water solution using a HANNA pH meter (Hanna Instruments, CA, USA) (Thomas, 1996). Organic matter content was measured by weight loss-on-ignition method (Schulte and Hopkins, 2015) that placed in 450°C muffle furnace for 4 hours. Soil total C and N contents were measured using a LECO CN-628 analyzer (LECO Inc., St. Joseph, MI, USA). Plant-available P, K, Ca and Mg were extracted using Mehlich-3 solution (Mehlich, 1984), then determined by the ICP-OES technique (Soltanpour et al., 2018) using a Perkin Elmer Avio 200 instrument (PerkinElmer, Inc., MA, USA). The soil properties across wetland treatments can be checked in Figure D-1.

Soil subsamples for identifying of bacterial and fungal communities were also collected in March and June 2019 at each sampling site. Microbial samples across different sites from the same wetland were mixed and homogenized thoroughly to represent the community condition of the entire wetland. Collected samples for microbial analyses were immediately placed on dry ice for transportation, then stored at – 80 °C until DNA extraction. We assessed the composition of bacterial and fungal communities using high-throughput sequencing of bacterial 16S ribosomal RNA (16S rRNA) and fungal ribosomal internal transcribed spacer (ITS) genes (Edgar, 2018). A detailed processing protocol can be found in (Y. Guo et al., 2022). Briefly, genomic DNA of soil microbes was extracted using the DNeasy PowerSoil extraction kit (Qiagen, Norway). The universal primer sets of 341F (5´-CCTACGGGNGGCWGCAG-3´) / 806R (5´-GGACTACHVGGGTWTCTAAT-3´) and ITS1F (5´-CTTGGTCATTTAGAGGAAGTAA-3´) / ITS4 (5´-TCCTCCGCTTATTGATATGC-3´) were used to target the V3-V4 region of bacterial 16S rRNA and fungal ITS4 genes. A two-step polymerase chain reaction (PCR) (K.-H. Chen et al., 2021) was conducted to amplify target genes. Then the verified PCR products were pooled and sequenced at the Duke Center for Genomic and Computational Biology (GCB, Durham, NC, USA) in a single library using Illumina Miseq v3 kits (paired-end 2 × 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) and denoised using DADA2 (Callahan et al., 2016). A total of 1,735,406 quality-filtered 16S rRNA gene and 561,066 ITS gene reads were obtained. The SILVA version 132 (Quast et al., 2013) and UNITE version 8.0 (Kõljalg et al., 2013) databases were used for bacterial and fungal taxonomic identification, respectively. Operational taxonomic units (OTUs) were determined at the 99% similarity level of the nucleotide sequences. Bacterial functional annotations were assigned according to FAPROTAX (Louca et al., 2016) databases. 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. Details on the microbial traits across wetland treatments are shown in Figures. D-2 and D-3.

Statistical Analyses

All statistical analyses were performed in R version 4.1.2 (R Core Team, 2021) using RStudio platform. We used linear mixed-effects models to determine individual and interactive effects of wetland management on decomposition rate k and recalcitrant fraction A, respectively (i.e., *Question 1*). Wetland treatments (i.e., upland land-use intensification × grazing × fire) were treated as fixed factors, while sampling site and block were treated as random factors. Assumptions of all specified model were checked with visually inspection of residual plots, and no violations were detected. We estimated *P*-values using Type III Sums of Squares, because our variables were non-sequential and the interactions among variables were considered. We used pairwise contrasts for post-hoc tests on any significant interactions. Linear mixed-effects models and pairwise comparisons were performed using “lmer” and “contrast” functions from *lme4* (Bates et al., 2014) and *lsmeans* (Lenth, 2016) packages, respectively.

Multiple regression and model selection were performed to rank variables related to litter traits, soil properties, and soil microbial attributes, and identify the most important factors in driving *in situ* plant litter decomposition (i.e., *Question 2*). According to (Carrillo et al., 2017), our analysis included two rounds of model selection: the first round model selection aimed to select best significant variables within each predictor category (i.e., litter traits vs. soil properties vs. microbial attributes vs. litter-microbe interactions vs. soil-microbe interactions); and the second round model selection aimed to select most important variables across all categories of significant predictors. All possible multiple regression models of *k* and *A* were compared based on Akaike Information Criterion (AICs), then four best models of each type of variables were presented in Table D-3. Model selection and ranking was performed using “dredge” function from *MuMln* package (Barton and Barton, 2015).

Based on the results from above multiple regressions and model selection, we constructed structural equation modeling (SEM) to identify direct and indirect pathways through which land management affect decomposition (i.e., *Question 3*). SEM were constructed separately for *k* and *A*. Prior to SEM, hypothetical path diagrams were conceptualized (Figure D-4), and all included predictive variables were standardized to 0-1 scale for comparing magnitude of effects across predictors. Non-significant terms were removed iteratively during model fitting until the fitness of SEM model was adequate with low Fisher's C value and non-significant chi-squared tests. SEM was performed using "psem" function in *piecewiseSEM* package (Lefcheck, 2016).

Results

Overall, our results showed that land management exerted significant individual or interactive effects on *in situ* plant litter decomposition processes. Firstly, upland land-use intensification and cattle grazing interactively affected decomposition rate *k* (Table 5-2). Land-use intensification increased *k* by 31.1% in ungrazed wetlands, but decreased *k* by 13.0% in grazed wetlands (Figure 5-2). In other words, cattle grazing resulted in a 15.1% higher *k* in SN wetlands, but a 23.6% lower *k* in IM wetlands (Figure 5-2). Secondly, prescribed fire exerted individual effects on both *k* and recalcitrant fraction *A* (Table 5-2), in which burned wetlands had a 17.5% lower *k* (Figure 5-2) and a 40.9% higher *A* than unburned wetlands (Figure 5-2). Finally, land-use intensification reduced *A* by 24.3%, regardless of grazing or fire treatments (Table 5-2, Figure 5-2). Additionally, we performed similar and complementary analyses and found that no significant spatial variations in litterbag decomposition within a wetland or across blocks (Figure D-5), suggesting that individual wetland is an appropriate spatial scale for inference.

Among sets of predictive variables from each category, regressions against litter traits produced the models with the highest explanatory powers for both decomposition parameters (*k*: $0.551<R^2<0.561$; *A*: $0.332<R^2<0.424$), followed by regressions against soil properties (*k*: $0.201<R^2<0.404$; *A*: $0.256<R^2<0.335$). Measurements of soil bacterial and fungal communities did not independently generate any significant models for predicting decomposition processes (i.e., *P* value>0.05) (Table 5-3). However, certain interaction terms between microbial and litter or soil variables were significant (Table 5-3).

Selected best predictors from multiple regressions (Table 5-3) varied across categories of litter, soil, and microbial variables for *k* and *A*, indicating that factors controlling decomposition rates and recalcitrant fractions were different (Table 5-3). Comparison of multiple regressions with all significant factors showed that the most significant factors driving *k* were litter P, litter Mg, soil K, and interactions between litter Mg and bacterial species richness, litter Mg and relative abundance of chemoheterotrophs, and soil K and relative abundance of cellulolytic bacteria. In contrast, the most significant factors driving *A* were litter Ca, litter lignin, soil N, soil pH, soil water content, and interactions between soil N and bacterial functional Shannon index, and soil water content and relative abundance of chemoheterotrophs.

Our identified best SEM demonstrated that land management affected decomposition processes via both direct and indirect pathways through altering litter traits, soil properties, and interactions with soil and microbial variables (Figures 5-3, 5-4). Our SEM of *k* (Fisher's C=17.4, p=0.90) explained 83% of the variation in decomposition rates (Figure 5-3). Upland land-use intensification resulted in wetland plant litter containing higher P but lower Mg, both of which were negatively associated with *k* (both $p<0.001$; effect size= –0.0012, –0.0023). Land-use intensification decreased relative abundance of cellulolytic bacteria, which had a positive effect on *k* ($p<0.05$; effect size=0.0006). Cattle grazing resulted in higher soil K, which had a positive effect on relative abundance of cellulolytic bacteria ($p<0.001$; effect size=0.43), and interactively affected *k* with cellulolytic bacteria ($p<0.001$; effect size= –0.0015). In addition to the above indirect effects, cattle grazing also exerted a direct negative effect on *k* ($p<0.001$; effect size= –

0.0002). Prescribed fire effects on decomposition k were mostly through affecting litter P content (Figure 5-3).

Our SEM of A (Fisher's C=18.0, p=0.80) explained 56% of the variation in recalcitrant fractions (Figure 5-4). Land-use intensification and prescribe fire led to a higher plant litter lignin, which was positively associated with A ($p<0.01$; effect size=0.212). Land-use intensification increased soil pH, making the soil pH more neutral, which promoted soil bacterial functional diversity ($p<0.001$; effect size=0.57). Moreover, land-use intensification increased soil water content of seasonal wetlands, which resulted in higher A ($p<0.001$; effect size=0.569) and was positively correlated with soil N ($p<0.001$). Soil N and bacterial functional diversity interactively led to a lower A ($p<0.05$; effect size=–0.376). Furthermore, land-use intensification exerted a direct negative effect on A ($p<0.001$; effect size=–0.134).

Discussions

In this study, we investigated individual and interactive effects of land-use intensification, cattle grazing, and prescribed fire on processes of *in situ* plant litter decomposition in subtropical seasonal wetlands. We also evaluated both direct and indirect pathways of land management effects through changes in wetland biotic and abiotic conditions. Overall, our results showed that wetland litter decomposition rates and recalcitrant fractions were either individually or interactively affected by land management. While certain land management effects were direct, most significant and pronounced effects were manifested indirectly through alterations in litter traits, soil properties, and microbial attributes. Specifically, we found: (1) land-use intensification increased k in ungrazed wetlands and decreased k in grazed wetlands, but consistently reducing A regardless of grazing or fire treatment; (2) prescribed fire individually suppressed litter decomposition by reducing k and increasing A; (3) litter traits were the strongest predictors of *in situ* wetland plant litter decomposition as compared to soil and microbial properties, and the best predictors for *k* and *A* varied; (4) microbial traits only exhibited interactive effects with litter and soil variables to affect wetland litter decomposition.

Land Management Affected Litter Decomposition by Altering Litter Traits and Associated Interactions

Consistent with prior research (Ge et al., 2013; Krishna and Mohan, 2017), not surprisingly, litter traits were the most dominant factor influencing the rates and completeness of decomposition processes in subtropical wetlands, as compared to other biotic and abiotic factors (Table 5-3). It is also the major pathways through which wetland management manifested their effects on litter decomposition (Figures 5-3, 5-4). For example, land-use intensification and cattle grazing interactively affected *k* (Figure 5-2), primarily because they exerted opposite effects on litter Mg concentration, which was one of the strongest predictors of *k*-values (Figure 5-3). Mg is an essential element in plant cells and involved in building conformational stabilization of macromolecules, including cell walls, cell membranes, proteins, and nucleic acids (Guo et al., 2016). Therefore, higher litter Mg concentrations indicate higher capability of stabilizing conformational structures in plant tissues that could later slow down its decomposition, which explains the negative relationship between litter Mg and *k*-values (Table 5-3, Yue et al., (2021)). Negative effects of land-use intensification on litter Mg concentration are likely due to fertilization of K (i.e., as a part of common practice of agricultural intensification), which can cause plant Mg deficiency (Guo et al., 2016), because K ions are antagonists for the absorptions of Mg ions in plants (K. Xie et al., 2021). Such antagonistic effects of K ions on Mg ions uptake of plants also explained why land-use intensification increased contents of both plant-available K and Mg in wetland soils (Table D-4), but only promoted litter K concentration and reversely decreased litter Mg concentration (Table D-3).

Positive effects of cattle grazing on litter Mg concentration is likely driven by grazing-induced shifts in wetland plant community composition. Indeed, Boughton et al., (2016) found that, in the same experimental wetlands, cattle grazing promoted growth of short graminoids and forbs (e.g., *Eleocharis vivipara*, *Luziola fluitans*, *Bacopa caroliniana*, and *Pontederia cordata*), which were shown to have higher content in Mg than other common grasses and shrubs in these wetlands (Table D-1) and also in grasslands of other geographic regions (Reiné et al., 2020).

Prescribed fire exhibited a strong suppression effect of litter decomposition by promoting growth of plants with high concentrations of P and lignin, which was negatively associated with *k*-values (i.e., reduced decomposition rates) and positively associated with *A*-values (i.e., increased fraction of recalcitrant litter and thus decreased completeness of decomposition), respectively (Figures 5-3, 5-4). Prescribed fire has been found to increase plant tissue P concentration and thus alter litter quality in multiple other studies (Butler et al., 2018; Cui et al., 2010; Wang et al., 2014), because burning could increase soil labile P content (as also shown in our data; Table D-4) and thereby facilitating plant P absorption (Butler et al., 2018). Unlike many studies measuring responses of plant nutrient status to fire disturbances, very few studies examined responses of plant lignin biosynthesis to periodically burning (Xie et al., 2018). Our results showed that vegetation in burned wetlands contained more lignin, possibly because lignin in cell wall provides structural support and functions as physical barrier. Thus plants with high level of lignin tend to be favored evolutionarily to build resistance and tolerance of heat stress under repeated burns (Q. Liu et al., 2018). It is as expected that higher litter lignin content led to higher *A*-values, since lignin is a well-known contributor to the recalcitrance of organic matter residues (Chapin et al., 2002; Swift et al., 1979). However, we surprisingly found higher litter P concentration resulted in slower decomposition rates, which is contradictory to many other studies, such as Wardle et al., (2002) and Zheng et al., (2017). Nevertheless, results from Chen et al., (2013) and van Huysen et al., (2016) in naturally nutrient-rich systems were consistent with our observations. We suspect that these ‘seemingly’ divergent results might arise from the complex interaction of litter traits with soil properties or microbial decomposers on litter decomposition (Carrillo et al., 2017; Zhan et al., 2021), as also shown in our results (Table 5-3). Due to the severe P legacy in Florida’s agricultural soils (Sharpley et al., 2013), wetlands embedded in IM pastures were not P-limited systems (Ho et al., 2018). Thus, litter with increased P concentration would not necessarily stimulate decomposers’ activities, and could even indicate that P-enriched soils likely already satisfied the microbial P demands and then inhibit the microbial P mining from fresh litter (H. Chen et al., 2013).

Land Management Affected Litter Decomposition by Altering Soil Properties and Associated Interactions

Soil properties were the second most important predictors in explaining variations of decomposition processes. Specifically, we found that soil plant-available P and K content negatively affected *k*-values, whereas soil TN and pH negatively but water content positively regulated *A*-values (Table 5-3). Our SEM results further showed that certain effects of soil properties were manifested through their interactions with other soil or microbial variables (Figures 5-3, 5-4). In wetlands, upland land-use intensification and wetland on-site disturbances significantly altered soil physiochemical properties, particularly soil plant-available P and K content, pH, and water content (Table D-3). These results suggested that changes in soil properties induced by land management are the additional indirect pathways through which land management affects wetland litter decomposition.

Our results regarding the overall inhibitory effect of nutrient enrichment (due to land-use intensification or grazing) on decomposition rates (Table 5-3; Figure 5-3) are consistent with findings from the teabag experiment in these same wetlands (Y. Guo et al., 2021) and other decomposition studies (H. Chen et al., 2013; Tie et al., 2022; Zheng et al., 2017). Further, our results also aligns with the teabag observations Guo et al., (2021) that higher soil nutrient levels only slowed down decomposition rate (i.e., lower *k*), which represents the initial stage of decomposition process that are mainly controlled by labile organic matter. However, nutrient addition facilitated the decomposition completeness by reducing recalcitrant fractions (i.e., lower *A*) (Table 5-3), which represents the relative long-term decomposition process that are mainly controlled by complex macromolecules (Chapin et al., 2002). Collectively, these results suggest that nutrient enrichment induced by land management could exhibit mixed effects on different stages in decomposition depending upon molecular composition in plant litter.

Moreover, we found less soil water content and more neutral pH environment that were due to upland intensification (Figure 5-4) were conducive to a more complete decomposition of recalcitrant organic compounds in wetlands, which are consistent with many studies (Middleton, 2020; Mueller et al., 2018; Petraglia et al., 2019; Wright et al., 2013). The suppression effect of high soil water content on decomposition completeness in wetlands could be caused either by water's high heat capacity that results in low temperature in hot climates and small temperature variation during the entire incubation (Petraglia et al., 2019) or by longer inundation and subsequently low oxygen availability, thus reducing the activities of aerobic decomposers (Mueller et al., 2018). Promotion effect on decomposition completeness at neutral soil pH was likely because that pH-neutral environment favors the microbial diversity and activities (Yang et al., 2021). Our microbial data collected from these wetlands confirmed this proposed mechanism by showing that more neutral soil pH was significantly correlated with higher bacterial functional diversity (Y. Guo et al., 2022), thus contributing to a more thorough litter decomposition.

In comparison, our results showed that dominant drivers for each decomposition stage (i.e., *k* vs. *A*) were different (Table 5-3), suggesting varying effects of external factors on different litter molecular components. For example, we only observed significant effects of soil water content and pH on *A*-values instead of *k*-values (Table 5-3). It was probably because, compared to labile organic material, the breakdown of aged and recalcitrant organic matter was more dependent on the actions of heterotrophic microorganisms and thus being more sensitive to the absence of oxygen and level of pH (Kristensen et al., 1995; Mueller et al., 2018). Such results allude to the complexity and nuances in the decomposition processes, and highlight the importance of linking the decomposition of specific litter components to their corresponding predictors.

Finally, our results showed relatively weak or no individual effects of community-level microbial attributes on wetland litter decomposition. Rather, their effects manifested through interactions with soil properties (Table 5-3; Figures 5-3, 5-4). Specifically, soil K content and relative abundance of cellulolytic bacteria interacted to negatively affected decomposition rates, while cellulolytic bacteria independently showed a positive effect on *k*-values (Figure 5-3). These results suggest that higher relative abundance of cellulolytic bacteria could indicate a more active cellulose-degrading activities, but only when the level of soil K was low. It is expected that more cellulolytic bacteria could contribute to faster early-stage decomposition rates because cellulose is considered a labile litter component (Stagg et al., 2018). We suspected that soil K enrichment might exhibit similar inhibitory effect as soil N addition on the decomposition of labile organic compounds (Z. Chen et al., 2019). However, whether and how that soil K inhibited the enzyme expressions of cellulolytic bacteria would need further molecular studies to confirm. In addition, soil N and bacterial functional diversity showed a synergic effect on reducing litter recalcitrant fractions (Figure 5-4), suggesting functional traits of soil microbes and nutrient status of soil collectively determined the level of organic matter depletion.

Management Implications

Our research provides scientific bases for the sustainable management of tropical and subtropical wetlands that are experiencing increasing pressures from land management and anthropogenic environmental changes. Our results indicate that human-induced disturbances in wetlands, particularly upland agricultural intensification, could accelerate litter decomposition rates and promote long-term litter decomposition completeness (Figure 5-2), which would consequently go against the formation of stable organic matter and decrease the capacity of seasonal wetlands to retain nutrients and sequester carbon. However, there are nuanced interactive effects from these management practices, in which cattle grazing could help buffer against the stimulating effect of land-use intensification on decomposition rates and thus avoid nutrient release pulses. Further, periodic prescribed fire showed profound inhibitory effects on wetland decomposition, suggesting that fire suppression could potentially further accelerate decomposition. Therefore, to better maintain regulating and supporting services provided by wetlands (e.g., water purification, nutrient retention, and carbon sequestration), it is important to reduce anthropogenic disturbances from agricultural activities to wetland ecosystems (Y. Guo et al., 2021). For instance, we should consider avoiding agricultural intensification in low-lying grasslands where more seasonal wetlands are concentrated, and avoiding fire suppression and adopting prescribed fire practices to wetlands embedded in tropical/subtropical agroecosystems. Moreover, we found that cattle grazing exhibited weaker effects on litter decomposition than upland land-use intensification and prescribed fire (Table 5-2). Therefore, it might not be highly necessary to build livestock exclosures for wetlands in agroecosystems, at least for the purpose of maintaining natural decomposition processes.

Table 5-1. Dominant plant species and their relative abundance in the field and in the litterbags of each wetland type

Wetland type	Plant species	Frequency in fields	Relative abundance in fields	Relative abundance in litterbags
IM × fenced × burned	*Panicum hemitomon*	0.52	0.39	1.00
IM × fenced × unburned	*Panicum hemitomon*	0.61	0.6	0.78
	Hymenachne amplexicaulis	0.17	0.17	0.22
IM × grazed × burned	*Panicum hemitomon*	0.40	0.14	0.42
	Paspalum notatum	0.19	0.11	0.33
	Juncus effusus	0.48	0.08	0.24
IM × grazed × unburned	*Luziola fluitans*	0.60	0.23	0.41
	Juncus effusus	0.53	0.18	0.32
	Panicum hemitomon	0.45	0.15	0.27
SN × fenced × burned	*Panicum hemitomon*	0.67	0.39	0.85
	Rhynchospora inundata	0.32	0.07	0.15
SN × fenced × unburned	*Panicum hemitomon*	0.45	0.4	0.60
	Rhynchospora inundata	0.51	0.21	0.31
	Amphicarpum muehlenbergianum	0.23	0.06	0.09
SN × grazed × burned	*Rhynchospora inundata*	0.48	0.15	0.27
	Panicum hemitomon	0.33	0.14	0.25
	Eleocharis vivipara	0.17	0.1	0.18
	Pontederia cordata	0.21	0.07	0.13
	Bacopa carodiniana	0.28	0.07	0.13
	Luziola fluitans	0.20	0.03	0.05
SN × grazed × unburned	*Panicum hemitomon*	0.50	0.18	0.31
	Pontederia cordata	0.28	0.15	0.26
	Luziola fluitans	0.36	0.14	0.24
	Rhynchospora inundata	0.27	0.11	0.19

Table 5-2. Linear mixed-effects model results on litterbag decomposition in response to management factors

Management treatment	Estimate ± SE	F value	*P* value
		Decomposition rate *k*	
Land-use intensity (IM)	0.0003 ± 0.0002	2.321	0.131
Cattle grazing (Grazed)	0.0001 ± 0.0002	3.245	0.075
Prescribed fire (Burned)	–0.0009 ± 0.0002	20.512	**<0.001*** **
IM × Grazed	–0.0007 ± 0.0003	22.564	**<0.001*** **
IM × Burned	0.0005 ± 0.0003	2.313	0.132
Grazed × Burned	0.0005 ± 0.0003	2.570	0.112
IM × Grazed × Burned	–0.0005 ± 0.0004	1.352	0.248
		Recalcitrant fraction *A*	
Land-use intensity (IM)	0.0060 ± 0.0793	4.264	**0.042***
Cattle grazing (Grazed)	0.0647 ± 0.0848	0.965	0.328
Prescribed fire (Burned)	0.2412 ± 0.0780	6.423	**0.013***
IM × Grazed	–0.0212 ± 0.1142	1.018	0.315
IM × Burned	–0.2526 ± 0.1092	3.794	0.054
Grazed × Burned	–0.1314 ± 0.1143	0.159	0.691
IM × Grazed × Burned	0.2003 ± 0.1567	1.634	0.204

Notes. Estimates represent differences relative to wetlands embedded in semi-natural pastures, and/or that are ungrazed and/or unburned. Bold values indicate significant coefficients. * indicates $p \leq 0.05$, ** indicates $p \leq 0.01$, *** indicates $p \leq 0.001$.

Table 5-3. Summary of best four multiple regression models of litterbag decomposition against grouped sets of potential predictors

Model No. / Model term	Decomposition rate *k*				Recalcitrant fraction *A*			
	1	2	3	4	1	2	3	4
	R^2=0.551	R^2=0.561	R^2=0.561	R^2=0.556	R^2=0.408	R^2=0.424	R^2=0.332	R^2=0.410
Litter traits								
C/N ratio								0.042
N			0.0002					
P	–0.0013***	–0.0011**	–0.0011**	–0.0014***				
K				0.0002		–0.076		
Mg	–0.0021***	–0.0018***	–0.0018***	–0.0021***			0.233**	
Ca					0.230**	0.261***		0.214**
Lignin					0.270**	0.252**		0.249**
N/lignin ratio		0.0002						
	R^2=0.404	R^2=0.310	R^2=0.201	R^2=0.351	R^2=0.317	R^2=0.256	R^2=0.335	R^2=0.278
Soil properties								
C/N ratio				–0.0003				
TN					–0.284*		–0.361*	
Mehlich-3 P	–0.0013***	–0.0009**		–0.0011**				
Mehlich-3 K			–0.0007*				0.128	
Mehlich-3 Ca	0.0006.			0.0006.		–0.226*		–0.181
pH					–0.275**		–0.266**	–0.118
Water content					0.562**	0.372**	0.523**	0.343**
	R^2=0.060	R^2=0.040	R^2=0.036	R^2=0.036	R^2=0.133	R^2=0.110	R^2=0.142	R^2=0.089
Microbial diversity	All models below were insignificant				All models below were insignificant			
Fungal richness	0.0005							–0.089
Fungal Shannon		0.0005						
Fungal evenness			0.0004			–0.111		
Bacterial richness				–0.0005				
Bacterial functional Shannon index					–0.169		–0.318	

Table 5-3. Continued

	Decomposition rate *k*				Recalcitrant fraction *A*			
Model No. / Model term	1	2	3	4	1	2	3	4
Bacterial functional richness							0.201	
	R^2=0.051	R^2=0.012	R^2=0.011	R^2=0.011	R^2=0.132	R^2=0.178	R^2=0.110	R^2=0.109
Microbial group abundance	All models below were insignificant				All models below were insignificant			
Cellulolytic bacteria	–0.0004					0.162		
Photoheterotrophs		0.0003					–0.184	
Chemoheterotrophs			–0.0003		0.209			
Phototrophs				0.0003				–0.185
	R^2=0.253	R^2=0.224	R^2=0.208	R^2=0.399	R^2=0.490	R^2=0.464	R^2=0.489	R^2=0.413
Interactions between litter traits and microbial features								
Litter P: bacterial richness				–0.0014**				
Litter Mg: cellulolytic bacteria			–0.0009**					
Litter Mg: chemoheterotrophs	–0.0011**			–0.0019***				
Litter Mg: bacterial richness		–0.0010**						
Litter Ca: bacterial functional richness					0.423***		0.382.	
Litter Ca: photoheterotrophs						0.649**	0.093	
Litter Ca: cellulolytic bacteria								0.224**
Lignin: chemoheterotrophs					0.405***	0.433***	0.415***	0.374***

Table 5-3. Continued

	Decomposition rate *k*				Recalcitrant fraction *A*			
Model No. / Model term	1	2	3	4	1	2	3	4
	R^2=0.157	R^2=0.170	R^2=0.211	R^2=0.228	R^2=0.299	R^2=0.345	R^2=0.350	R^2=0.280
Interactions between soil properties and microbial features								
Soil K: bacterial richness	–0.0011*							
Soil P: bacterial richness		–0.0011*						
Soil K: cellulolytic bacteria			–0.0008*					
Soil P: cellulolytic bacteria				–0.0009*				
Soil N: bacterial functional richness					–0.399*	–0.349*		
Soil N: bacterial Shannon index							–0.351*	–0.346*
Soil water: cellulolytic bacteria					0.779			0.653***
Soil water: chemoheterotrophs						0.916***	0.824***	
Soil pH: chemoheterotrophs						–0.321.	–0.320.	
	R^2=0.720	R^2=0.714	R^2=0.720	R^2=0.726	R^2=0.512	R^2=0.538	R^2=0.504	R^2=0.523
Combined significant variables								
Litter P	–0.0011***	–0.0011***	–0.0011***	–0.0011***				
Litter Mg	–0.0019***	–0.0016***	–0.0019***	–0.0016***				
Litter Ca					0.197**	0.183**	0.203**	0175*
Lignin					0.246**	0.236**	0.249**	0.242**
Litter Mg: bacterial richness		–0.0004						

Table 5-3. Continued

Model term \ Model No.	Decomposition rate *k* 1	2	3	4	Recalcitrant fraction *A* 1	2	3	4
Litter Mg: chemoheterotrophs				–0.0004				
Soil K	–0.0007***	–0.0007***	–0.0005	–0.0007***				
Soil N					–0.234*	–0.178*	–0.012	–0.096
Soil pH					–0.187*	–0.148.		–0.144
Soil water					0.527***		0.524***	0.553***
Soil K: cellulolytic bacteria			–0.0002					
Soil N: bacterial functional Shannon							–0.420.	–0.301
Soil water: chemoheterotrophs						0.804***		

Notes. The selected models were presented in the order of increasing AIC. Direction of effects were indicated with plus or minus symbol (* indicates $p<0.05$; ** indicates $p<0.01$; *** indicates $p<0.001$).

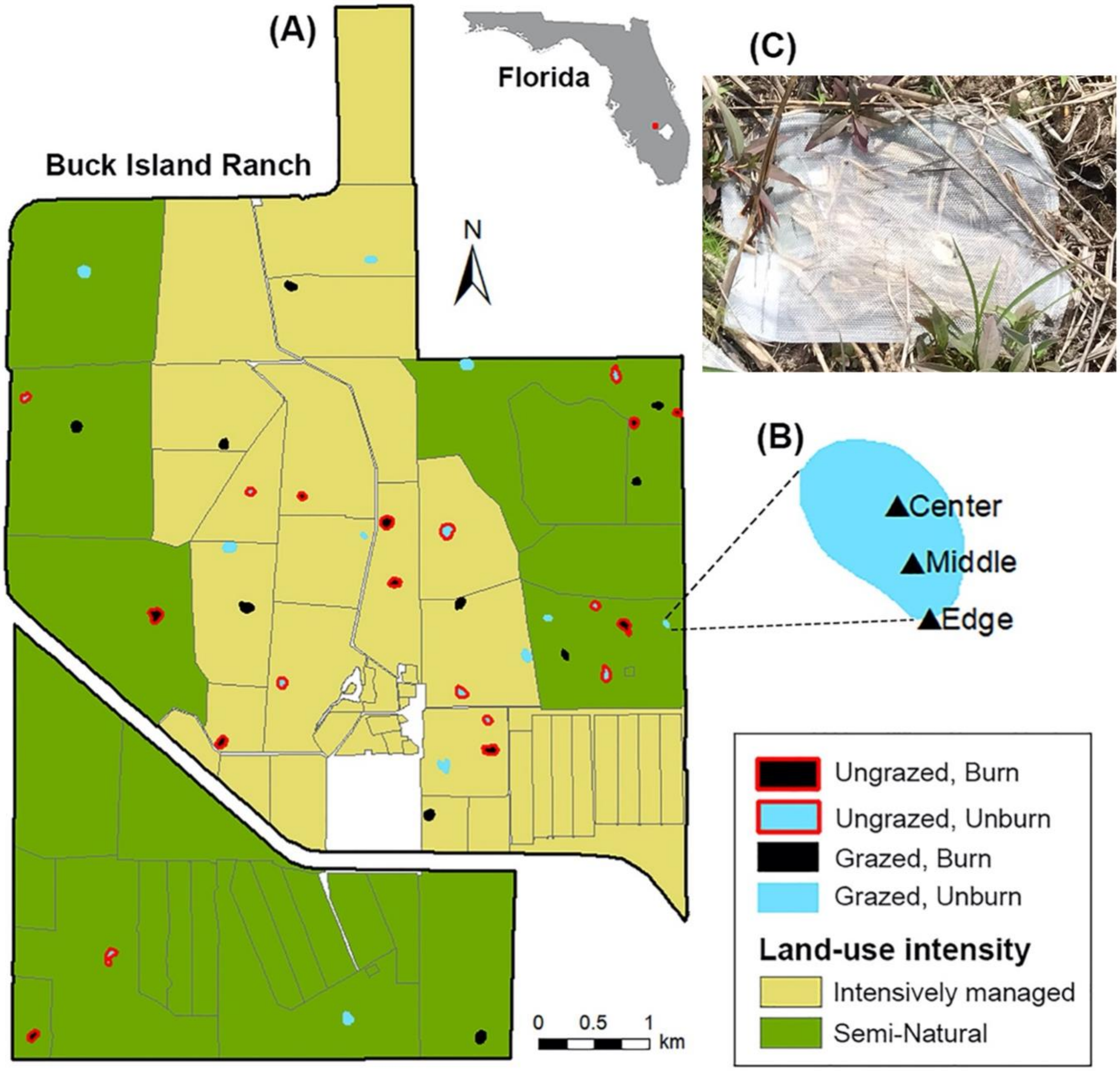

Figure 5-1. (A) Map of the 40 wetlands in a whole-ecosystem experiment at Archbold Biological Station Buck Island Ranch, Lake Placid, Florida, USA. Intensively managed (IM) pastures are shown in yellow, and semi-natural (SN) pastures are shown in green. Fenced (ungrazed) wetlands are illustrated with red outlines, and grazed wetlands with no outlines. Wetlands with prescribed fire treatment are shown in black color, and wetlands with no fire treatment are filled with light blue color. (B) A set four identical litterbags were deployed at sampling sites – center, middle, and edge – for each wetland to capture the spatial variation along the hydrological gradient. (C) Photo is shown as an example of litterbag deployed in the field.

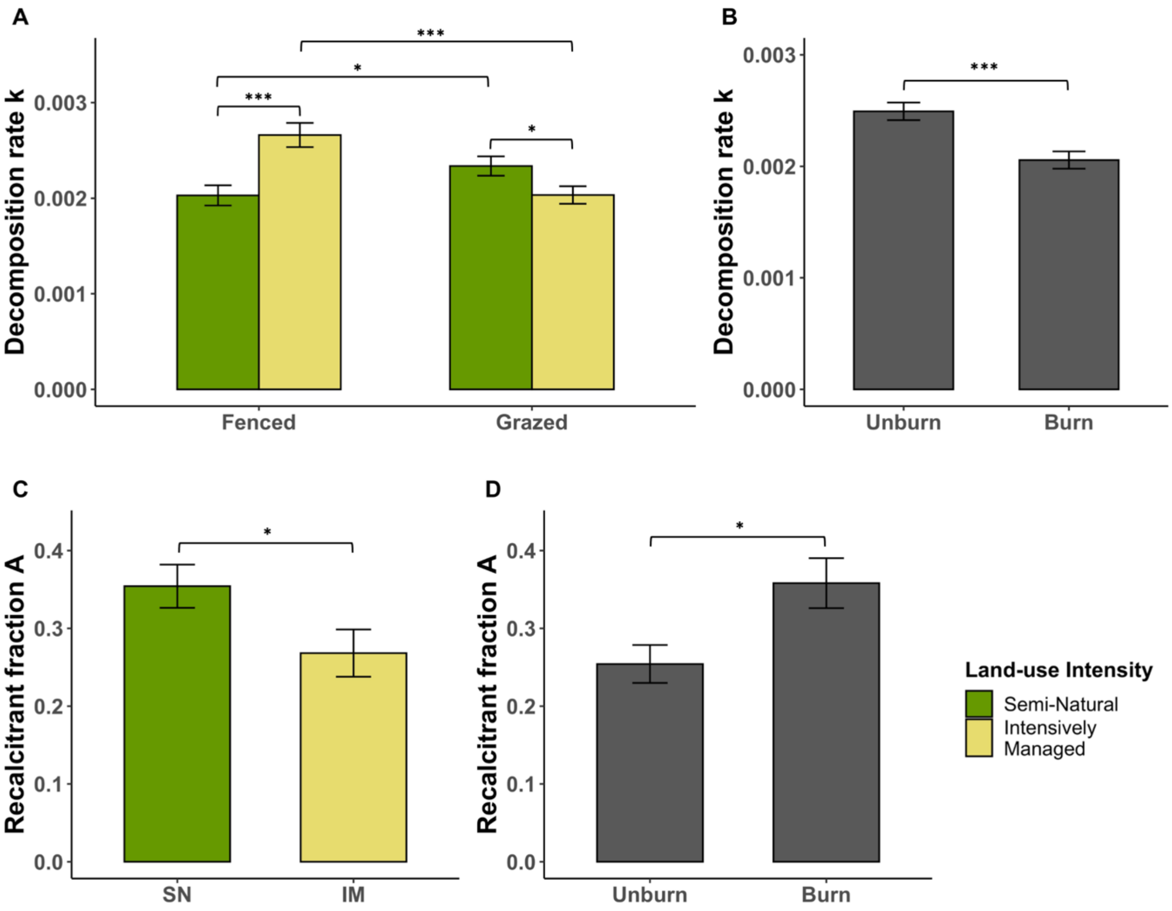

Figure 5-2. Significant differences in litterbag decomposition rate *k* (A, B) and recalcitrant fraction *Asym* (C, D) across wetland treatments (from embedded in intensively managed (IM) and semi-natural (SN) pastures, grazed and fenced (ungrazed) wetlands, burned and unburned wetlands). Visualizations of bar plots were simplified to illustrate differences among treatment with significant individual or interactive effects. Brackets indicate the pairwise comparison (* indicates $p<0.05$; ** indicates $p<0.01$; *** indicates $p<0.001$). Error bars represent SEs, and brackets across bars indicate significant differences based on pairwise post-hoc tests. Full results of model fitting are shown in Table 1.

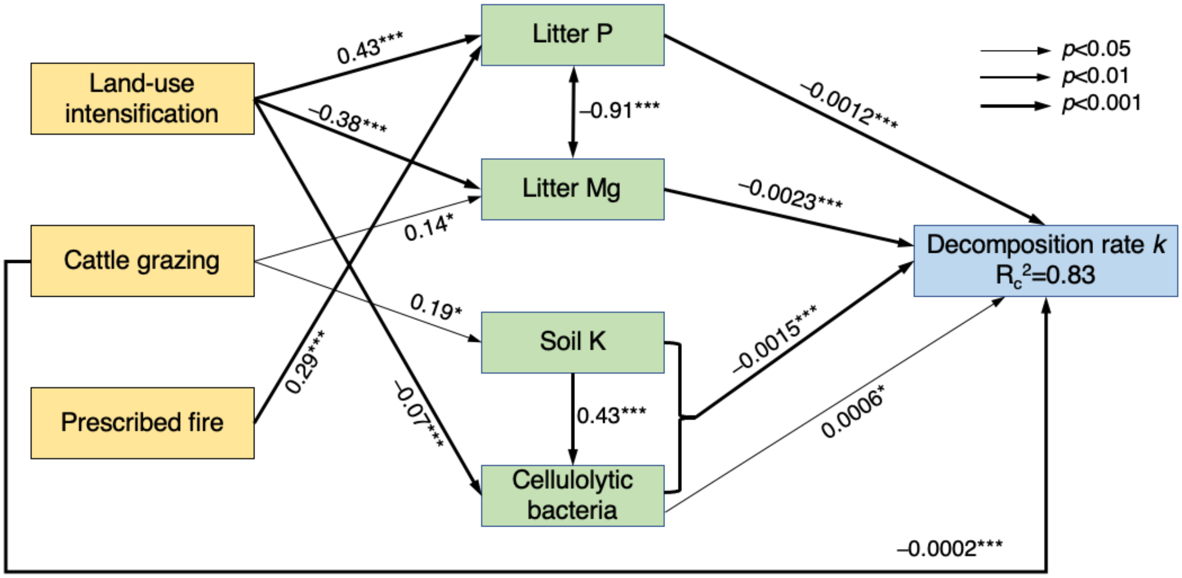

Figure 5-3. Structural equation modeling results to illustrate direct and indirect pathways of land management effects on decomposition rate *k*. Black arrows represented each significant path (* indicates *p*<0.05; ** indicates *p*<0.01; *** indicates *p*<0.001), with brackets indicating interaction effects. Nonsignificant treatment effects or relationships (*p*>0.05) are omitted in the figure for clarity. The thickness of the arrow is proportional to the magnitude of effect size.

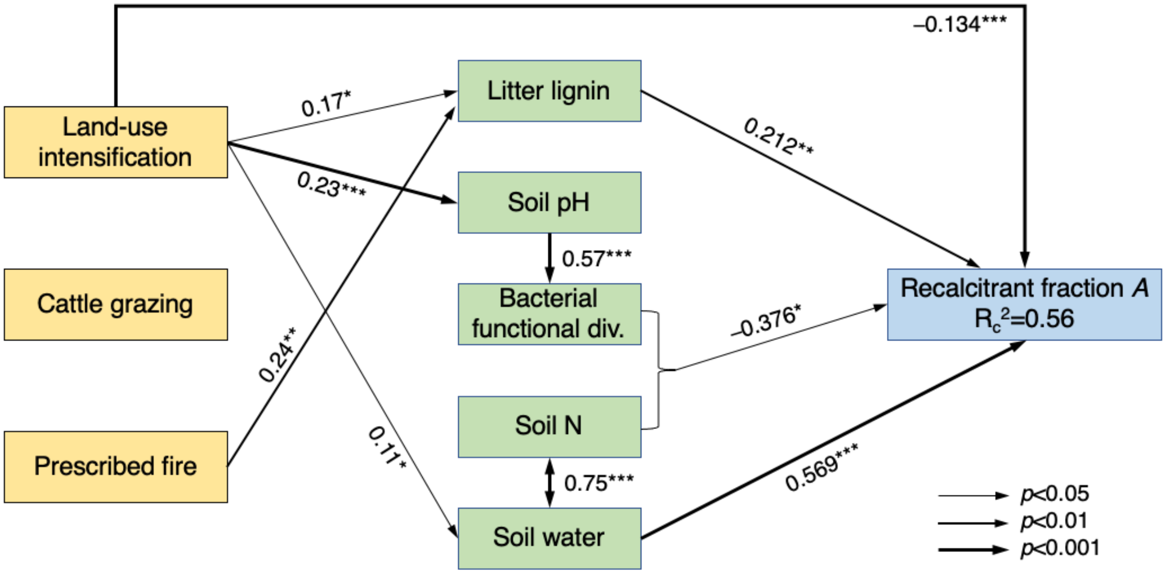

Figure 5-4. Structural equation modeling results to illustrate direct and indirect pathways of land management effects on recalcitrant fraction *A*. Black arrows represented each significant path (* indicates $p<0.05$; ** indicates $p<0.01$; *** indicates $p<0.001$), with brackets indicating interaction effects. Nonsignificant relationships ($p>0.05$) have been omitted for clarity. The thickness of the arrow is proportional to the magnitude of effect size.

CHAPTER 6
CONCLUSIONS

This dissertation addressed several issues related to global change drivers (i.e., land-use intensification, livestock grazing, and prescribed fire) and their consequences for biological communities, ecosystem processes, and underlying functions and services in subtropical agriculture-dominated landscapes. In this work, I integrated diverse approaches that include data synthesis, field expeirment, and laboratory soil, plant, and microbiome analyses. The unified theme across all chapters of this dissertation was to improve understanding on the consequences of land management on multiple ecosystem functions and services for the sustainability of land, water, and other natural resources in subtropical agricultural landscapes.

Results from Chapter 2 indicated that current agricultural land-use intensification promoted provisioning services including forage and livestock production, but at the expense of regulating and supporting services, such as declined water quality regulation, greenhouse gas mitigation, and biodiversity maintenance. Land-use intensification not only altered the magnitude, but also the relationship of multiple ecosystem services in grazing grasslands. Moreover, such effects crossed boundaries from grasslands to embedded natural seasonal wetlands in the landscape. These results revealed differential and cascading effects of intensified management on multiple ecosystem functions and services in managed grasslands and embedded natural wetlands, and demonstrated the importance of landscape-level strategies to achieve multifunctional and sustainable agriculture intensification. These findings highlighted that the two land management approaches (i.e., SN and IM) were complementary in their supported ecosystem services, and should be implemented and spatially optimized at the landscape scale to achieve sustainable intensification that balance agricultural production, conservation, multifunctionality, and rural prosperity.

Results from Chapter 4 on microbial communities demonstrated that upland land-use intensification and two common agricultural disturbances substantially altered the composition and structure of bacterial and fungal communities in subtropical wetlands. However, responses of composition and diversity of bacterial and fungal communities to land management intensity and disturbances were not consistent, and differed across taxonomic level and functional potential. 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, I found that pH and secondary nutrients (i.e., Ca and Mg) availability were the most important soil properties that explained how agricultural land use and wetland management drive the composition of bacterial and fungal communities. These findings suggested that integration of lime application into intensified land uses to neutralize soil pH could facilitate maintenance of microbial diversity and associated functions. Chapter 4 highlighted the importance of assessing microbial responses comprehensively to better evaluate the impacts of agricultural land use and wetland management on belowground biological communities, associated ecosystem functions, and agricultural sustainability.

Results from teabag (Chapter 3) and litterbag decomposition (Chapter 5) demonstrated that human-induced environmental changes in wetlands, particularly upland agricultural intensification, could accelerate organic matter decomposition rates and reduce their stabilization. Such effects could lead to reduced formation of stable organic matter, or decreased capacity of subtropical wetlands to retain nutrients and sequester carbon. However, there were nuanced interactive effects from these management practices, in which cattle grazing could help buffer against the stimulating effect of land-use intensification on decomposition rates and thus avoid nutrient release pulses. Further, periodical prescribed fire showed inhibitory effects on wetland decomposition, suggesting that fire suppression in subtropical wetlands could promote organic matter depletion and thus nutrient loss. Hence, if a management goal is to promote carbon sequestration and nutrient retention services in wetlands, my results highlighted the need to avoid agricultural intensification in surrounding pastures but maintain natural fire dynamics in seasonal wetlands to sustain their capacity of providing associated regulating and supporting services.

In summary, this dissertation filled multiple key empirical data and knowledge gaps on the responses of soil microbial communities, organic matter decomposition, and multiple ecosystem functions and services to interacting land management and disturbances in subtropical agroecosystems. Results from this work work could help inform Earth System Models to quantify and predict anthropogenic effects on carbon and nitrogen cycling, wetland ecosystem functions and services, and agroecosystem multifunctionality at regional to global scales. Future research could link these multifunctionality results with socio-economic data and approaches to provide further insights into multifunctional, profitable, and equitable agricultural landscapes, to guide sustainable management and intensification of agroecosystems, and to solve human-environment conflicts in an era of Anthropocene with dwindling natural resources and rapid environmental changes.

APPENDIX A
SUPPLEMENTARY MATERIAL FOR DATA SYNTHESIS

Study Site Description

Our study area is Archbold Biological Research Station, located in south-central Florida (27°09’N, 81°11W) on Buck Island Ranch (BIR). BIR is a commercial cow-calf production ranch and a long-term agro-ecological field station. The total area of BIR is 4,252 ha, where is dominated by wet prairie (1,771 ha), dry prairie (1,604 ha) and wetland (544 ha) (Swain et al., 2013a). The landscape of Florida prairie ecosystem has adapted to natural fires at 1- to 4-years return intervals. Thus, most pasture areas in BIR are prescribed burned every 2 to 3 years to maintain the pyrogenic system as well as to manage forage. BIR is under a humid subtropical climate with distinct hot wet (June-October) and mild dry (November-May) seasons. Average daily temperatures during two seasons are 26.1°C and 19.4 °C, respectively. Average annual precipitation is about 1,300 mm, approximately 70% of it fall during the wet season.

After being heavily drained and converted to forage grass (*Paspalum notatum* Flugge), the central and north-central areas of BIR are classified as intensively managed (IM) pastures (Fig. 1). IM pastures also received nitrogen (N), phosphorus (P) and potassium (K) fertilizers (P and K were up until 1987), regular lime, and intense grazing activities during wet seasons from 1970s. N fertilization is applied using NH_4SO_4 or NH_4NO_3 at a rate of 56 kg/ha annually or semiannually, while P and K were historically applied using P_2O_5 and K_2O at a rate of 34-90 kg/ha (Boughton et al., 2016). Cattle density in IM pastures is 0.57-1.7 cows per hectare. The IM pastures are at elevation of ~ 7.9 to 10.7 m, gently sloping from north to the south and draining into Harney Pond Canal. Soils in IM pastures are mainly Felda fine sand (Swain et al., 2007), which is a sandy or loamy, siliceous, and hyperthermic alfisol.

The surrounding prairie and savannas are less drained, never fertilized, partially converted to *P. notatum*, and moderately grazed during dry seasons, which are classified as semi-natural (SN, e.g., less intensively managed) pastures. In addition to *P. notatum*, other common plants in SN pastures are bunchgrasses (*Andropogon virginicus*, *Axonopus affinis*, and *Panicum longifolium*), forbs (*Lachnanthes caroliniana* and *Eupatorium mohrii*), and woody dicots (*Eupatorium capillifolium* and *Euthamia graminifolia*). SN pasture elevation is at ~ 8.0-10.0 m. In the area north of the Harney Pond Canal, drainage is gradually to the southeast; while in the area south of the Harney Pond Canal, the water drains from south to north. Cattle density in SN pastures is 0.15-1.12 cows per hectare. Soils in SN pastures are primarily Pineda fine sand underneath a thin layer of muck. These two pasture types represent the two principal ranch managing regimes in south Florida (Capece et al., 2007).

Both types of pastures include many small wetlands that are seasonally flooded emergent/shrub freshwater marshes (Boughton et al., 2019). These wetlands were historically considered isolated because they were geographically distinct and separated from other aquatic bodies. However, with the construction of extensive ditches, some wetlands are connected via ditches during wet seasons, especially in IM pastures. BIR has total more than 600 such wetlands with a mean size of 0.87 ha and hydroperiod from 2 to 10 months. These wetlands are scattered across the ranch and represent ~ 15% of total land area, which is typical for the Lake Okeechobee Basin (Gathumbi et al., 2005). Cattle have free access to the wetlands within the same pasture for cooling and feeding. Embedded wetlands are exposed to prescribed burns ignited in pastures and sometimes get burned when conditions are dry enough. Wetlands located in different pasture types are given distinct biotic and abiotic attributes and thereby classified as IM and SN wetlands. For example, dominant plants in IM wetlands are *Juncus effusus*, *Pontedaria cordata*, and *Panicum hemitomon*. While in SN wetlands common plants are more diverse, which include *Pontedaria cordata*, *Panicum hemitomon*, *Amphicarpum muehlenbergianum*, *Justicia angusta*, *Sagittaria lancifolia*, and *Bacopa caroliana*.

Existing Experimental Infrastructure

The BIR has been an active location for research work on ecosystem services provided by subtropical grasslands since 2001. There is a dense sensor network including 5 eddy covariance flux towers, 32 groundwater wells, and many water sampling locations. BIR has accumulated co-located long-term datasets (e.g., climate, plant community and functional traits, productivity, soil nutrients, hydrology, GHG fluxes, phenology). These data could serve as the foundational baseline for us to understand the long-term agro-ecological responses and dynamics of subtropical grasslands to different management intensities.

In addition to long-term monitoring, BIR has several existing experimental infrastructures available for research. These infrastructures also demonstrate the Archbold BIR's commitment and interests in long-term agricultural and ecological research, as well as contributions to cross-site comparisons and large-scale syntheses. First is sixteen 16-ha experimental pastures and experimental design for a USDA NIFA funded project (2016-2020), which was established in 2016. These experimental grassland units were originally designed to assess the interaction of management intensity and grazing-fire pattern on greenhouse gas flux, water use efficiency, forage productivity and quality and plant diversity. The experiment was set up as a randomized block design with four blocks for a 2×2 cross of grassland type (intensively managed and semi-natural) and fire-grazing pattern (Patch Burn Grazing - PBG vs. Large Burn Grazing - LBG). The presented study took advantage of this design and leverage previously surveyed data by only analyzing the difference between intensively managed and semi-natural grasslands within a subset of LBG pastures. The second infrastructure is fire and grazing 40 wetland experiment from two types of grasslands. This experiment was established in 2006 and originally funded by USDA from 2006-2009, to understand the interactive effects of upland intensification, grazing and fire on wetland communities and ecosystems. This is a randomized block design with a 2x2x2 cross of pasture management (intensively managed and semi-natural), grazing (grazed and not grazed), and prescribed fire (burned and unburned). There are 5 replicates of each treatment. The presented study took advantage of this design and leverage previously surveyed data by only analyzing the wetland embedded in intensively managed and semi-natural grasslands within a subset of grazed wetlands.

Table A-1. Land-use intensification refers to the differences in management practices from semi-natural to intensively managed grasslands.

Semi-natural grasslands	Intensively managed grasslands
1. No fertilization	1. N, P, K (P and K were up until 1987) fertilizers received
2. Partially converted to non-native forage grasses	2. Completely converted to non-native forage grasses (e.g., Bahiagrass)
3. No lime application	3. Lime applied
4. Constructed less extensive drainage ditches	4. Constructed extensive drainage ditches
5. No water retention infrastructure	5. Included water retention infrastructure (e.g., riser board)
6. Introduced light cattle grazing in dry seasons	6. Introduced heavy cattle grazing in wet seasons

Table A-2. Summary of grassland ecosystem service indicators included in this synthesis.

Ecosystem services	Biophysical indicators	Temporal scale	Independent sample size	Independent observations	Measurement unit	Whether have been published
Soil nutrient maintenance	Soil total nitrogen (TN)	2016	63	63	%	No
	Soil ammonium (NH_4^+)	2019	72	72	ug g^{-1}	No
	Soil nitrate (NO_3^-)	2016, 2019	144	72	ug g^{-1}	No
	Soil total phosphorus (TP)	2016, 2019	144	72	ug g^{-1}	No
	Soil Mehlich3-P	2016	67	67	lb ac^{-1}	No
	Soil C/N ratio	2016	61	61	unitless	No
	Soil organic matter (OM)	2016, 2019	144	72	%	No
Water quality regulation	Water TN	2008-2015	557	8	mg L^{-1}	No
	Water NH_4^+	2006-2015	714	8	mg L^{-1}	No
	Water NO_3^-	2008-2015	557	8	mg L^{-1}	No
	Water TP	2003-2015	978	8	mg L^{-1}	No
	Water orthophosphate (PO_4^{3-})	2003-2015	976	8	mg L^{-1}	No
Carbon storage and climate regulation	Soil total carbon (TC)	2016	63	63	%	No
	Root biomass	2016, 2019	144	72	g	No
	Annual net primary productivity (ANPP)	2017-2019	54	18	g m^{-2}	No
	CO_2 flux	2013-2015	1685	2	mol m^{-2} s^{-1}	Yes, Chamberlain et al., (2017); Gomez-Casanovas et al., (2018)
	CH_4 flux	2013-2015	1620	2	mol m^{-2} s^{-1}	Yes, Chamberlain et al., (2017); Gomez-Casanovas et al., (2018)

Table A-2. Continued

Ecosystem services	Biophysical indicators	Temporal scale	Independent sample size	Independent observations	Measurement unit	Whether have been published
Biodiversity	Total plant richness	2016-2019	48	8	unitless	No
	Plant α-diversity	2018-2019	32	8	unitless	No
	Plant β-diversity	2016-2019	48	8	unitless	No
	Vegetative height CV	2016-2019	48	8	%	No
	Vertebrate richness	2016-2018	44	44	unitless	Yes, Tabak et al., (2019)
	Vertebrate α-diversity	2016-2018	44	44	unitless	Yes, Tabak et al., (2019)
	Bird richness	2010	8	2	unitless	No
	Bird α-diversity	2010	8	2	unitless	No
Invasion resistance	Non-native plant richness	2016-2019	48	8	unitless	No
	Non-native vertebrate α-diversity	2016-2018	44	44	unitless	No
Forage and livestock production	ANPP	2017-2019	54	18	g m^{-2}	No
	Forage N	2017-2019	286	8	%	No
	Forage P	2017-2019	285	8	%	No
	Forage in vitro organic matter digestibility (IVODM)	2017-2019	286	8	%	No
	Cattle stocking density	2017-2018	192	8	d ac^{-1} $month^{-1}$	No

Table A-3. Summary of wetland ecosystem service indicators included in this synthesis.

Ecosystem services	Biophysical indicators	Temporal scale	Independent sample size	Independent observations	Measurement unit	Whether have been published
Soil nutrient maintenance	Soil total nitrogen (TN)	2007, 2016	199	100	%	Yes, Ho et al., (2018)
	Soil total phosphorus (TP)	2007	100	100	ug g^{-1}	Yes, Ho et al., (2018)
	Soil C/N ratio	2007, 2016	199	100	unitless	Yes, Ho et al., (2018)
	Soil organic matter (OM)	2007, 2016	198	100	%	Yes, Ho et al., (2018)
Water quality regulation	Water TN	2006/08/09/14	80	20	mg L^{-1}	Yes, Jansen et al., (2019)
	Water NH_4^+	2006/08/09/14	80	20	mg L^{-1}	Yes, Jansen et al., (2019)
	Water NO_3^-	2008/09/14	60	20	mg L^{-1}	No
	Water TP	2006/08/09/14	80	20	mg L^{-1}	Yes, Jansen et al., (2019)
	Water PO_4^{3-}	2006/08/09/14	80	20	mg L^{-1}	Yes, Jansen et al., (2019)
Carbon storage and climate regulation	Soil total carbon (TC)	2007, 2016	199	100	%	Yes, Ho et al., (2018)
	Root biomass	NA	25	NA	g	Yes, DeLucia et al., (2019)
	Annual net primary productivity (ANPP)	2016, 2017	19	10	g m^{-2}	Partial data published in Sonnier et al., (2020)
	CH_4 flux	2013-2015	188	16	umol m^{-2} s^{-1}	Yes, DeLucia et al., (2019)
Biodiversity	Plant richness	2006-2016/18	40	20	unitless	No
	Plant α-diversity	2018	20	20	unitless	No
	Plant β-diversity	2018	20	20	unitless	No
	Vegetative height CV	2018	20	20	%	No
	Invertebrate richness	2006	20	20	unitless	Yes, Medley et al., (2015)
	Invertebrate α-diversity	2006	20	20	unitless	Yes, Medley et al., (2015)
	Vertebrate richness	2006	20	20	unitless	Yes, Medley et al., (2015)
	Vertebrate α-diversity	2006	20	20	unitless	Yes, Medley et al., (2015)
Invasion resistance	Non-native plant richness	2018	20	20	unitless	No

Table A-4. Effects of land-use intensification on grassland and embedded wetland multifunctionality.

Ecosystem / Approach	Grassland		Wetland	
	t	*P*	t	*P*
Simple averaging	–2.40	0.061.	–3.29	0.004**
Service-based weighted averaging	–3.17	0.025*	–5.41	<0.001***
Cluster-based weighted averaging	–3.23	0.023*	–2.81	0.012*
50% quantile threshold	–0.78	0.47	–3.17	0.006**

Table A-5. Model specification of best models for each response variable of ecosystem function and service and overall model performance that include AIC, BIC, marginal and conditional R^2.

Dataset	Indicator	AIC	BIC	Marginal R^2	Conditional R^2
Model specification					
Grassland soil	TN	291.34	302.05	0.262	0.502
lmer((1/value) ~ Pasture_Type + Elevation + (1|Pasture), data = pas.soil.tn)					
Grassland soil	NH_4^+	178.32	189.71	0.143	0.370
lmer(sqrt(value) ~ Pasture_Type + Elevation + (1|Pasture), data = pas.soil.nh4)					
Grassland soil	NO_3^-	634.03	651.85	0.077	0.602
lmer(log(value+0.001) ~ Pasture_Type + Elevation + (1|Pasture/Year), data = pas.soil.no3)					
Grassland soil	TP	146.42	164.24	0.328	0.602
lmer(log(value) ~ Pasture_Type + Elevation + (1|Pasture/Year), data = pas.soil.tp)					
Grassland soil	Mehlich3-P	117.70	128.72	0.122	0.122
lmer(log(value) ~ Pasture_Type + Elevation + (1|Pasture), data = pas.soil.mp)					
Grassland soil	C/N ratio	302.40	312.96	0.252	0.371
lmer(value ~ Pasture_Type + Elevation + (1|Pasture), data = pas.soil.cn)					
Grassland soil	OM	265.01	279.86	0.429	0.706
lmer(sqrt(value) ~ Pasture_Type + Elevation + (1|Pasture), data = pas.soil.om)					
Grassland soil	TC	-90.70	-79.99	0.256	0.504
lmer((1/value) ~ Pasture_Type + Elevation + (1|Pasture), data = pas.soil.tc)					
Ditch water	TN	945.85	976.10	0.030	0.331
lmer(log(value+0.2) ~ Pasture_Type + Elevation + (1|Ditch) + (1|Year/Month), data = pas.water.tn)					
Ditch water	NH_4^+	93.38	125.37	0.020	0.343
lmer(log(value+0.001) ~ Pasture_Type + Elevation + (1|Ditch) + (1|Year/Month), data = pas.water.nh4)					
Ditch water	NO_3^-	903.73	933.99	0.003	0.438

Table A-5. Continued

Dataset	Indicator	AIC	BIC	Marginal R^2	Conditional R^2
Model specification					
lmer(sqrt(value) ~ Pasture_Type + Elevation + (1|Ditch) + (1|Year/Month), data = pas.water.no3)					
Ditch water	TP	-563.94	-529.75	0.083	0.357
lmer((value)^(1/3) ~ Pasture_Type + Elevation + (1|Ditch) + (1|Year/Month), data = pas.water.tp)					
Ditch water	PO_4^{3-}	3104.85	3139.03	0.039	0.396
lmer(log(value+0.001) ~ Pasture_Type + Elevation + (1|Ditch) + (1|Year/Month), data = pas.water.op)					
Grassland plant	Total richness	285.92	299.01	0.485	0.774
lmer(value ~ Pasture_Type + Elevation + (1|Pasture) + (1|Year/Season), data = pas.plant.rich)					
Grassland plant	α-diversity	37.84	45.17	0.645	0.758
lmer((1/value) ~ Pasture_Type + Elevation + (1|Pasture), data = pas.plant.shan)					
Grassland plant	β-diversity	-157.93	-146.70	0.312	0.677
lmer(value ~ Pasture_Type + Elevation + (1|Pasture/Year), data = pas.plant.beta)					
Grassland plant	Non-native richness	201.01	214.11	0.342	0.823
lmer(value ~ Pasture_Type + Elevation + (1|Pasture) + (1|Year/Season), data = pas.plant.inv)					
Grassland plant	Height CV	45.29	58.39	0.303	0.555
lmer(log(value) ~ Pasture_Type + Elevation + (1|Pasture) + (1|Year/Season), data = pas.hveg)					
Grassland plant	ANPP	195.83	207.54	0.172	0.236
lmer((value)^(1/3) ~ Pasture_Type + Elevation + (1|Pasture) + (1|Year), data = pas.product)					
Grassland plant	Root biomass	290.55	308.37	0.183	0.365
lmer(sqrt(value) ~ Pasture_Type + Elevation + (1|Pasture/Year), data = pas.soil.rb)					
Grassland forage	N	-407.21	-381.62	0.046	0.484
lmer(sqrt(value) ~ Pasture_Type + Elevation + (1|Pasture) + (1|Year/Month), data = pas.forage.ndm)					
Grassland forage	P	180.99	206.55	0.089	0.582
lmer(log(value) ~ Pasture_Type + Elevation + (1|Pasture) + (1|Year/Month), data = pas.forage.pdm)					
Grassland forage	IVOMD	1922.88	1948.47	0.028	0.561
lmer(value ~ Pasture_Type + Elevation + (1|Pasture) + (1|Year/Month), data = pas.forage.ivomd)					
Animal use	Stocking density	714.42	733.97	0.289	0.523
lmer(sqrt(value) ~ Pasture_Type + (1|Pasture) + (1|Year/Month), data = stocking.density)					
Grassland animal	Native vertebrate richness	148.49	153.85	0.043	0.043

Table A-5. Continued

<table>
<thead>
<tr><th>Dataset</th><th>Indicator</th><th rowspan="2">AIC</th><th rowspan="2">BIC</th><th rowspan="2">Marginal R^2</th><th rowspan="2">Conditional R^2</th></tr>
<tr><th colspan="2">Model specification</th></tr>
</thead>
<tbody>
<tr><td colspan="6">lm(richness ~ Pasture_Type, data = vert.native)</td></tr>
<tr><td>Grassland animal</td><td>Native vertebrate diversity</td><td>-48.11</td><td>-42.76</td><td>0.067</td><td>0.067</td></tr>
<tr><td colspan="6">lm(shannon ~ Pasture_Type, data = vert.native)</td></tr>
<tr><td>Grassland animal</td><td>Non-native vertebrate diversity</td><td>-60.95</td><td>-55.60</td><td>0.065</td><td>0.065</td></tr>
<tr><td colspan="6">lm((1/shannon) ~ Pasture_Type, data = vert.non)</td></tr>
<tr><td>Grassland animal</td><td>Bird richness</td><td>0.14</td><td>0.26</td><td>0.007</td><td>0.956</td></tr>
<tr><td colspan="6">lmer(log(richness) ~ Pasture_Type + (1|Season), data = bird.t)</td></tr>
<tr><td>Grassland animal</td><td>Bird diversity</td><td>0.71</td><td>1.03</td><td>0.093</td><td>0.496</td></tr>
<tr><td colspan="6">lmer(log(shannon) ~ Pasture_Type + (1|Season), data = bird.t)</td></tr>
<tr><td>Grassland gas</td><td>CO_2</td><td>114.65</td><td>135.90</td><td>0.008</td><td>0.107</td></tr>
<tr><td colspan="6">lmer((value)^(1/3) ~ Pasture_Type + (1|Year/Month), data = pas.co2.date)</td></tr>
<tr><td>Grassland gas</td><td>CH_4</td><td>-3052.3</td><td>-3025.8</td><td>0.060</td><td>0.607</td></tr>
<tr><td colspan="6">lmer((value)^(1/3) ~ Pasture_Type + (1|Year/Month), data = pas.ch4.date)</td></tr>
<tr><td>Wetland soil</td><td>TN</td><td>406.06</td><td>425.82</td><td>0.024</td><td>0.447</td></tr>
<tr><td colspan="6">lmer(log(value) ~ Pasture_Type + Elevation + (1|Wetland/Year), data = wet.soil.tn)</td></tr>
<tr><td>Wetland soil</td><td>TP</td><td>257.88</td><td>270.90</td><td>0.062</td><td>0.504</td></tr>
<tr><td colspan="6">lmer(log(value) ~ Pasture_Type + Elevation + (1|Wetland), data = wet.soil.tp)</td></tr>
<tr><td>Wetland soil</td><td>C/N ratio</td><td>782.56</td><td>802.29</td><td>0.322</td><td>0.667</td></tr>
<tr><td colspan="6">lmer(value ~ Pasture_Type + Elevation + (1| Wetland/Year), data = wet.soil.cn)</td></tr>
<tr><td>Wetland soil</td><td>OM</td><td>343.18</td><td>359.62</td><td>0.046</td><td>0.443</td></tr>
<tr><td colspan="6">lmer((value)^(1/3) ~ Pasture_Type + Elevation + (1|Wetland), data = wet.soil.om)</td></tr>
<tr><td>Wetland soil</td><td>TC</td><td>401.05</td><td>420.81</td><td>0.054</td><td>0.502</td></tr>
<tr><td colspan="6">lmer(log(value) ~ Pasture_Type + Elevation + (1|Wetland/Year), data = wet.soil.tc)</td></tr>
<tr><td>Wetland water</td><td>TN</td><td>-15.80</td><td>-1.51</td><td>0.085</td><td>0.632</td></tr>
<tr><td colspan="6">lmer(sqrt(value) ~ Pasture_Type + Elevation + (1|Wetland) + (1|Year), data = wet.water.tn)</td></tr>
<tr><td>Wetland water</td><td>NH_4^+</td><td>-142.31</td><td>-128.32</td><td>0.002</td><td>0.546</td></tr>
<tr><td colspan="6">lmer(sqrt(value) ~ Pasture_Type + Elevation + (1|Wetland) + (1|Year), data = wet.water.nh4)</td></tr>
<tr><td>Wetland water</td><td>NO_3^-</td><td>-162.53</td><td>-150.37</td><td>0.024</td><td>0.392</td></tr>
<tr><td colspan="6">lmer(sqrt(value) ~ Pasture_Type + Elevation + (1| Wetland) + (1|Year), data = wet.water.no3)</td></tr>
<tr><td>Wetland water</td><td>TP</td><td>208.46</td><td>220.37</td><td>0.385</td><td>0.641</td></tr>
<tr><td colspan="6">lmer(log(value) ~ Pasture_Type + Elevation + (1|Wetland), data = wet.water.tp)</td></tr>
<tr><td>Wetland water</td><td>PO_4^{3-}</td><td>-15.48</td><td>-1.19</td><td>0.178</td><td>0.575</td></tr>
<tr><td colspan="6">lmer((value)^(1/3) ~ Pasture_Type + Elevation + (1|Wetland) + (1|Year), data = wet.water.op)</td></tr>
</tbody>
</table>

Table A-5. Continued

Dataset	Indicator	AIC	BIC	Marginal R^2	Conditional R^2
Model specification					
Wetland plant	Total richness	-5.44	4.69	0.208	0.639
lmer((value)^(1/3) ~ Pasture_Type + Elevation + (1|Wetland) + (1|Year), data = wet.plant.rich)					
Wetland plant	α-diversity	-13.19	-9.21	0.357	0.357
lm(log(value) ~ Pasture_Type + Elevation, data = wet.plant.shan)					
Wetland plant	β-diversity	-85.25	-81.27	0.513	0.513
lm(value ~ Pasture_Type + Elevation, data = wet.plant.beta)					
Wetland plant	Non-native richness	26.91	30.90	0.577	0.577
lm(sqrt(value) ~ Pasture_Type + Elevation, data = wet.plant.inv)					
Wetland plant	Height CV	67.04	70.82	0.186	0.186
lm(sqrt(value) ~ Pasture_Type + Elevation, data = wet.hveg)					
Wetland plant	ANPP	278.97	288.96	0.108	0.298
lmer(sqrt(value) ~ Pasture_Type + Elevation + (1|Wetland) + (1|Year), data = wet.product)					
Wetland plant	Root biomass	79.90	86.00	0.058	0.706
lmer((value)^(1/3) ~ Pasture_Type + (1|Soil_Depth/Root_Size), data = wet.root)					
Wetland animal	Invertebrate richness	22.90	26.68	0.138	0.138
lm(log(sr_invert) ~ Pasture_Type + Elevation, data = wet.wild)					
Wetland animal	Vertebrate richness	19.19	23.17	0.052	0.052
lm(log(sr_vert) ~ Pasture_Type + Elevation, data = wet.wild)					
Wetland gas	CH_4	148.93	162.82	0.111	0.421
lmer((value)^(1/3) ~ Pasture_Type + (1|Location/Season), data = wet.ch4)					

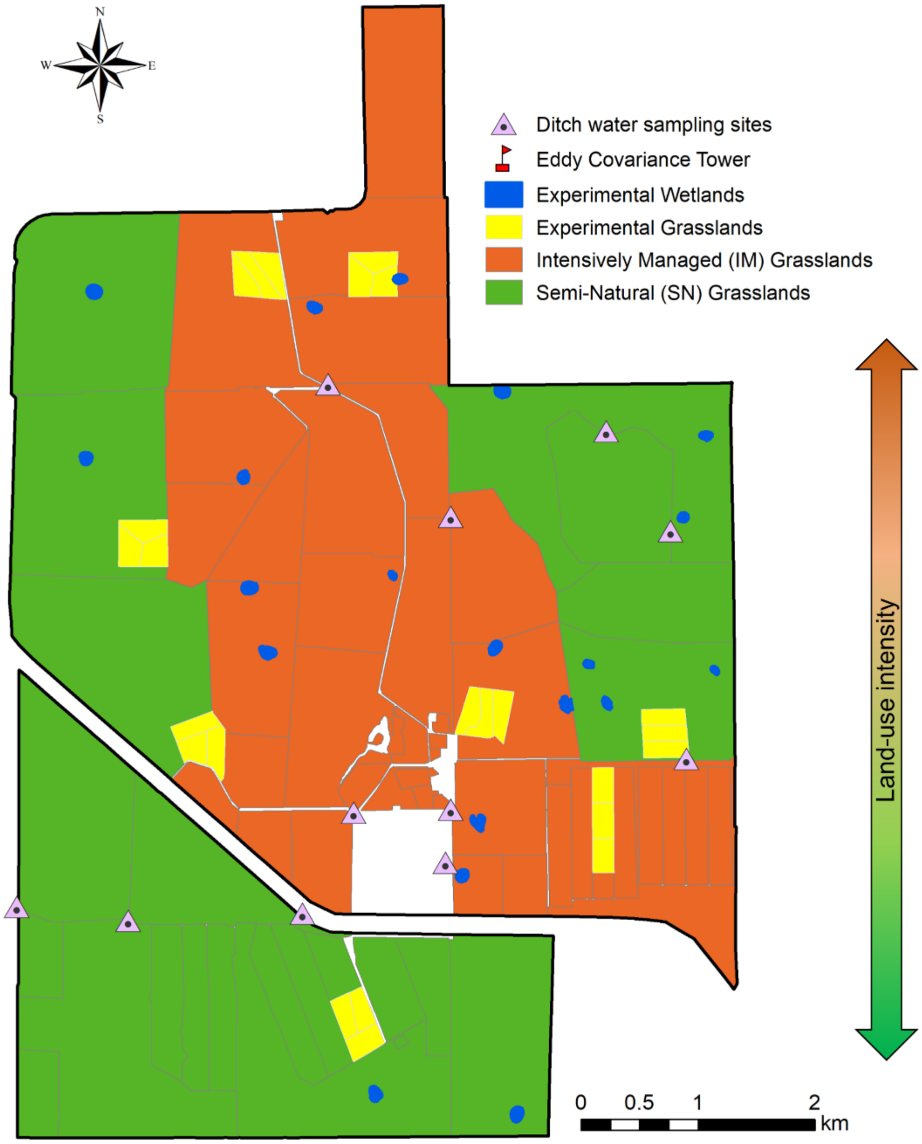

Figure A-1. Map of the Buck Island Ranch at Archbold Biological Station with 8 experimental pastures (yellow polygons), 20 wetlands (blue circles), 8 ditch water sampling sites (purple triangles), and 2 eddy covariance towers in intensified managed and semi-natural grasslands.

APPENDIX B
SUPPLEMENTARY MATERIAL FOR TEABAG DECOMPOSITION

Principal Component Analysis (PCA) on Soil Attributes

Differences in soil attributes across land use and management treatments, incubation periods, and sampling sites were observed (Figures B-2, B-3). Due to the multicollinearity among the soil attributes (Figure B-1), we performed PCA on 11 measured soil attributes before using them as explanatory variables on predicting TBI parameters. The first three principle components (PCs) produced from PCA totally explained 81.54% variance. The first PC (PC1) explained 59.05% of the total variance that was negatively defined by soil water content, soil C and other nutrients, including N, P, K, Ca, and OM (Figure 3-4). The second PC (PC2) explained 12.17% of the total variance and was mainly defined by negative soil pH, and positive C/N and C/P ratio. The third PC (PC3) explained 10.32% of the total variance and was positively defined by soil pH and C/P ratio. PC2 was important predictor of k (p=0.002, Table 3-2), while PC1 and PC3 were determinant predictors of S (p<0.001; p=0.003 respectively, Table 3-2).

Table B-1. Linear mixed-effects model results showing the estimates of Tea Bag Index at the wetland level.

Driver	Estimate	SE	P value
	Decomposition rate *k*		
Pasture (IM)	0.0004	0.0017	0.128
Grazing (Grazed)	–0.0015	0.0017	**0.021***
Burn (Burned)	0.0031	0.0016	0.427
Period (March-June)	–0.0101	0.0008	**< 0.001*****
IM × Grazed	–0.0003	0.0024	0.397
IM × Burned	–0.0045	0.0024	**0.089.**
Grazed × Burned	–0.0022	0.0023	0.722
IM × Grazed × Burned	0.0033	0.0033	0.318
	Stabilization factor *S*		
Pasture (IM)	–0.158	0.030	**< 0.001*****
Grazing (Grazed)	–0.036	0.029	0.669
Burn (Burned)	0.010	0.029	0.434
Period (March-June)	–0.020	0.015	0.172
IM × Grazed	0.143	0.042	**0.017***
IM × Burned	0.084	0.042	0.704
Grazed × Burned	–0.006	0.041	**0.009****
IM × Grazed × Burned	–0.143	0.058	**0.014***

Notes. Bold values indicate significant coefficients. * indicates $p \leqslant 0.05$, ** indicates $p \leqslant 0.01$, *** indicates $p \leqslant 0.001$.

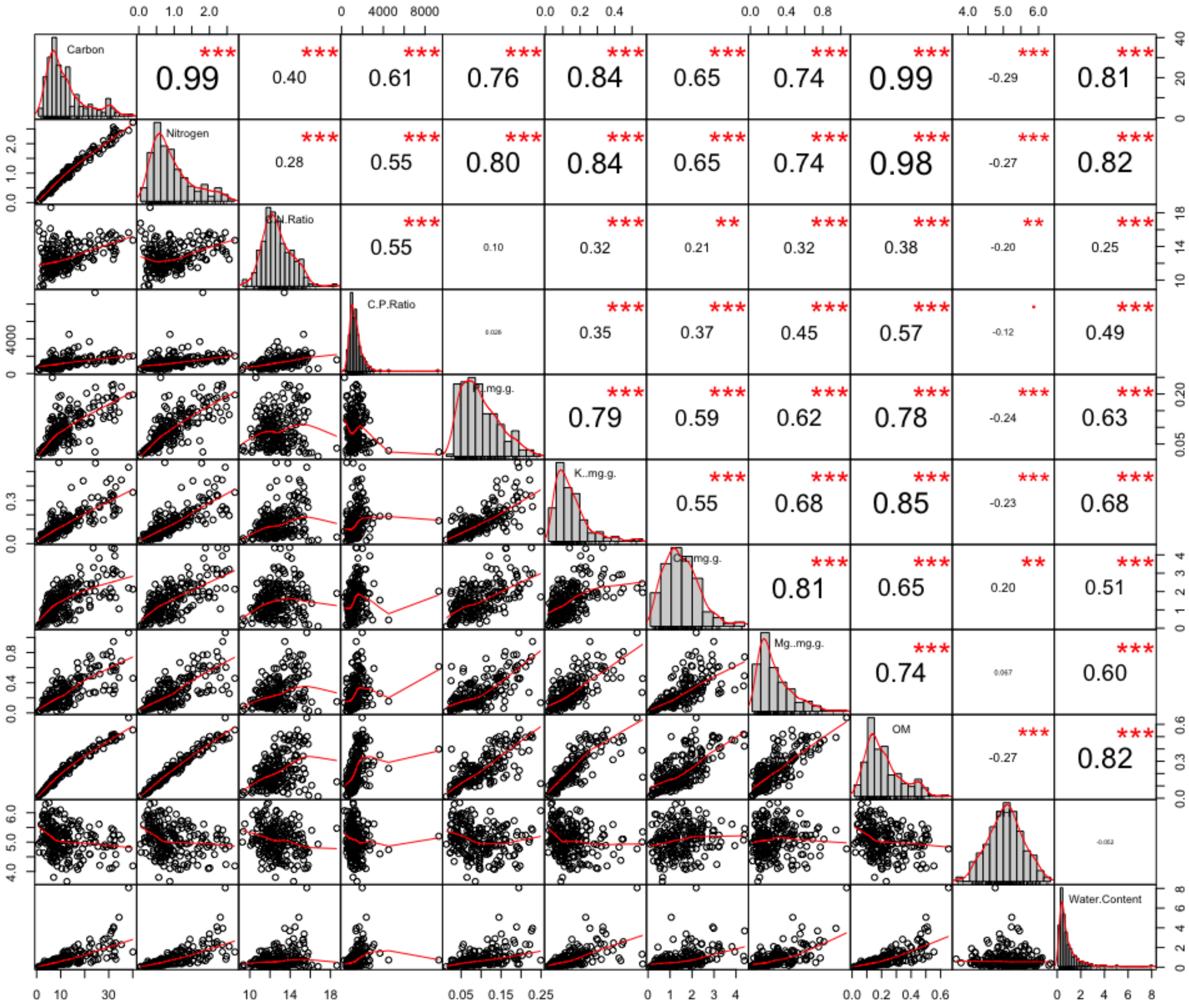

Figure B-1. Spearman correlation between soil physical and chemical properties.

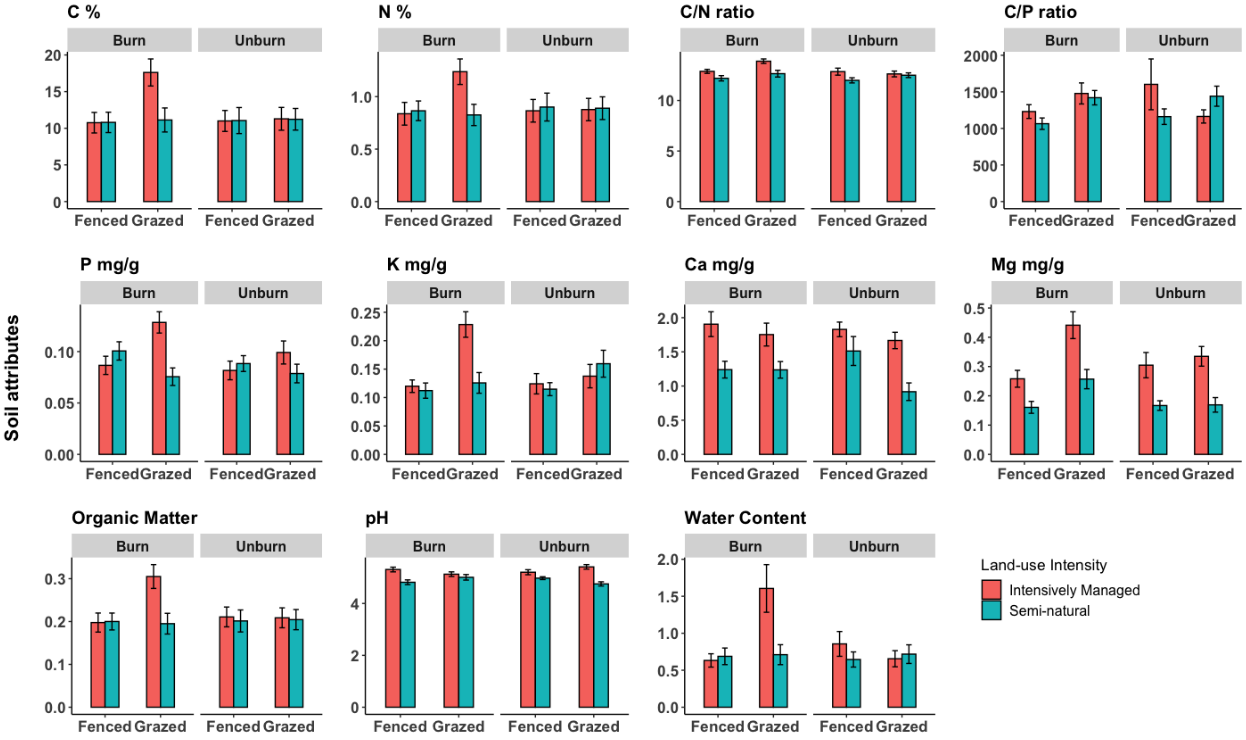

Figure B-2. Differences in soil attributes across wetland treatments. Bars represent means (n = 30), and error bars represent standard errors (SEs).

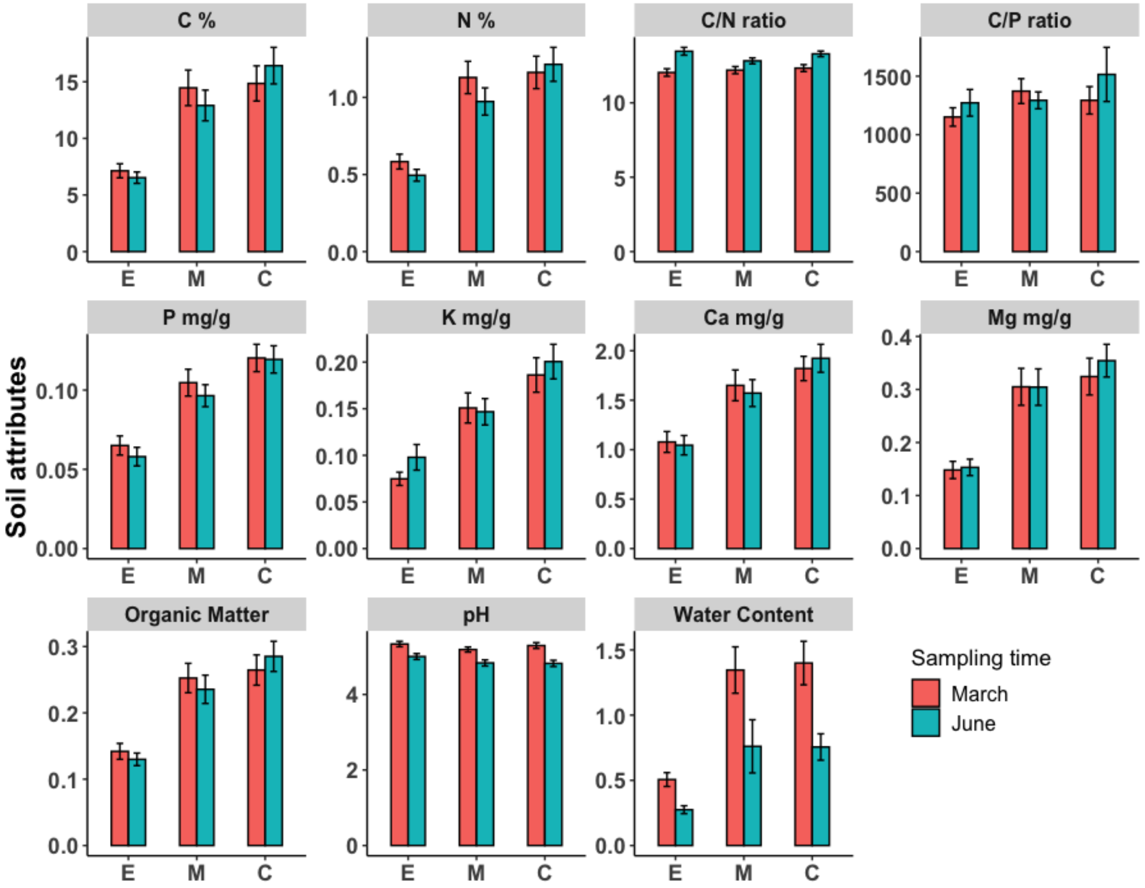

Figure B-3. Differences in soil attributes across wetland sampling sites and incubation periods. Bars represent means (n = 40), and error bars represent standard errors (SEs).

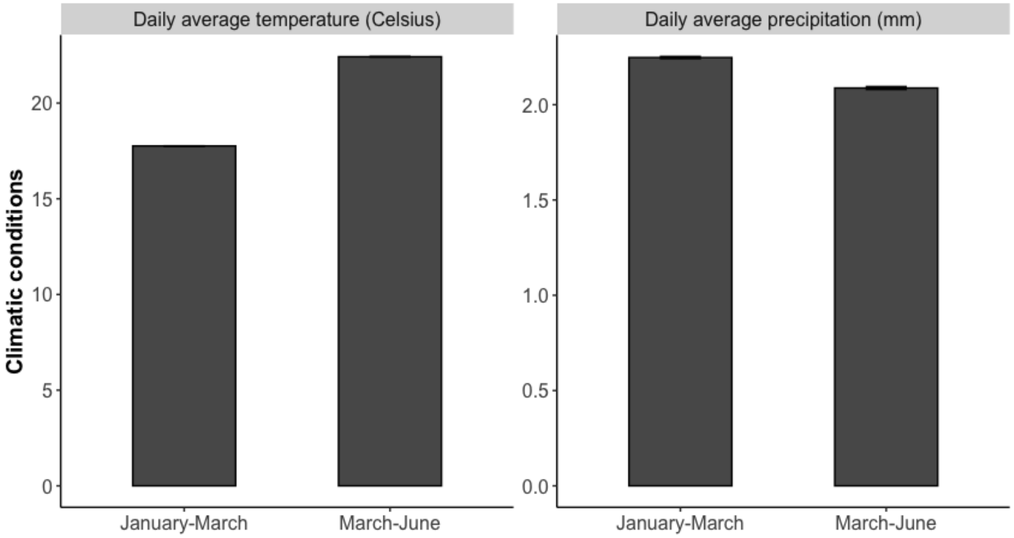

Figure B-4. Differences in daily average temperature (°C) and daily average precipitation (mm). Bars represent means (n = 40), and error bars represent standard errors (SEs).

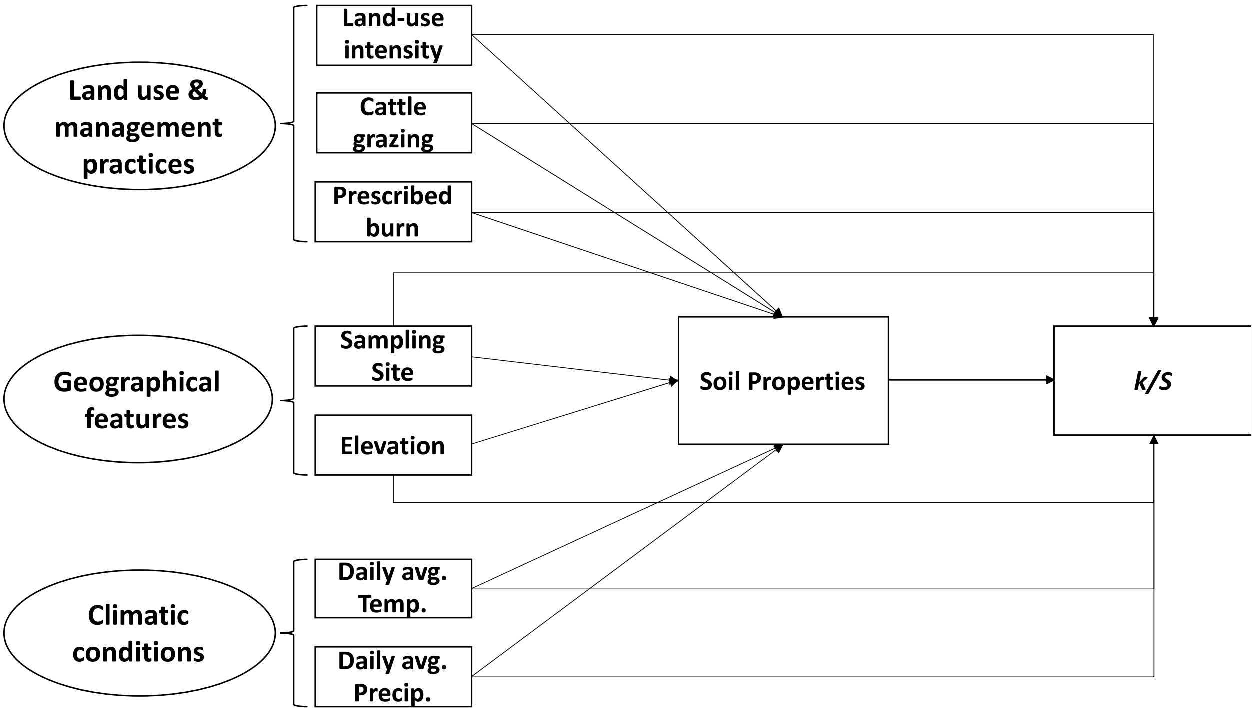

Figure B-5. Hypothesized path diagram for the structural equation modeling for Tea Bag Index.

APPENDIX C
SUPPLEMENTARY MATERIAL FOR SOIL MICROBIAL COMMUNITIES

Table C-1. List of identified bacterial functional group and their mean relative abundance across samples.

Relative abundance	Functional group name
32.0017%	chemoheterotrophy
31.1178%	aerobic_chemoheterotrophy
17.0245%	cellulolysis
4.1326%	phototrophy
4.1112%	photoheterotrophy
1.6379%	predatory_or_exoparasitic
1.6322%	nitrogen_fixation
1.6023%	nitrification
1.4047%	aerobic_ammonia_oxidation
1.0801%	chloroplasts
0.8257%	intracellular_parasites
0.5999%	fermentation
0.3596%	animal_parasites_or_symbionts
0.2929%	sulfate_respiration
0.2929%	respiration_of_sulfur_compounds
0.2777%	methanotrophy
0.2777%	methylotrophy
0.2777%	hydrocarbon_degradation
0.1976%	aerobic_nitrite_oxidation
0.1784%	aromatic_compound_degradation
0.1155%	nitrate_reduction
0.0821%	iron_respiration
0.0757%	ureolysis
0.0710%	human_associated
0.0428%	invertebrate_parasites
0.0405%	human_gut
0.0405%	mammal_gut
0.0376%	manganese_oxidation
0.0305%	human_pathogens_all
0.0213%	photosynthetic_cyanobacteria
0.0213%	oxygenic_photoautotrophy
0.0213%	photoautotrophy
0.0209%	nitrate_respiration
0.0209%	nitrogen_respiration
0.0168%	nonphotosynthetic_cyanobacteria
0.0090%	human_pathogens_pneumonia
0.0074%	dark_oxidation_of_sulfur_compounds

Table C-2. List of identified fungal guilds and their trophic modes and their mean relative abundance across samples.

Relative abundance	Functional guild name	Trophic mode
12.3501%	Plant Pathogen	Pathotroph
11.5615%	Undefined Saprotroph	Saprotroph
10.5607%	Dung Saprotroph	Saprotroph
9.5290%	Soil Saprotroph	Saprotroph
6.9287%	Animal/Plant Pathogen, Undefined Saprotroph	Pathotroph-Saprotroph
5.8093%	Endophyte, Lichen Parasite, Undefined Saprotroph	Symbiotroph-Saprotroph, Saprotroph
5.2973%	Arbuscular Mycorrhizal	Symbiotroph
3.9295%	Animal/Plant Pathogen, Endophyte, Lichen Parasite, Soil/Wood Saprotroph	Pathotroph-Symbiotroph-Saprotroph
2.8339%	Endophyte	Symbiotroph
2.5038%	Endophyte, Litter/Soil Saprotroph	Symbiotroph-Saprotroph
0.7500%	Animal Pathogen, Undefined Saprotroph	Pathotroph-Saprotroph
0.6250%	Endophyte, Fungal/Lichen Parasite, Plant Pathogen, Wood Saprotroph	Symbiotroph-Pathotroph-Saprotroph
0.5678%	Animal/Plant Pathogen, Endophyte, Fungal Parasite, Wood Saprotroph	Pathotroph-Symbiotroph-Saprotroph
0.2089%	Plant Pathogen, Wood Saprotroph	Pathotroph-Saprotroph
0.0759%	Animal/Plant Pathogen, Endophyte, Wood Saprotroph	Pathotroph-Symbiotroph-Saprotroph
0.0694%	Plant Pathogen, Undefined Saprotroph	Pathotroph-Saprotroph
0.0539%	Endophyte, Dung/Litter Saprotroph	Symbiotroph-Saprotroph
0.0498%	Animal/Plant Pathogen, Endophyte, Fungal/Lichen Parasite, Wood Saprotroph	Pathotroph-Symbiotroph-Saprotroph
0.0194%	Dung/Litter Saprotroph	Saprotroph
0.0143%	Dung/Wood Saprotroph	Saprotroph
0.0116%	Fungal Parasite, Plant Pathogen, Litter Saprotroph	Pathotroph-Saprotroph
0.0002%	Endophyte, Plant Pathogen	Symbiotroph-Pathotroph

Table C-3. Model fixed-effects coefficients for soil physiochemical properties.

Soil properties	Land-use intensification (L)	Grazing (G)	Fire (F)	L × G	L × F	G × F	L × G × F
TC	0.18	0.17	0.10	–0.29	–0.11	–0.26	0.81
TN	0.12	0.13	0.09	–0.24	–0.11	–0.25	0.71
C/N ratio	**0.90*** **	**0.46***	0.13	–0.68	–0.18	–0.01	1.22
Mehlich-3 P	**–0.01***	–0.01	**0.01***	**0.02****	0	–0.02	0.04
Mehlich-3 K	**0.02***	**0.05****	0	–0.05	0	–0.03	**0.12***
Mehlich-3 Ca	**0.40*****	**–0.53***	–0.21	0.29	0.36	0.48	–0.53
Mehlich-3 Mg	**0.68*****	**0.05***	0	0.02	–0.16	0.28	0.16
OM	0.02	0.01	0	–0.03	–0.02	–0.02	0.13
pH	**0.23*****	–0.19	–0.16	0.38	0.30	0.39	**–0.80***
Water content	**0.46***	0.20	0.01	–0.66	–0.26	–0.18	**1.19***

Notes. Coefficients represent differences relative to wetlands embedded in semi-natural pastures, and/or that are ungrazed and/or unburned. Bold values indicate significant coefficients. * indicates $p \leq 0.05$, ** indicates $p \leq 0.01$, *** indicates $p \leq 0.001$.

Table C-4. Results of PERMANOVA on weighted and unweighted UniFrac distance of the bacterial and fungal community.

			weighted UniFrac			unweighted UniFrac		
	Treatment	DF	Sum of sq	Pseudo-F	*P*	Sum of sq	Pseudo-F	*P*
Bacteria	Land-use intensity (L)	1	0.069	3.77	**0.001*****	0.839	2.93	**0.001*****
	Grazing (G)	1	0.018	0.98	0.446	0.298	1.04	0.355
	Fire (F)	1	0.025	1.36	0.176	0.324	1.13	0.184
	L × G	1	0.014	0.77	0.644	0.297	1.03	0.359
	L × F	1	0.011	0.59	0.836	0.249	0.87	0.793
	G × F	1	0.017	0.91	0.475	0.318	1.11	0.190
	L × G × F	1	0.015	0.81	0.592	0.324	1.13	0.192
Fungi	Land-use intensity (L)	1	0.029	1.51	0.167	0.419	2.17	**0.002****
	Grazing (G)	1	0.082	4.26	**0.004****	0.233	1.21	0.189
	Fire (F)	1	0.017	0.90	0.421	0.154	0.80	0.783
	L × G	1	0.027	1.39	0.210	0.282	1.46	0.062
	L × F	1	0.015	0.79	0.485	0.209	1.08	0.356
	G × F	1	0.022	1.15	0.284	0.217	1.12	0.265
	L × G × F	1	0.017	0.90	0.432	0.228	1.18	0.187

Notes. Bold values indicate significant coefficients. * indicates $p \leq 0.05$, ** indicates $p \leq 0.01$, *** indicates $p \leq 0.001$.

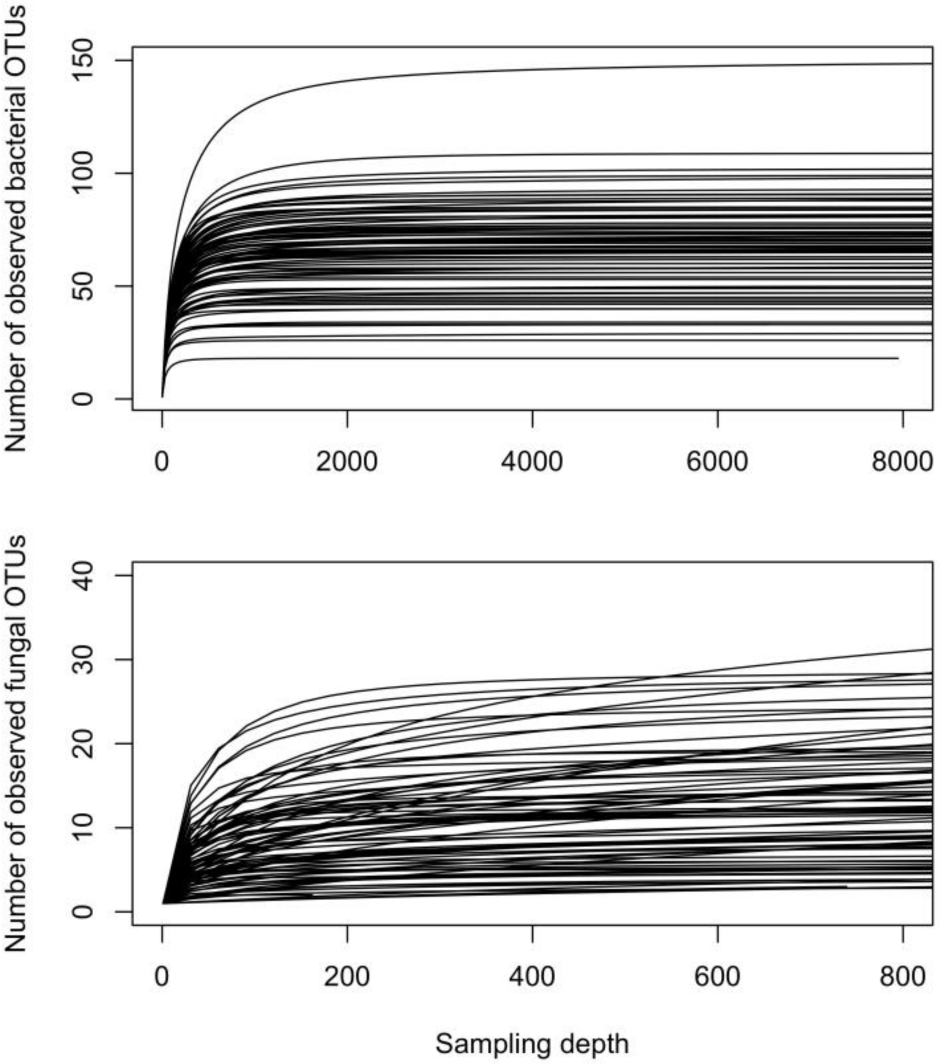

Figure C-1. Relationship between sampling depth and the observed number of bacterial and fungal operational taxonomic units (OTUs).

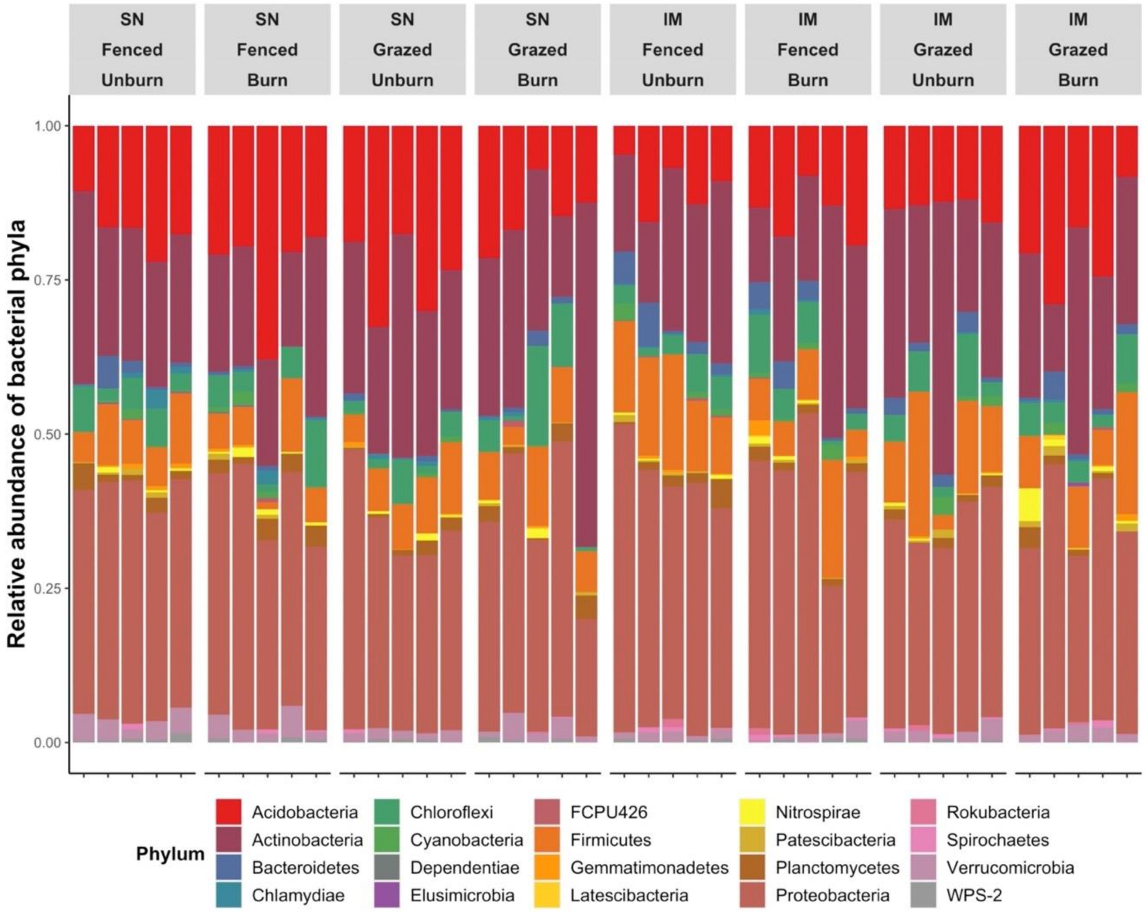

Figure C-2. Relative abundance of primary bacterial phyla that account more than 0.1% of total OTUs in each wetland grouped by wetland treatments. Each bar represents one wetland and bars are clustered by wetland treatment; SN=wetlands embedded in semi-natural pastures, IM=wetlands embedded in intensively managed pastures, Fenced=ungrazed wetlands, Grazed=grazed wetlands, Unburn=unburned wetlands, and Burn=burned wetlands.

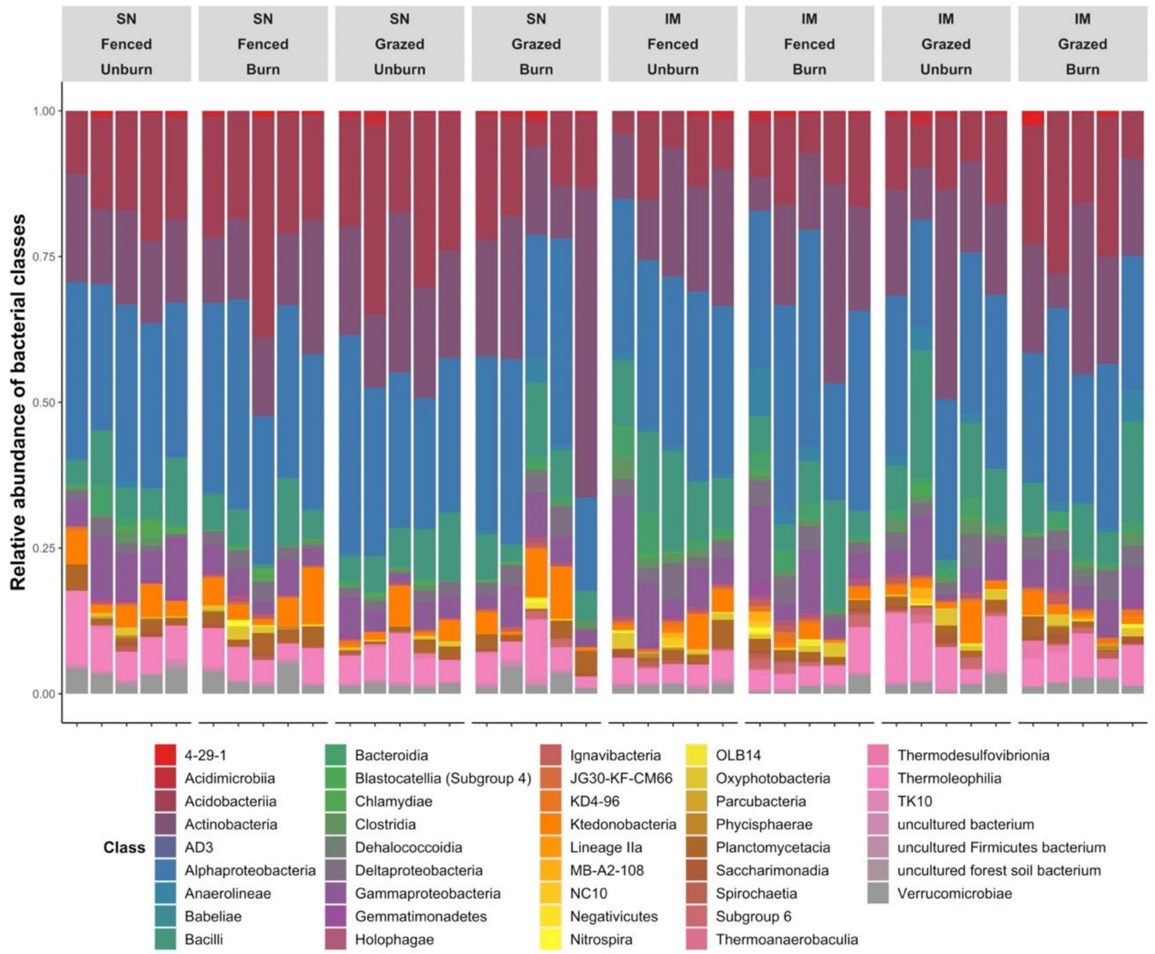

Figure C-3. Relative abundance of primary bacterial classes that account more than 0.1% of total OTUs in each wetland grouped by wetland treatments. Each bar represents one wetland and bars are clustered by wetland treatment; SN=wetlands embedded in semi-natural pastures, IM=wetlands embedded in intensively managed pastures, Fenced=ungrazed wetlands, Grazed=grazed wetlands, Unburn=unburned wetlands, and Burn=burned wetlands.

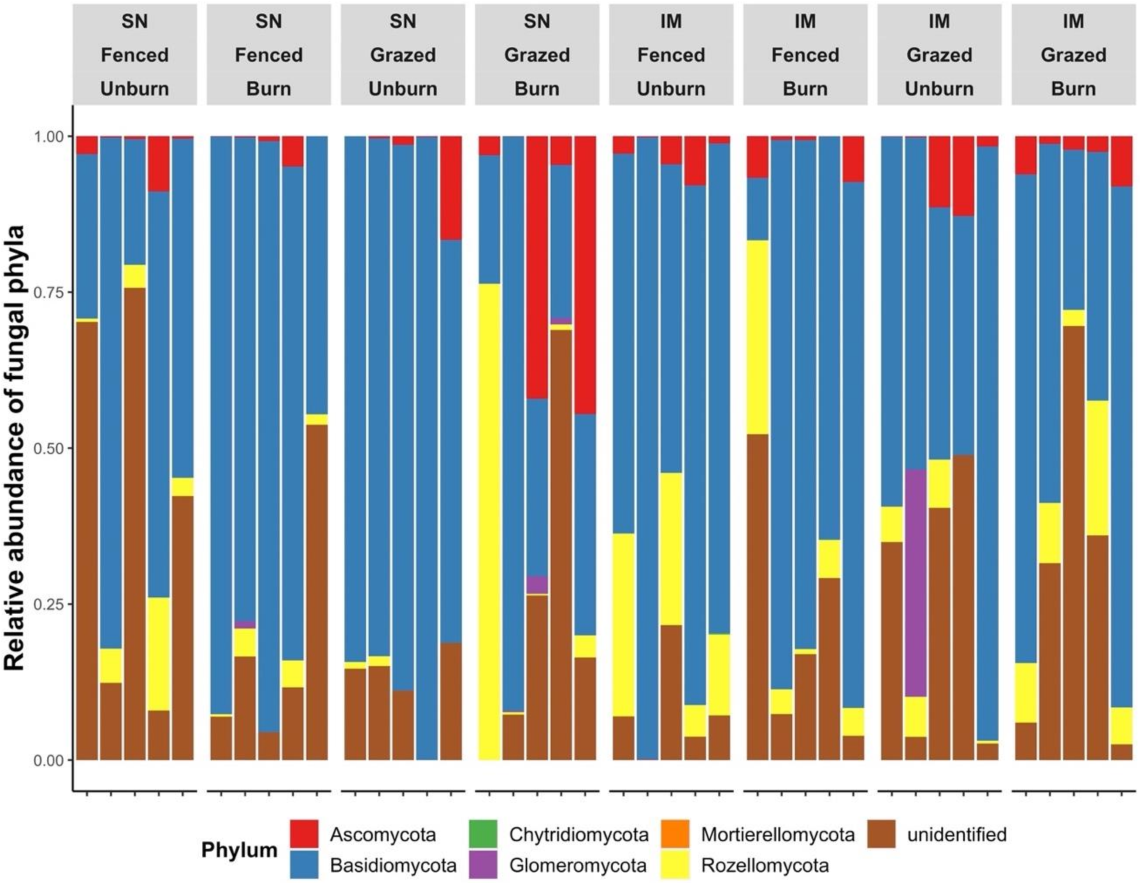

Figure C-4. Relative abundance of primary fungal phyla in each wetland grouped by wetland treatments. Each bar represents one wetland and bars are clustered by wetland treatment; SN=wetlands embedded in semi-natural pastures, IM=wetlands embedded in intensively managed pastures, Fenced=ungrazed wetlands, Grazed=grazed wetlands, Unburn=unburned wetlands, and Burn=burned wetlands.

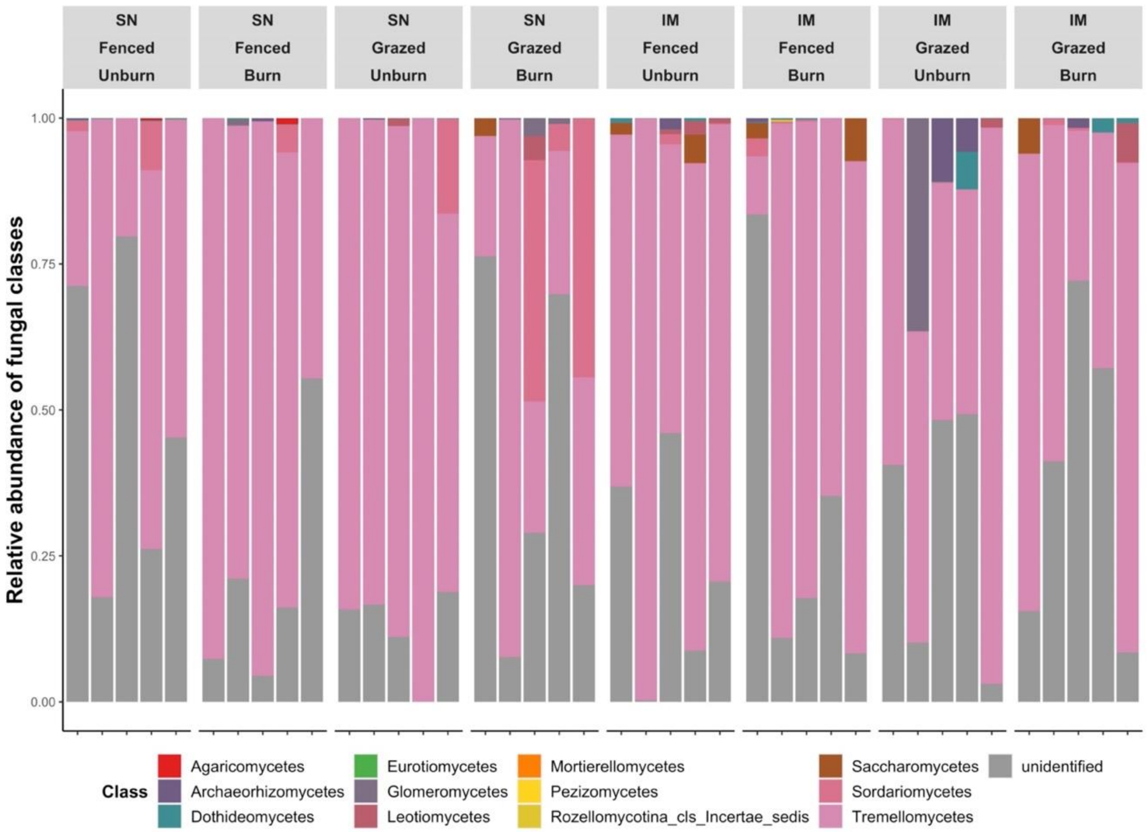

Figure C-5. Relative abundance of primary fungal classes in each wetland grouped by wetland treatments. Each bar represents one wetland and bars are clustered by wetland treatment; SN=wetlands embedded in semi-natural pastures, IM=wetlands embedded in intensively managed pastures, Fenced=ungrazed wetlands, Grazed=grazed wetlands, Unburn=unburned wetlands, and Burn=burned wetlands.

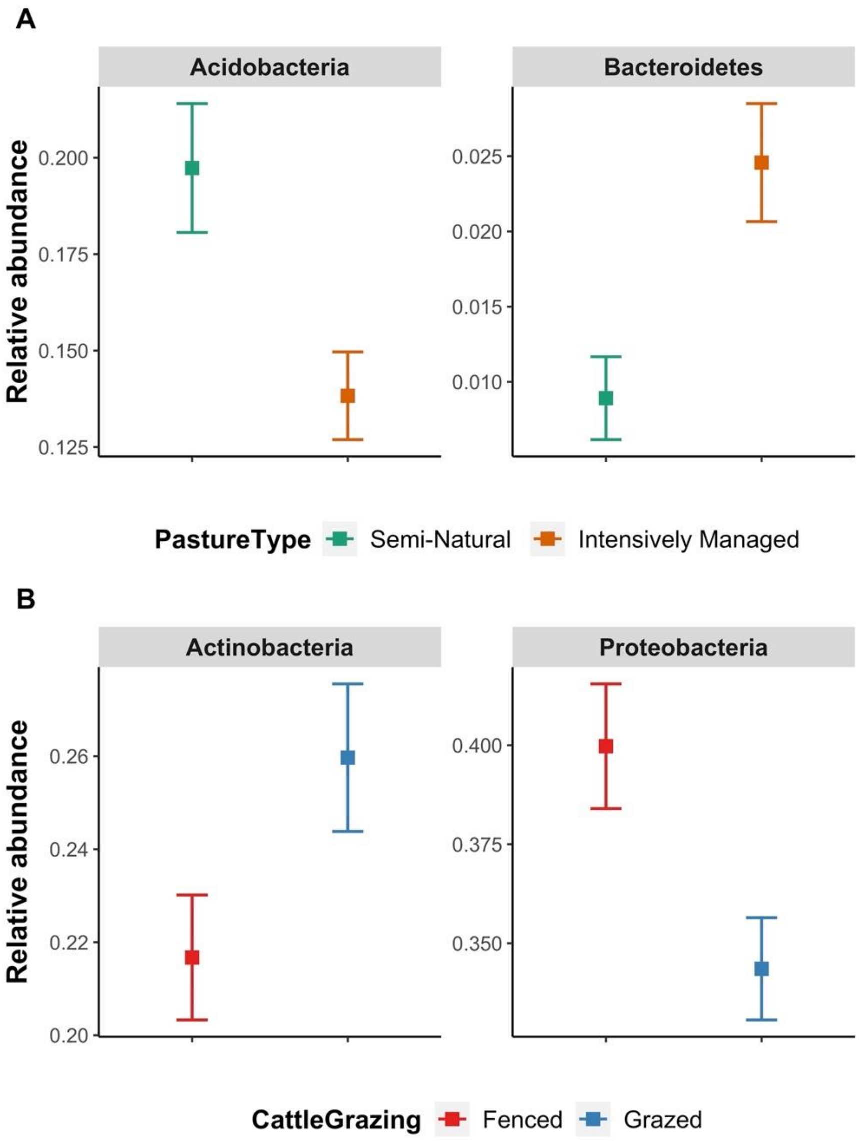

Figure C-6. Relative abundance of bacterial phyla that showed a significant response (as resulted from ANCOM test with W-values higher than 0.7) to the land-use intensity (A) or cattle grazing (B) treatments (mean ± SE; N=40).

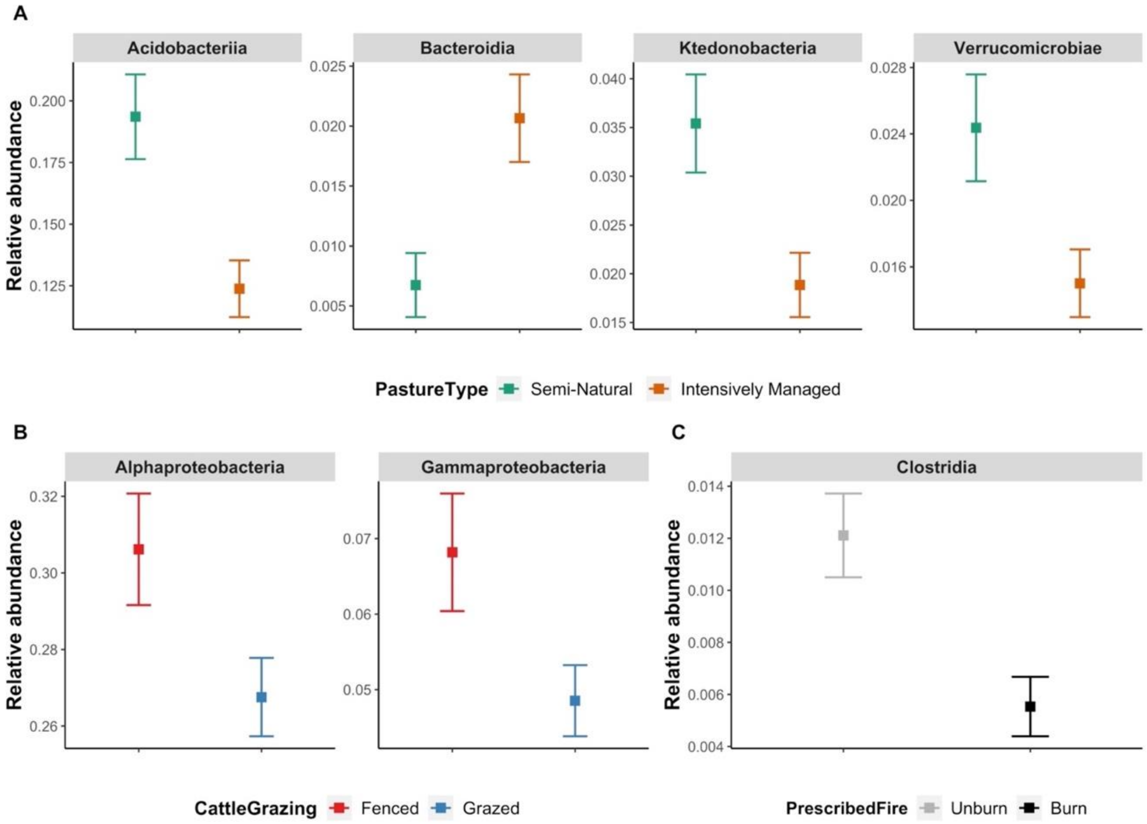

Figure C-7. Relative abundance of bacterial classes that showed a significant response (as resulted from ANCOM test with W-values higher than 0.7) to the land-use intensity (A) or cattle grazing (B) or prescribed fire (C) treatments (mean ± SE; N=40).

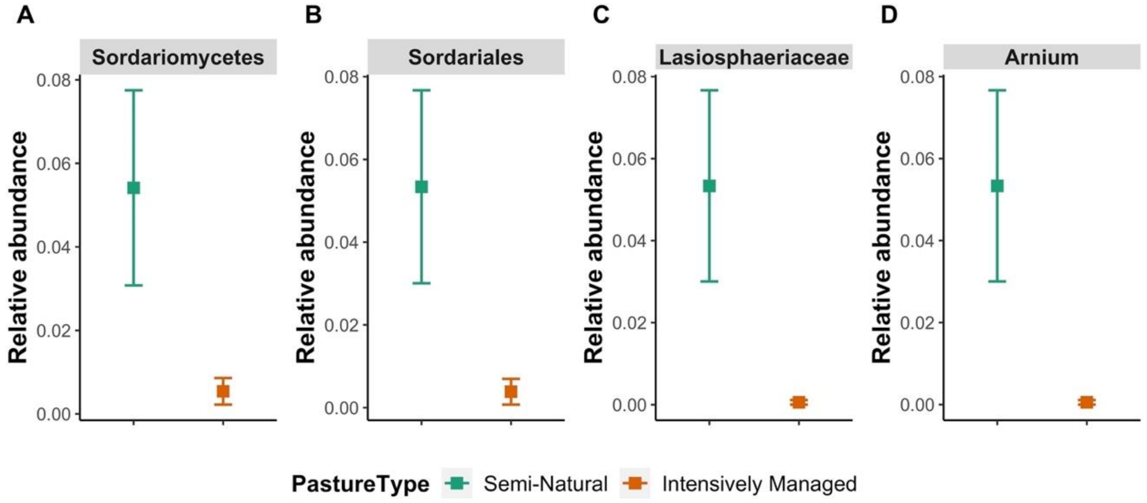

Figure C-8. Relative abundance of fungal taxa (A: class, B: order, C: family, D: genus) that showed a significant response (as resulted from ANCOM test with W-values higher than 0.7) to the land-use intensity treatment (mean ± SE; N=40).

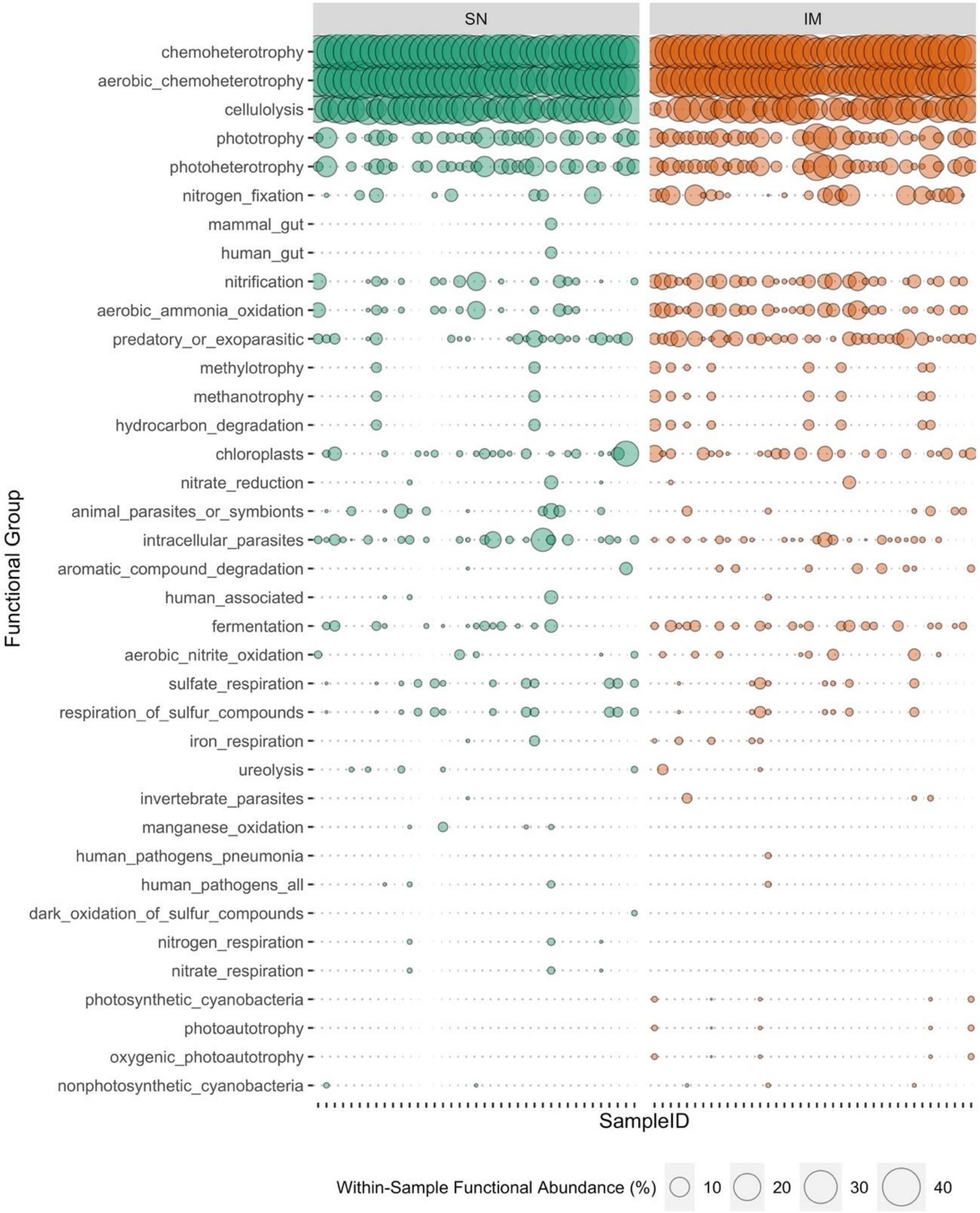

Figure C-9. Relative abundance of all detected bacterial functional groups between IM and SN wetlands.

APPENDIX D
SUPPLEMENTARY MATERIAL FOR WETLAND LITTERBAG DECOMPOSITION

Table D-1. Dominant plant species in each type of wetland and their trait value in average.

Wetland type	Plant species	C %	N %	P %	K %	Ca %	Mg %	lignin %	C/N	C/P	N/lignin
IM × fenced × burned	*Panicum hemitomon*	43.61	1.32	0.13	0.67	0.13	0.05	8.05	33.04	333.24	0.16
IM × fenced × unburned	*Panicum hemitomon*	42.29	1.29	0.06	0.29	0.11	0.06	8.93	32.73	755.04	0.14
	Hymenachne amplexicaulis	41.09	1.03	0.12	1.07	0.07	0.15	6.13	39.86	356.20	0.17
IM × grazed × burned	*Panicum hemitomon*	44.06	1.29	0.11	0.63	0.11	0.08	6.91	34.04	412.34	0.19
	Paspalum notatum	41.25	0.77	0.11	0.51	0.32	0.17	8.58	53.29	384.92	0.09
	Juncus effusus	45.30	0.78	0.06	0.78	0.16	0.08	10.56	58.15	779.44	0.07
IM × grazed × unburned	*Luziola fluitans*	43.15	1.67	0.09	0.44	0.28	0.14	8.74	25.85	469.12	0.19
	Juncus effusus	44.90	0.89	0.05	1.16	0.11	0.10	8.17	50.53	867.02	0.11
	Panicum hemitomon	44.22	1.09	0.06	0.71	0.10	0.11	8.36	40.63	693.62	0.13
SN × fenced × burned	*Panicum hemitomon*	44.12	1.16	0.05	0.28	0.20	0.16	8.62	38.00	936.44	0.13
	Rhynchospora inundata	43.71	0.54	0.06	0.19	0.38	0.12	10.10	81.70	713.58	0.05
SN × fenced × unburned	*Panicum hemitomon*	44.12	1.91	0.06	0.39	0.20	0.14	6.82	23.08	704.89	0.28
	Rhynchospora inundata	40.78	0.60	0.03	0.27	0.21	0.08	9.87	68.45	1421.82	0.06
	Amphicarpum muehlenbergianum	44.22	0.90	0.05	0.81	0.12	0.08	6.40	48.95	877.94	0.14
SN × grazed × burned	*Rhynchospora inundata*	41.98	0.84	0.04	0.38	0.13	0.03	9.61	49.70	953.11	0.09
	Panicum hemitomon	42.29	1.55	0.05	0.48	0.09	0.08	6.74	27.36	770.75	0.23
	Eleocharis vivipara	41.55	1.03	0.10	0.80	0.17	0.16	7.77	40.35	428.35	0.13
	Pontederia cordata	39.38	1.67	0.08	2.33	0.95	0.18	6.99	23.54	465.79	0.24
	Bacopa carodiniana	41.05	1.44	0.07	1.55	0.94	0.29	9.78	28.54	548.15	0.15
	Luziola fluitans	42.11	2.78	0.10	0.66	0.39	0.20	7.42	15.13	428.19	0.38
SN × grazed × unburned	*Panicum hemitomon*	44.12	0.65	0.02	0.29	0.10	0.05	6.88	67.96	1781.02	0.09
	Pontederia cordata	40.89	1.73	0.09	1.71	0.96	0.15	7.49	23.58	464.48	0.23
	Luziola fluitans	42.76	1.72	0.08	0.80	0.37	0.14	6.59	24.85	532.43	0.26
	Rhynchospora inundata	43.86	1.20	0.06	0.32	0.18	0.11	8.45	36.68	734.44	0.14

Table D-2. Abundance-weighted average litter traits of each wetland type.

Wetland type	C %	N %	P %	K %	Ca %	Mg %	lignin %	C/N	C/P	N/lignin
IM × fenced × burned	43.09	1.41	0.05	0.39	0.19	0.12	7.73	39.47	942.71	0.20
IM × fenced × unburned	44.06	1.07	0.05	0.26	0.23	0.16	8.84	44.55	903.01	0.12
IM × grazed × burned	42.91	1.29	0.06	0.79	0.40	0.11	7.27	40.13	940.21	0.18
IM × grazed × unburned	41.53	1.33	0.07	0.90	0.35	0.13	8.15	34.70	673.62	0.17
SN × fenced × burned	42.03	1.23	0.07	0.46	0.10	0.08	8.31	34.30	667.30	0.15
SN × fenced × unburned	43.61	1.32	0.13	0.67	0.13	0.05	8.05	33.04	333.24	0.16
SN × grazed × burned	44.00	1.26	0.07	0.75	0.18	0.12	8.46	37.74	657.06	0.15
SN × grazed × unburned	43.43	1.00	0.10	0.63	0.19	0.11	8.35	46.30	492.19	0.13

Table D-3. Multiple linear regression coefficients for litter traits.

Litter traits	Land-use intensification (L)	Grazing (G)	Fire (F)
C	0.38	–0.24	0.16
N	–0.07	–0.04	**–0.12****
Log(P)	**0.45*****	0.05	**0.26*****
Log(K)	**0.18***	**0.58*****	–0.01
Ca	**–0.14*****	**0.11*****	0.01
Log(Mg)	**–0.45*****	**0.25****	–0.05
Lignin	**0.27***	–0.15	**0.38****
C/N ratio	–1.85	1.86	1.76
C/P ratio	**–328.27*****	–19.97	**–202.14*****
N/lignin ratio	**–0.02****	0	**–0.02****

Notes. Coefficients represent differences relative to wetlands embedded in semi-natural pastures, and/or that are ungrazed and/or unburned. Bold values indicate significant coefficients. * indicates $p \leq 0.05$, ** indicates $p \leq 0.01$, *** indicates $p \leq 0.001$.

Table D-4. Model fixed-effects coefficients for soil physiochemical properties.

Soil properties	Land-use intensification (L)	Grazing (G)	Fire (F)	L × G	L × F	G × F	L × G × F
TC	0.18	0.17	0.10	–0.29	–0.11	–0.26	0.81
TN	0.12	0.13	0.09	–0.24	–0.11	–0.25	0.71
C/N ratio	**0.90*****	**0.46***	0.13	–0.68	–0.18	–0.01	1.22
Mehlich-3 P	**0.01***	–0.01	**0.01***	**0.02****	0	–0.02	0.04
Mehlich-3 K	**0.02***	**0.05****	0	–0.05	0	–0.03	**0.12***
Mehlich-3 Ca	**0.40*****	**–0.53***	–0.21	0.29	0.36	0.48	–0.53
Mehlich-3 Mg	**0.68*****	**0.05***	0	0.02	–0.16	0.28	0.16
OM	0.02	0.01	0	–0.03	–0.02	–0.02	0.13
pH	**0.23*****	–0.19	–0.16	0.38	0.30	0.39	**–0.80***
Water content	**0.46***	0.20	0.01	–0.66	–0.26	–0.18	**1.19***

Notes. Coefficients represent differences relative to wetlands embedded in semi-natural pastures, and/or that are ungrazed and/or unburned. Bold values indicate significant coefficients. * indicates $p \leq 0.05$, ** indicates $p \leq 0.01$, *** indicates $p \leq 0.001$.

Table D-5. Model fixed-effects coefficients for soil microbial attributes.

Microbial attributes	Land Intensification (L)	Grazing (G)	Fire (F)	L × G	L × F	G × F	L × G × F
Bacterial OTU richness	–10.8	–17.9	–7.2	17.5	3.1	17.4	–11.8
Bacterial OTU Shannon index	–0.16	–0.26	–0.21	0.23	0.11	0.31	–0.14
Bacterial OTU evenness	–0.009	–0.002	–0.009	–0.004	0.006	0.001	0.015
Fungal OTU richness	–0.60	–1.60	–1.59	1.39	1.50	**2.39***	**–2.31***
Fungal OTU Shannon index	–0.42	–0.84	–0.85	0.95	0.93	**1.32***	**–1.27***
Fungal OTU evenness	–0.12	–0.16	–0.19	0.24	0.27	0.28	–0.30
Bacterial functional richness	3.2	–1.3	–2.6	–1.4	–0.1	**4.6****	–1.6
Bacterial functional Shannon index	**0.16*****	–0.04	–0.06	–0.01	–0.01	0.16	–0.10
Bacterial functional evenness	0.02	0.02	0.03	–0.01	–0.02	–0.02	0.01
Fungal functional richness	**0.26***	–0.69	–0.69	0.19	0.56	1.06	–0.96
Fungal functional Shannon index	–0.03	–0.25	–0.34	0.17	0.25	0.35	–0.48
Fungal functional evenness	–0.01	–0.15	–0.28	0.21	0.13	0.23	–0.34
Chemoheterotrophs	–1.41	1.30	2.60	–0.82	–1.56	–4.85	3.54
Aerobic chemoheterotrophs	**–2.35***	1.11	2.55	–0.17	–1.56	–5.27	3.53
Cellulolytic bacteria	**–9.03*****	0.75	–3.22	3.10	5.36	2.13	–3.56
Phototrophs	**0.74***	0.35	–0.04	–0.23	–0.46	0.01	0.45
Photoheterotrophs	**0.74***	0.35	–0.04	–0.22	–0.46	0.01	0.42
Predatory bacteria	**0.46****	–0.28	0.28	0.45	0.06	0.20	–0.48

Notes. Coefficients represent differences relative to wetlands embedded in semi-natural pastures, and/or that are ungrazed and/or unburned. Bold values indicate significant coefficients. * indicates $p \leq 0.05$, ** indicates $p \leq 0.01$, *** indicates $p \leq 0.001$.

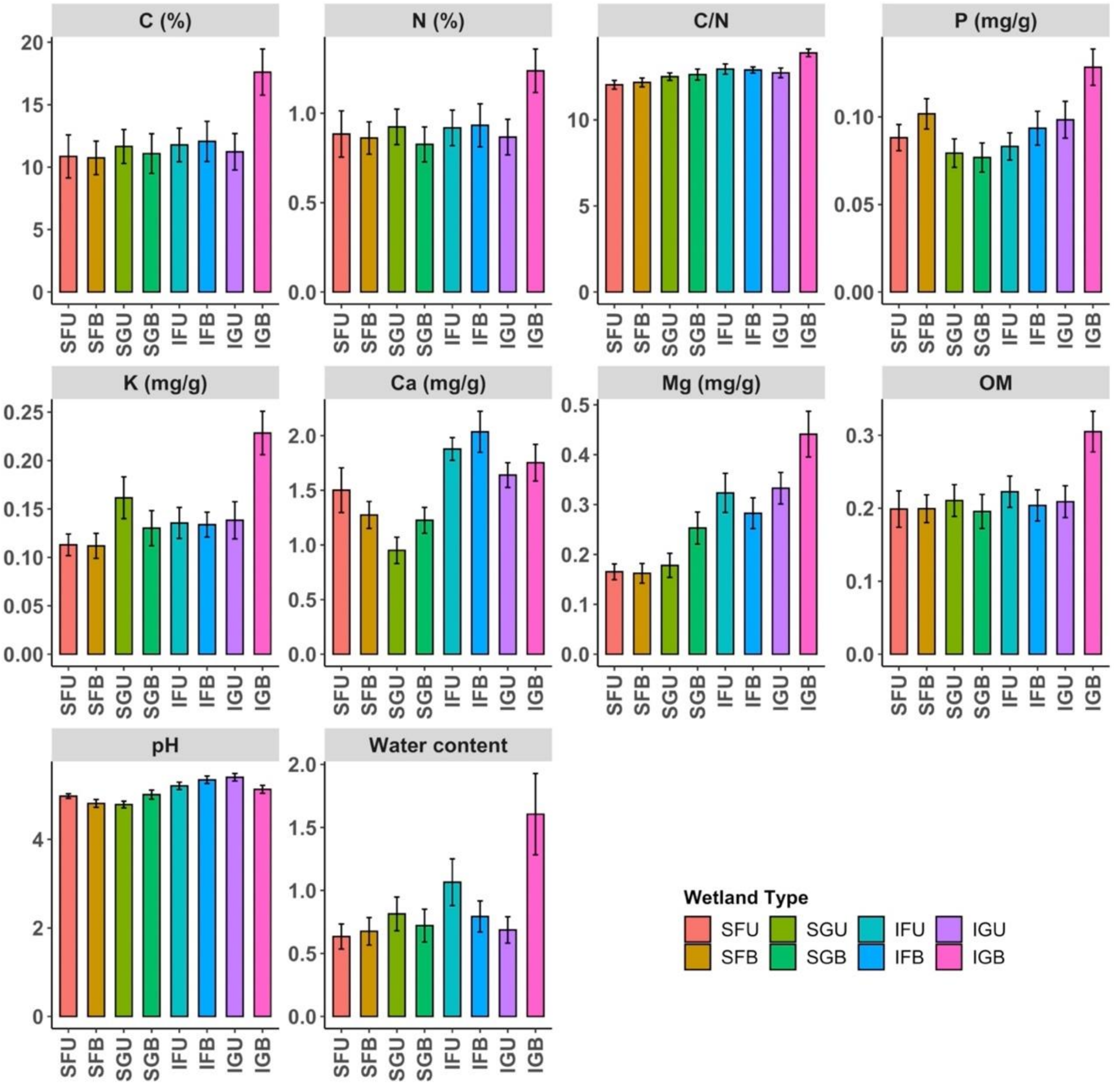

Figure D-1. Soil properties in each type of wetland. SFU refers to semi-natural, fenced, unburned wetlands; SFB refers to semi-natural, fenced, burned wetlands; SGU refers to semi-natural, grazed, unburned wetlands; SGB refers to semi-natural, grazed, burned wetlands; IFU refers to intensively managed, fenced, unburned wetlands; IFB refers to intensively managed, fenced, burned wetlands; IGU refers to intensively managed, grazed, unburned wetlands; IGB refers to intensively managed, grazed, burned wetlands.

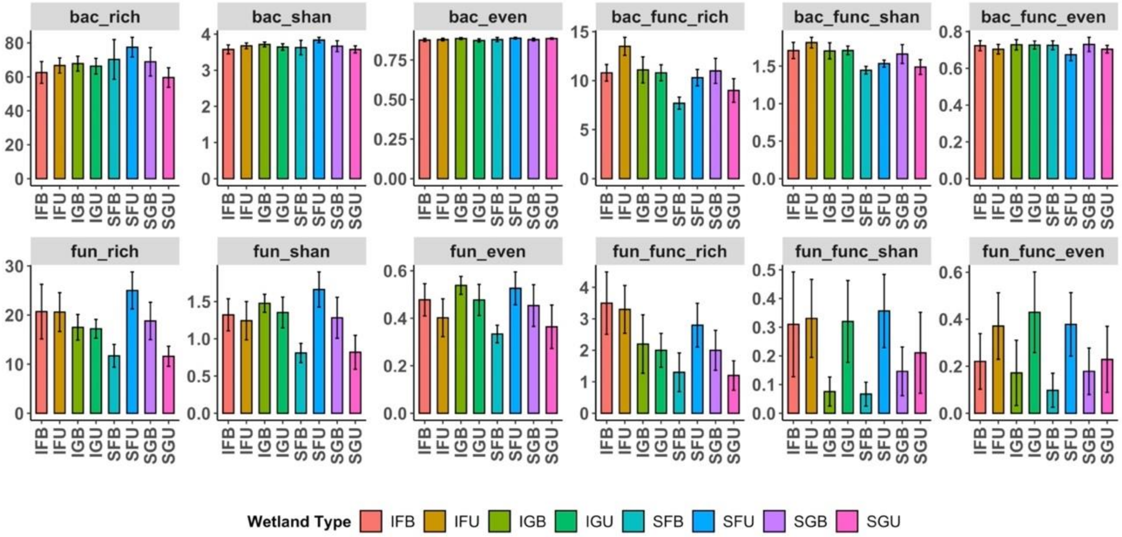

Figure D-2. Microbial alpha diversities in each type of wetland. Bac_rich refers to bacterial OTU richness; bac_shan refers to bacterial OTU Shannon diversity; bac_even refers to bacterial OTU evenness; bac_func_rich refers to bacterial functional group richness; bac_func_shan refers to bacterial functional group Shannon diversity; bac_func_even refers to bacterial functional group evenness; fun_rich refers to fungal OTU richness; fun_shan refers to fungal OTU Shannon diversity; fun_even refers to fungal OTU evenness; fun_func_rich refers to fungal functional group richness; fun_func_shan refers to fungal functional group Shannon diversity; fun_func_even refers to fungal functional group evenness.

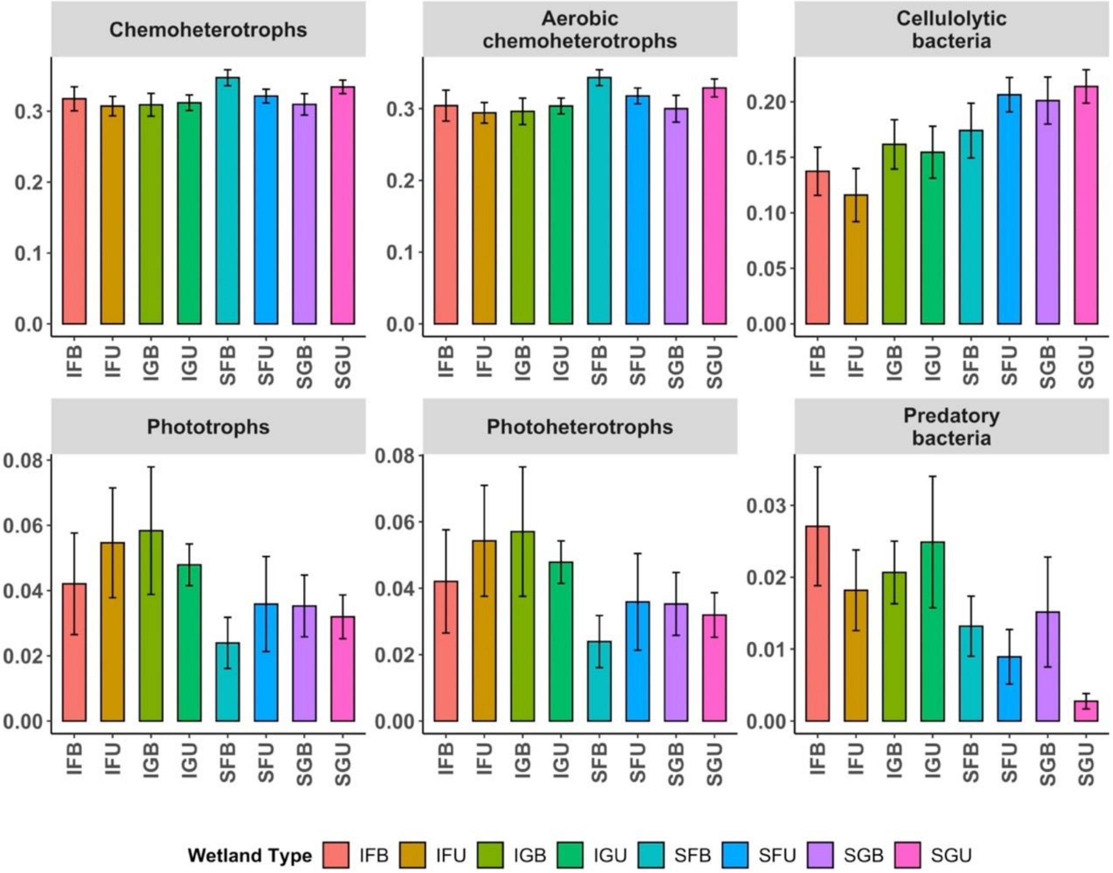

Figure D-3. Relative abundance of bacterial functional groups in each type of wetland. SFU refers to semi-natural, fenced, unburned wetlands; SFB refers to semi-natural, fenced, burned wetlands; SGU refers to semi-natural, grazed, unburned wetlands; SGB refers to semi-natural, grazed, burned wetlands; IFU refers to intensively managed, fenced, unburned wetlands; IFB refers to intensively managed, fenced, burned wetlands; IGU refers to intensively managed, grazed, unburned wetlands; IGB refers to intensively managed, grazed, burned wetlands.

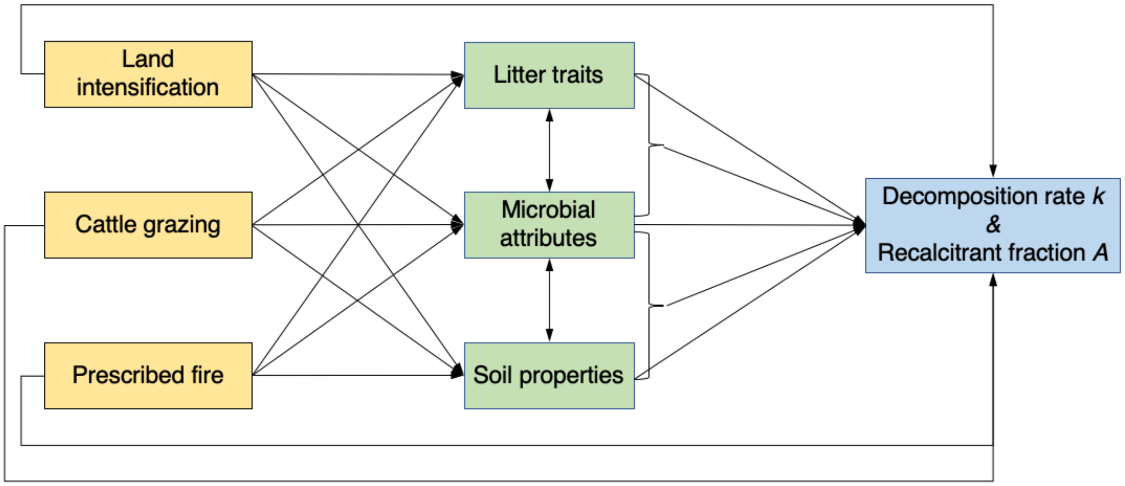

Figure D-4. Hypothesized path diagram for structural equation models for decomposition rate *k* and recalcitrant fraction *A*.

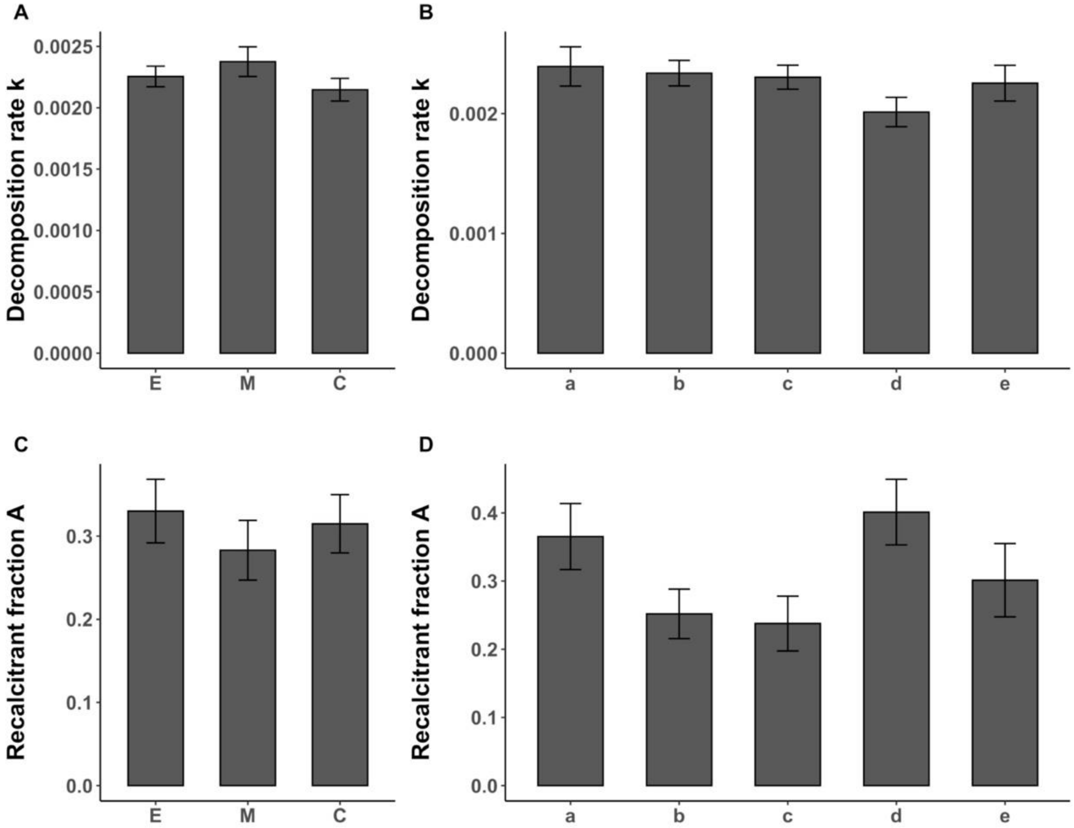

Figure D-5. Differences in litterbag decomposition rate k (A, B) and recalcitrant fraction Asym (C, D) across three wetland sampling sites (edge, middle and center) and five blocks (a, b, c, d, e). Error bars represent SEs.

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BIOGRAPHICAL SKETCH

Yuxi Guo was born and raised in Beijing, China. Yuxi completed her Bachelor of Science, majoring in Wildlife Conservation and Nature Reserve Management, at the Beijing Forestry University in 2015. Then she left home, came to United States, and joined Dr. Bohlman's lab conducting her master's research, which was about using hyperspectral data to estimate the coverage of an invasive grass and its ecological impacts. Yuxi earned her Master of Science degree at the School of Forest Resources and Conservation in the University of Florida in 2017. Later, Yuxi moved down from University of Florida's Gainesville campus to Fort Lauderdale Research and Education Center to join Dr. Qiu's lab in 2018. She conducted her doctoral research studying the responses of ecosystem functions and services to agricultural land management. Yuxi Guo received her Doctor of Philosophy degree in Forest Resources and Conservation in 2022.