2023-08-01

The Ecological Effects Of Nitrogen Enrichment In Aridlands

Jennifer Holguin
University of Texas at El Paso

Recommended Citation

Holguin, Jennifer, "The Ecological Effects Of Nitrogen Enrichment In Aridlands" (2023). *Open Access Theses & Dissertations*. 3914.
https://scholarworks.utep.edu/open_etd/3914

THE ECOLOGICAL EFFECTS OF NITROGEN ENRICHMENT IN ARIDLANDS

JENNIFER HOLGUIN

Doctoral Program in Ecology and Evolutionary Biology

APPROVED:

Jennie R. McLaren, Ph.D., Chair

Anthony J. Darrouzet-Nardi, Ph.D.

Marguerite E. Mauritz, Ph.D.

Sasha C. Reed, Ph.D.

Stephen L. Crites, Jr., Ph.D.
Dean of the Graduate School

DEDICATION

To Jennie and Miguel, thank you for always believing in me.

THE ECOLOGICAL EFFECTS OF NITROGEN ENRICHMENT IN ARIDLANDS

by

JENNIFER HOLGUIN, B.S.

DISSERTATION

Presented to the Faculty of the Graduate School of
The University of Texas at El Paso
in Partial Fulfillment
of the Requirements
for the Degree of

DOCTOR OF PHILOSOPHY

Department of Biological Sciences
THE UNIVERSITY OF TEXAS AT EL PASO
August 2023

ACKNOWLEDGMENTS

Funds for this research were provided by the United States Department of the Interior, the National Park Service Cooperative Agreement with the University of Texas at El Paso (UTEP) [Cooperative Agreement P17AC01014], the National Science Foundation: Drylands Critical Zone Network Grant [EAR-2012475], UTEP Graduate Excellence Fellowship, and the AAUW American Dissertation Fellowship. I want to thank my collaborators, Michael D. Bell, Ph.D., Emmi Felker-Quinn, Ph.D., Anthony J. Darrouzet-Nardi, Ph.D., Sasha C. Reed, Ph.D., and Marguerite Mauritz-Tozer Ph.D. who not only provided me with their invaluable insight but also were sources of inspiration. Special thank you to the army of undergraduates and peers who directly supported me and this research, especially Violeta Mendoza-Martinez, Carina Sanchez, Anna Maxwell, Mariana Lafon, Michael Bush, Diana Marquez, Cynthia Rae-Moreno, Kathleen Schaeffer, Daniela Aguirre, Catherine Cort, Kristina Young, Anna Pina, Andrew Uccello, Austin Roy, Dylan Stover, Sydne Spinella, and Miguel A. Bocanegra. Lastly, thank you to Scott L. Collins and Jennie R. McLaren for starting me on this journey.

ABSTRACT

Anthropogenic nitrogen (N) deposition is one of the most prominent factors driving global change. Across the globe, N deposition has driven major changes in terrestrial ecosystems, such as declines in plant biodiversity, enhanced exotic plant growth, and changes to biogeochemical processes involved in carbon and nutrient cycling. While noteworthy effort has been put forth to investigate the effects of N deposition on terrestrial ecosystems, a disproportionate number of N addition studies have been conducted in temperate mesic systems. Thus, we lack a holistic and mechanistic understanding of how N deposition impact aridland ecosystems. Additionally, our predictions of the effects of anthropogenic N deposition in more remote aridlands receiving elevated but low N deposition are constrained due to the relatively high abundance of aridland N addition studies adding N at high rates. To improve our understanding and predictive ability of the effects of N deposition in aridlands, here I $^{1.}$ review the literature examining the effects of N inputs on aridlands from microbial to ecosystem scales (Chapter 2), $^{2.}$ examine how multiple resources may limit soil microbial processes in a semi-arid northern Chihuauhan Desert grassland ecosystem (Chapter 3), $^{3.}$ assess the effects of simulated N deposition on plant communities, ecosystem properties, and biogeochemical processes in three semi-arid northern Chihuauhan Desert grassland sites (Chapter 4), and lastly, $^{4.}$ investigate how invasive exotic grass (*Eragrostis lehmanniana)* management (i.e., removal) and N inputs may interact to affect ecosystem structure and function, as well as exotic grass management in a semi-arid northern Chihuahuan Desert grassland dominated by *E. lehmanniana* (Chapter 5). Overall, this dissertation adds to the growing evidence revealing the complex nature of N deposition effects in aridland ecosystems, including context dependencies linked to water availability, multiple resource limitations, and experimental N dose.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1: THE ECOLOGICAL EFFECTS OF NITROGEN DEPOSITION IN TERRESTRIAL ECOSYSTEMS

BACKGROUND OF NITROGEN IN NATURE AND HUMAN INFLUENCE

Nitrogen (N) is a critical element of life, forming biological components such as nucleic acids and proteins. On Earth, N, in the form of dinitrogen gas (N_2), constitutes ~78% of Earth's atmosphere (by volume). However, despite its high abundance, N in N_2 is bonded by a triple covalent bond, making it highly stable and unavailable to ~99% of organisms (Galloway et al., 2003). In nature, N_2 can be transformed into reactive forms (i.e., biologically available N) of N via biotic or abiotic N fixation. However, biological N fixation, which is almost entirely mediated by bacteria and archaea known as diazotrophs, is rare and requires significant energy, specialized enzymes, and specific environmental conditions, such as low oxygen levels. Abiotic fixation via lightning also occurs; however, its importance to N fixation globally is low relative to biological N fixation (Chapin et al., 2002).

In the second half of the 20th century, human activities, most notably oil and gas and industrialized agricultural operations, have dramatically increased the amount of reactive N (Galloway et al., 2008). Oil and gas activities primarily produce inorganic oxidized forms of N, such as nitrogen oxides (NO_x), which include nitric oxide (NO) and nitrogen dioxide (NO_2). Agricultural activities, most notably the use of synthetic N fertilizers produced via the Haber Bosch process, produce reduced forms of N, such as ammonia (NH_3) and ammonium (NH_4^+). Anthropogenic reactive N production can result in the direct deposition of biologically available N to surrounding terrestrial or aquatic ecosystems via wet (e.g., rain, snow, and fog), dry (e.g., dust), or gaseous deposition. N deposition can result in a myriad of ecological consequences with cascading effects to higher trophic levels (Throop and Lerdau, 2004; Meunier et al., 2016; Stevens et al., 2018).

NITROGEN DEPOSITION EFFECTS ON TERRESTRIAL ECOSYSTEMS

Plant Communities

N is a key limiting resource to plant primary production in many terrestrial ecosystems (LeBauer and Treseder, 2008; Song et al., 2019). However, anthropogenic N deposition is a major threat to biodiversity worldwide (Phoenix et al., 2006). Across the globe, N enrichment has been shown to drive dramatic changes to plant communities, such as declines in plant biodiversity (Simkin et al., 2016; Payne et al., 2017; Borer and Stevens, 2022), reduced abundance of N-sensitive plants, such as legumes and non-vascular plants (Clark et al., 2019; Midolo et al., 2019; Borer and Stevens, 2022), and enhanced growth of exotic species (Jia et al., 2016; Guo et al., 2023).

N-induced changes to plant communities can be attributed to several factors. For instance, N enrichment can drive changes in competitive interactions between plants, which may favor nitrophilic plant species (Grime, 1973; Tilman, 1987). N-induced soil acidification (i.e., the decline in soil pH) can also result in base cation depletion, e.g., Ca^{2+}, Mg^{2+}, K^{+}, and $Na^{+,}$ and increased solubility of toxic soil metal ions, e.g., Al^{3+}, Fe^{3+}, and Mn^{2+} (Vitousk et al., 1997; Horswill et al., 2008; Tian and Niu, 2015). Though, N enrichment effects on plants can often vary widely due to differences in climate, plant functional types, soil characteristics, and study design, most notably N dose and duration (Midolo et al., 2019; Song et al., 2019).

Soil Microbial Community Structure and Function

Soil microorganisms play a critical role in ecosystem functioning, such as by mediating processes involved in C and nutrient cycling and forming mutualistic relationships that support plant growth. In a wide variety of ecosystems, N inputs often lead to changes in soil microbial community structure, such as declines in fungal-to-bacterial ratios (Zhou et al., 2017) and shifts from oligotrophic to copiotrophic groups (Ramirez et al., 2012). Common changes to microbial function are also observed as declines in soil microbial biomass and respiration (Liu and Greaver, 2010; Ramirez et al., 2010; Ramirez et al., 2012) and changes to extracellular enzyme activities, particularly enzymes involved in N and phosphorus (P) cycling, and those which degrade complex C compounds (Waldrop and Zak, 2006; Ramirez et al., 2012).

There are several direct and indirect ways in which N enrichment can drive changes to soil microbial community structure and function. For instance, N inputs may accentuate or alleviate soil microbial C limitation by reducing plant investment in belowground structures (Treseder, 2008) or enhancing aboveground productivity and plant litter inputs (Treseder, 2008; Liu and Greaver, 2010). Like plants, soil N-induced acidification can also constrain soil microbial growth due to base cation limitation and increased mobilization of toxic soil metals (Treseder, 2008). However, soil microbial response to N inputs can also vary due to differences across biomes with different climate and soil characteristics, as well as differences in experimental approaches, including N dose and duration (Yue et al., 2016; Zhou et al., 2017; Jia et al., 2020).

DISSERTATION: NITROGEN DEPOSITION EFFECTS ON ARIDLAND ECOSYSTEMS

Aridlands (arid and semi-arid ecosystems) are regions with an aridity index (i.e., the ratio of total annual precipitation to potential evapotranspiration; Sellers, 1964), which ranges from 0.03- 0.20 $\mathrm{mm\,mm^{-1}}$ for arid zones and 0.20-0.50 $\mathrm{mm\,mm^{-1}}$ for semi-arid zones (Steven, 2017). Aridlands cover 1/3rd of Earth's terrestrial surface (Plaza et al., 2018) and play a dominant role in the interannual variability of the global C-cycle (Poulter et al., 2014; Ahlström et al., 2015). While noteworthy effort has been put forth to investigate the possible effects of N deposition on terrestrial ecosystem structure and function, a disproportionate amount of N addition studies have been conducted in temperate mesic systems (Song et al., 2019). This focus on temperate mesic systems has left us with a relatively incomplete understanding of how N enrichment can affect water-limited ecosystems, such as arid and semi-arid ecosystems.

Aridland responses to N enrichment are likely to differ from their more extensively studied mesic counterparts. For instance, because water is the primary driver of biological activities in aridlands (Noy-Meir, 1973; Reynolds et al., 2004; Collins et al., 2014), N enrichment effects are likely to be more strongly associated with water availability in aridlands than in more mesic systems. Plant cover is also often discontinuous in aridlands; thus, N-induced changes to plant communities, such as increased competition for nutrients, space, and light (Borer et al., 2014; Cleland et al., 2019), may be less important in these ecosystems. Aridland soils also have markedly higher pH, lower cation exchange capacity, and lower soil organic C, total N, and organic P concentrations relative to more humid regions (Plaza et al., 2018). Therefore, beyond N, multiple biogenic resources, such as C and phosphorous (P), may be limiting for organisms in aridlands.

Beyond the differences between aridlands and more mesic ecosystems, predictions of the effects of anthropogenic N deposition on aridlands are constrained due to the relatively high abundance of aridland N addition studies that apply N at high rates. At the global scale, N addition doses play an important role in the magnitude and direction of response to N inputs, with stronger effects observed with increasing N doses (Zhou et al., 2017; Midolo et al., 2019). In aridlands, stronger responses to N inputs are often observed at high N addition rates (e.g., >50 kg N ha^{-1} yr^{-1} in Li et al., 2010; Zhang et al., 2013; Wang et al., 2020). In contrast, some aridland N addition applying N at relatively low N addition rates, such as those conducted on the Colorado Plateau, demonstrate few effects on plants and soil microbial community and function (e.g., 2, 5, and 8 kg N ha^{-1} yr^{-1}; McHugh et al., 2017; Philips et al., 2021; Osborne et al., 2022a). The Colorado Plateau and other more remote aridland regions are experiencing elevated but much lower levels of N deposition (e.g., 3 and 5 kg N ha^{-1} yr^{-1} in the Colorado Plateau; Philips et al., 2021), other aridland regions adjacent to large urban centers, e.g., Southern California (20- 45 kg N ha^{-1} yr^{-1}; Fenn et al., 2003). Aridlands are expected to be sensitive to even low levels of N deposition (Pardo et al., 2011; Sinsabaugh et al., 2015). Thus, increasing our knowledge of the effects of N deposition at low levels may be particularly relevant for remote aridland regions experiencing elevated but relatively lower anthropogenic N inputs.

DISSERTATION SUMMARIES

This dissertation aims to strengthen our understanding of the effects of N deposition in aridlands. This central objective is addressed across four chapters. The first chapter is a review of experimental N addition effects from microbial to ecosystem scales in aridlands. Chapters three to five contain new data from an N simulation experiment performed in grassland sites within Carlsbad Caverns National Park (CAVE) in southeast New Mexico, USA.

CAVE is in the northern Chihuauhan Desert (Fig. 1.1) and is classified as semi-arid. The mean annual temperature (MAT) at CAVE is 16.8 ºC, with mean annual precipitation (MAP) of 378.7 mm, most of which occurs (~70% of MAP) during the summer monsoon season (normally from July to September). Similar to other regions throughout the western U.S. (Fenn et al., 2003; Dix et al., 2020), CAVE has been experiencing elevated levels of N deposition (modeled total N deposition ranging from 3.5 to 4.5 kg N ha^{-1} yr^{-1}; Fig. 1.2), associated with oil and gas operations immediately adjacent to the park (Sullivan et al., 2016). CAVE is also experiencing invasion by exotic C_4 South African grass *Eragrostis lehmanniana*, which since its introduction in the 1930s, has spread across 1,470,000 acres of southwestern rangelands (Gori and Enquist, 2003). In 2018, we established three experimental sites in three CAVE grassland sites located within the lower modeled range of N deposition (Fig. 1.2). One of the three sites was also dominated by exotic grass *E. lehmanniana.*

Fig. 1.1. Study location. Carlsbad Caverns National Park, Carlsbad, New Mexico, USA, Northern Chihuahuan Desert.

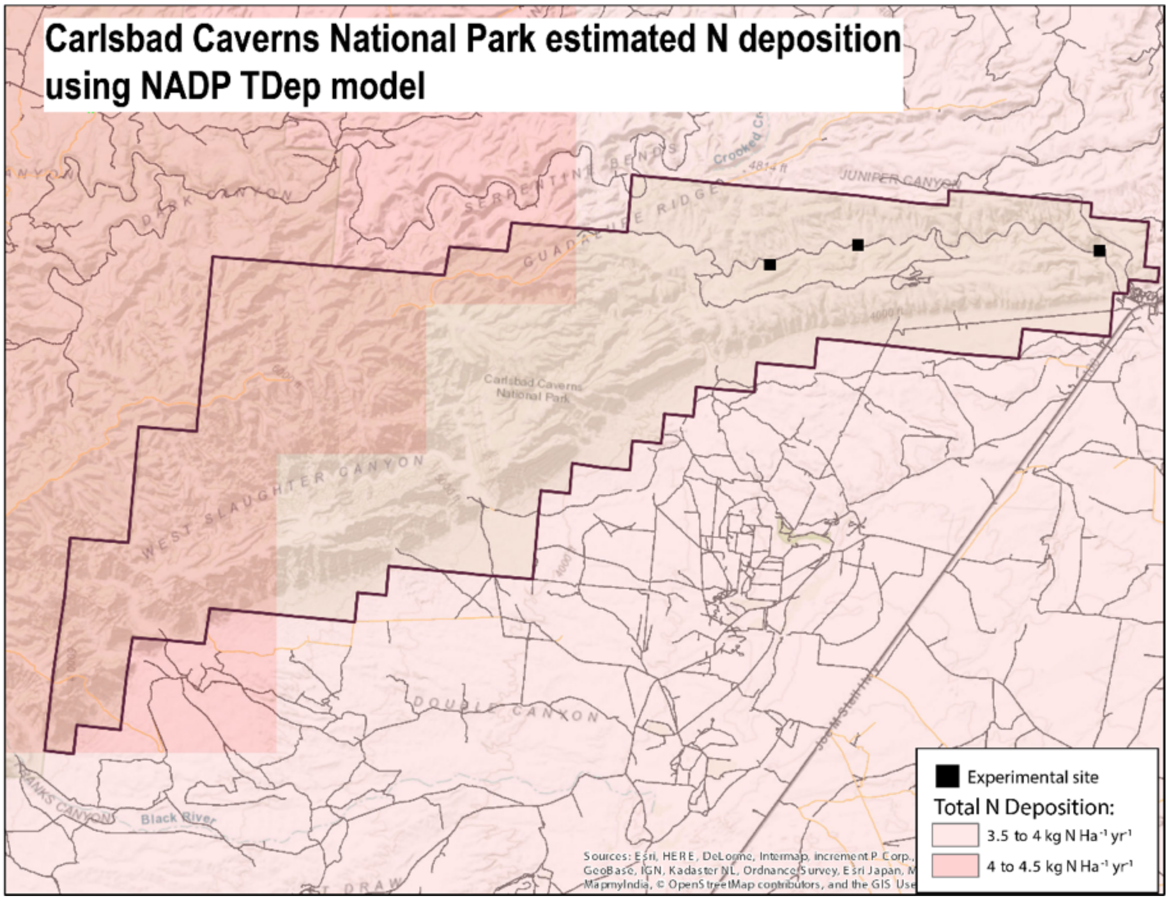

Fig. 1.2. Modeled nitrogen (N) deposition occurring at Carlsbad Caverns National Park, N.M. USA. National Atmospheric Deposition Program Total Deposition (NADP) Total deposition (TDep) estimates derived from summing wet and dry deposition. Figure courtesy of Michael D. Bell National Park Service Air Resources Division.

Chapter 2 is a review of N addition effects on aridland (arid and semi-arid) ecosystems that discusses aridland N addition studies from microbial to ecosystem scales. Our goal with this review is to $^{1.}$ provide general and contrasting responses to N additions in aridlands, $^{2.}$ identify important mediating factors related to N inputs that may be driving change, and $^{3.}$ discuss key limitations, knowledge gaps, and future research recommendations.

Chapter 3 is a factorial C, N, and P laboratory incubation experiment that tests if Chihuahuan Desert soil microbial biomass and function are individually or interactively limited by C, N, and P when given sufficient water.

Chapter 4 is a four-year N fertilization and water addition experiment conducted in three Chihuahuan Desert grassland sites modeled to be experiencing N deposition (~4 kg N ha^{-1}year^{-1}). We were interested in determining: $^{1.}$ if low but realistic N inputs (i.e., based on modeled N deposition; 0, 2, and 4 kg N ha^{-1} year^{-1}) impact aridland plant communities, soil ecosystem properties, and biogeochemical dynamics, and $^{2.}$ if effects of N depend on periods of above-average water availability.

Chapter 5 is a four-year exotic grass (*E. lehmanniana)* removal, recovery, and N addition field study in a Chihuauhan Desert grassland dominated by *E. lehmanniana*. We were interested in determining: $^{1.}$ How the presence and removal of *E. lehmanniana* impact plant community structure, soil ecosystem properties, and biogeochemical processes, and if effects persist when removals cease. 2. If N deposition intensifies the effects of *E. lehmanniana* and impedes *E. lehmanniana* removal efforts.

Chapter 6 provides a summary of our findings, conclusions, and future research directions for aridland N deposition research.

CHAPTER 2: NITROGEN ADDITION EFFECTS IN ARIDLAND ECOSYSTEMS: A REVIEW FROM MICROBIAL TO ECOSYSTEM SCALES

ABSTRACT

Anthropogenic nitrogen (N) deposition is a major driver of global change. However, aridlands (arid and semi-arid ecosystems) are among the least represented ecosystems in global change assessments, where responses can often vary due to climate and biome type differences. Changes to aridland ecosystem structure and function have important implications on biological and cultural diversity and global carbon and N biogeochemical cycling. Here, we provide an overview of microbial to ecosystem-scale responses to experimental N amendments in aridland ecosystems. Our main goals are to $^{1.}$ describe general and contrasting microbial to ecosystem-scale responses to N inputs in experimental aridland N addition studies, $^{2.}$ identify important mediating factors linked to change with experimental N inputs, and $^{3.}$ discuss fundamental limitations, knowledge gaps, and future research recommendations.

INTRODUCTION

Anthropogenic nitrogen (N) deposition is a major driver of global change (Phoenix et al., 2006; Bobbink et al., 2010; Stevens et al., 2018) and has been subject to numerous experiments and global meta-analyses (e.g., LeBauer and Treseder, 2008; Teseder, 2008; Song et al., 2019). At the global scale, N inputs can impact various ecosystem processes, e.g., by enhancing plant primary productivity (Song et al., 2019), reducing plant biodiversity (Payne et al., 2017), and altering soil microbial structure and function (Ramirez et al., 2012; Zhang et al., 2018; Wang et al., 2023). Experimental N dose and study duration are also important factors driving ecosystem response to experimental N inputs, with sensitivity often increasing with increasing experimental N dose and study duration (e.g., Midolo et al., 2019; Zhang et al., 2018; Song et al., 2019). However, responses to N inputs can often vary due to differences in biome type, climate, and edaphic properties (e.g., Midolo et al., 2019; Zhou et al., 2017; Borer and Stevens, 2022). Consequently, our predictions of the effects of N deposition in aridlands (arid and semi-arid ecosystems) are challenged, as aridlands are among the least represented ecosystems in global change assessments (Song et al., 2019; Xu et al., 2021). Aridland responses to N amendments have also often been shown to differ from responses observed in more mesic systems (e.g., LeBauer and Treseder, 2008; Zhou et al., 2017). Aridlands cover 1/3rd of Earth's terrestrial surface (Plaza et al., 2018), and changes to their structure and function have far-reaching consequences on biological and cultural diversity (Zhang et al., 2023a), and global carbon (C) and N cycling (Poulter et al., 2014; Ahlström et al., 2015; Eberwein et al., 2020).

Unlike in more mesic systems, water is the primary factor governing biological activities in aridlands, with rainfall and linked activities and resources commonly described as episodic in nature (Noy-Meir, 1973; Reynolds et al., 2004; Collins et al., 2008). The often limiting and variable dynamics of water in aridlands thus create conditions that may result in vastly different responses to N inputs than their more studied mesic counterparts. For instance, N-induced soil acidification is often cited as an important driver of change across ecosystems globally (Simkin et al., 2016; Song et al., 2019; Wang et al., 2023). However, in aridlands, soils are often high in pH due to the slow leaching of soluble salts (Plaza et al., 2018). Thus, N addition effects driven by soil acidification may be less important in aridlands than in more mesic systems. Additionally, unlike more mesic systems, low and highly variable water availability has resulted in soils that contain low concentrations of soil organic C, total N, and organic Phosphorus (P) (Plaza et al., 2018). Consequently, other critical biogenic resources, such as C and P, may be limiting in aridlands beyond N and water.

Global assessments provide critical insight into the effects of N deposition on ecosystem processes. However, aridlands possess unique environmental characteristics and conditions which may complicate predictions relative to more mesic systems. Additionally, while our understanding of the impacts of N inputs on aridland ecosystems is improving (see meta-analyses by Hooper et al., 1999; Yahdjian et al., 2011; Sinsabaugh et al., 2015), we still lack a holistic (e.g., below to aboveground effects) and mechanistic understanding of the potential impacts of N deposition in aridlands. Here we provide an overview of microbial to ecosystem-scale responses to experimental N amendments in aridland ecosystems. It is important to note that we did not intend to provide an exhaustive literature review. Therefore, we encourage readers to review meta-analyses of N addition effects on aboveground primary production across drylands (arid to subhumid regions) by Hooper and Johnson (1999) and Yahdjian et al. (2011), N addition effects on aridland soil microbial communities and function by Sinsabaugh et al. (2015), and a review of N addition effects in Mediterranean ecosystems by Ochoa-Hueso et al. (2011).

Our main goals are to:

1. Describe general and contrasting microbial to ecosystem-scale responses to N additions in experimental aridland N addition studies
2. Identify important mediating factors linked to change with experimental N inputs.
3. Discuss fundamental limitations, knowledge gaps, and future research recommendations.

COMMUNITIES: SOIL MICROBES

Soil microbes mediate processes that can drive ecosystem-scale responses to N inputs, such as C and nutrient cycling (Litchman et al., 2015; Zhang et al., 2018). N inputs can have a myriad of effects on soil microbial communities. For instance, across various ecosystems globally, N enrichment has shown to alter microbial community composition by reducing fungi-to-bacteria ratios (Zhou et al., 2017), reducing soil bacterial diversity (Wang et al., 2023), increasing the abundance of copiotrophic groups while decreasing oligotrophic groups (Ramirez et al., 2012), as well as enhancing (Zhou et al., 2017) but more commonly, inhibiting soil microbial biomass (Treseder, 2008; Ramirez et al., 2012). Microbial community and biomass changes can be attributed to several processes, such as shifts in competitive interactions between oligotrophic and copiotrophic groups (Ramirez et al., 2012), the direct or indirect alleviation of N or C limitation (Zhou et al., 2017), as well as negative effects associated with N-induced soil acidification (Treseder, 2008; Wang et al., 2023). However, soil microbial response to N amendments can vary due to differences in biome type and N fertilization rates (Zhou et al., 2017).

Overall, aridland N addition studies demonstrate similar changes to soil microbial community structure and biomass as in other global assessments (e.g., global assessments by Treseder, 2008, Ramirez et al., 2012, and Zhou et al., 2017). For instance, many aridland N addition studies report declines in fungal-to-bacterial ratios (e.g., Bi et al., 2012; Wei et al., 2013; Chen et al., 2015; Yang et al., 2017; Wang et al., 2020), declines in bacterial richness and diversity (e.g., Muller et al., 2015; Eberwein et al., 2020), enhanced relative abundance of copiotrophic groups and reduced abundance of oligotrophic groups (Ling et al., 2017), and soil microbial biomass declines (e.g., Zhu et al., 2016; Wang et al., 2020; Cui et al., 2021; Püspök et al., 2023). However, aridland N addition studies also report contrasting soil microbial responses to N inputs. For example, while soil fungi may be more sensitive to N enrichment than bacteria (i.e., by declining fungal/ bacterial ratios), in some aridlands studies, fungi appear insensitive to N amendments (e.g., Porras-Alfaro et al., 2011; Muller et al., 2015; Su et al., 2016). N inputs also do not always alter soil community structure or decrease soil microbial biomass in aridlands. For example, some aridland N addition studies show a lack of effect on soil microbial community structure (Sinsabaugh et al., 2015; Su et al., 2016; McHugh et al., 2017) and have shown to either increase (e.g., Zhang et al., 2014; Zhu et al., 2016 at lower N level only) or have no effect on soil microbial biomass (e.g., Zeglin et al., 2007; Osborne et al., 2022a).

Divergent microbial responses (e.g., positive vs. negative, weak or no effects vs. strong effects) to N amendments in aridlands may be primarily linked to N dose. For instance, in a meta-analysis of aridland N addition studies (N=14), N doses <70 kg ha^{-1} yr^{-1} and N loads <120 kg ha^{-1} exerted a positive effect on microbial biomass and metabolic rates, respectively, while greater N doses and loads negatively affected microbial biomass and metabolism (Sinsabaugh et al., 2015). Here, positive effects on soil may be linked to the release of N limitation (global meta-analysis by Chen et al., 2015; Zhou et al., 2017), while negative effects may be associated with the extent to which N inputs acidify soils (global meta-analysis by Wang et al., 2023). At the global scale, soil acidification intensifies as N doses increase (Song et al., 2019; Tian and Niu, 2015). Aridland soils are expected to be well-buffered against N-induced soil acidification due to their high pH (Bobbink et al., 2010). However, dryland soils also have markedly low cation exchange capacities (Plaza et al., 2018), which has been suggested to play a greater role in the ability to buffer N-induced soil acidification (global meta-analysis by Midolo et al., 2019). Here, we found that many aridland N addition studies reporting changes to soil microbial communities and biomass also report significant declines in soil pH (e.g., Li et al., 2010; Bi et al., 2012; Wei et al., 2013; Zhang et al., 2013; Wang et al., 2020). In contrast, studies reporting weak or non-significant effects of N on soil microbes also reported a lack of change to soil pH, usually at lower N doses (e.g., 2, 5, and 8 kg^{-1} N ha^{-1} yr^{-1} in McHugh et al., 2017 and Osborne et al., 2022a).

Beyond N, other factors that may explain conflicting responses between aridland N addition studies may be associated with limitations by other resources. For instance, growing evidence suggests that resources other than or in addition to N may limit soil microbial function in these ecosystems such as water and C (e.g., Schaeffer et al., 2003; Schaeffer and Evans, 2005;

Choi et al., 2022), and P (Cui et al., 2018). On the Colorado Plateau, while field N amendment studies demonstrated a lack of overall change to soil microbial community structure and function with N inputs (McHugh et al., 2017; Osborne et al., 2022a), a laboratory incubation experiment factorially adding C, N, and water to soils collected from the same study ecosystem in McHugh et al. (2017) and Osborne et al. (2022a) reported a greater net increase of microbial biomass with C and N added together, and evidence of serial limitation on soil C-cycling by water and C (Choi et al., 2022). Thus, multiple limiting resources (beyond N) may dictate how some aridland ecosystems respond to N inputs.

COMMUNITIES: BIOLOGICAL SOIL CRUST

Biocrust are soil surface-dwelling communities comprised of photosynthetic and heterotrophic organisms such as cyanobacteria, lichen, and mosses (Weber et al., 2022). Biocrusts occur in all drylands globally and play a significant role in ecosystem functioning, including C and N cycling (Darrouzet-Nardi et al., 2015; Barger et al., 2016; Weber et al., 2022). In aridland N addition studies, N inputs have been shown to alter biocrust composition (Wang et al., 2015; Rong et al., 2022) and reduce cover (Ochoa-Hueso et al., 2016; Benvenutto-Vargas and Ochoa-Hueso, 2020; Philips et al., 2021; She et al., 2022). However, biocrust response to N inputs can be highly variable due to differences in soil properties (particularly soil pH and soil organic C) (e.g., Wang et al., 2015; Ochoa-Hueso et al., 2016; Rong et al., 2022) successional stage and species (Ochoa-Hueso et al., 2016; Zhou et al., 2016; Baldarelli et al., 2021), aboveground plant cover (Ochoa-Hueso et al., 2016; Baldarelli et al., 2021; She et al., 2022), and interannual variation in precipitation (Ochoa-Hueso et al., 2016; Philips et al., 2021).

Additionally, while biocrust have been demonstrated to be sensitive to even low N inputs (e.g., Sinsabaugh et al., 2015; Philips et al., 2021), conflicting evidence from gradient N addition studies also suggest that biocrust may be relatively less sensitive to lower N inputs in some aridland ecosystems (e.g., Zhou et al., 2016; Benvenutto-Vargas and Ochoa-Hueso, 2020; Rong et al., 2022). The duration of the study may also influence biocrust response to N inputs. For instance, in a semi-arid Mediterranean ecosystem, two years of N inputs applied at a rate of 0, 10, 20, and 50 kg N ha^{-1} yr^{-1} resulted in no changes to foliose lichen *Cladonia foliacea* cover (Ochoa-Hueso and Manrique, 2011). Yet, after 10 years, *C. foliacea* cover markedly declined under the study's highest N addition treatment (50 kg N ha^{-1} yr^{-1}; Benvenutto-Vargas and Ochoa- Hueso, 2019), thus, highlighting the importance of long-term studies. Despite context dependencies, biocrust sensitivity to N inputs has important implications for an array of ecosystem functions, e.g., soil stability (Chaudhary et al., 2009) and soil C and nutrient availability (Ferrenberg et al., 2017; Young et al., 2022).

COMMUNITIES: PLANTS

In a wide variety of ecosystems, N inputs often drive changes in plant communities by reducing biodiversity (Bobbink et al., 2010; Payne et al., 2017; Borer and Stevens, 2022), species richness (Simkin et al., 2016; Clark et al., 2019; Stevens et al., 2022), and the abundance of N sensitive plant species, e.g., legumes and non-vascular plants (Midolo et al., 2019; Borer and Stevens, 2022). N enrichment generally alters plant communities by stimulating the growth of nitrophilic species, which can intensify the competitive interactions between plants for space, nutrients, and light (Borer and Stevens, 2022). N-induced soil acidification can also drive plant community changes by depleting base cations such as Ca^{2+}, Mg^{2+}, K^{+}, and Na^{+} and enhancing the solubility of toxic metals such as Al^{3+}, Fe^{3+}, and Mn^{2+} (Vitousk et al., 1997; Horswill et al., 2008; Tian and Niu, 2015). Although, the effects of N enrichment can often vary widely due to differences in climate, disturbance, and plant community composition (Midolo et al., 2019; Borer and Stevens, 2022).

In aridlands, experimental N inputs also often lead to declines in diversity (Mun and Whitford, 1989; Brooks, 2003; Nui et al., 2018; Wheeler et al., 2021) and species richness (Carpenter et al., 1991; Brooks, 2003; Allen et al., 2009; Zeng et al., 2010). Plant community changes with N inputs in these ecosystems are often linked to declines in perennial forbs (Carpenter et al., 1990) and legumes (Brooks, 2003; Baez et al., 2007; Zeng et al., 2010), enhanced foliar N concentrations (e.g., Schwinning et al., 2005; Hall et al., 2011; Reichmann et al., 2013; Yahdjian et al., 2014) as well as increases in the abundance of grasses, annual species (Mun and Whitford, 1989; Zeng et al., 2010; Nui et al., 2018; Wheeler et al., 2021), and nonnative species (Brooks, 2003; Schwinning et al., 2005; Allen et al., 2009; Rao and Allen, 2010; Vourlitis, 2017). Unsurprisingly, however, the effects of N on aridland plant communities regularly depend on water availability. For instance, in some experiments, aridland plant community responses have been only evident following periods of above-average precipitation (e.g., Brooks, 2003; Ludwig et al., 1989; Ladwig et al., 2012; Whitford and Steinberger, 2011; Wheeler et al., 2021) or following prolonged drought which increased the dieback of dominant species (e.g., native shrubs in Vourlitis, 2017).

Beyond water availability, the effects of N on plant communities may vary due to differences in experimental N dose. For instance, N addition effects on aridland plant communities are most evident under relatively high N fertilization doses (most studies apply N at a dose of >50 kg N ha^{-1} yr^{-1}). Plant communities change to simulated low but realistic rates of N deposition were not evident in either aridlands with either low background N deposition, such as the Colorado Plateau (N deposition ~3 kg N ha^{-1} yr^{-1}; Philips et al., 2021) or relatively high deposition regions such as Southern California (Allen et al., 2009) which can receive N deposition ranging from 20-45 kg N ha^{-1} yr^{-1} (Fenn et al., 2003). However, in a later study using the same experimental platform in Allen et al. (2009), effects on plant communities emerged with low N doses (5 kg N ha^{-1} yr^{-1}) with effects linked to a record wet year (Fenn et al., 2010). Thus, in addition to experimental N dose, it may also be essential to consider co-occurring background N deposition and water availability (e.g., above to below-average rainfall).

ECOSYSTEM PROCESSES: NET PRIMARY PRODUCTION

N inputs generally stimulate above-ground plant productivity (ANPP) across ecosystems globally (e.g., LeBauer and Treseder, 2008; Song et al., 2019; Feng et al., 2023). However, global analyses assessing the effects of N inputs on plant C sequestration focus on highly productive mesic ecosystems (Song et al., 2019). For instance, Elser et al. (2007) stated that N limitation in freshwater, marine, and terrestrial ecosystems is widely distributed. However, this analysis only included three sites below 650 mm mean annual precipitation, with no arid or desert ecosystems. In global meta-analysis by LeBauer and Treseder (2008) suggested primary production in desert ecosystems is insensitive to N relative to more mesic systems, but this analysis also included only three desert biome studies.

In dryland,specific (arid to sub-humid ecosystems) meta-analyses, aboveground net primary production (ANPP) has been suggested to be co-limited by water and N (Hooper and

Johnson, 1999) or increasingly sensitive to N across a precipitation gradient (Yahdjian et al., 2011). Later aridland N addition studies (not included in Hooper and Johnson, 1999 and Yahdjian et al., 2011) also suggest aridland plants are N limited with effects often depending on water availability (e.g., low vs. high water availability in Hall et al., 2011; Whitford and Steinberger, 2011; Ladwig et al., 2012; Nui et al., 2018; Ma et al., 2020). Climate differences (linked to differences in rainfall) have also been shown to result in contrasting responses to N. For instance, N has been shown to promote ANPP of the dominant perennial shrub *Larrea tridentata* in the Chihuahuan Desert (e.g., Ettershank et al., 1978; Lightfoot and Whitford, 1987; Fisher et al., 1988; Lajtha and Whitford, 1989) while exerting no significant effect on *L. tridentata* the Mojave and Sonoran Deserts, both of which receive less average summer rain than the Chihuahuan Desert (e.g., Romney et al., 1978; Sharifi et al., 1988; Barker et al., 2006; Hall et al., 2011). Beyond water, the effects of N inputs on aridland ANPP can also differ due to differences in plant functional types (e.g., shrubs vs. forbs in Hall et al., 2011; shrubs vs. grasses in Yahdjian et al., 2014; forbs vs. grasses in Niu et al., 2018), patch type (interspace vs. under shrub canopy in Hall et al., 2011), soil type (greater sensitivity of winter annuals growing in sandy Entisol vs. alluvium Aridisol soil in William and Bell, 1981), and N dose (e.g., greater sensitivity at higher N doses in Nui et al., 2018).

While ANPP is a commonly used metric to assess ecosystem response to global change factors such as N deposition (Song et al., 2019), below-ground net primary production (BNPP) represents a major component of NPP in stressful arid conditions where plant productivity tends to be allocated to roots for water and nutrient extraction (Sun et al., 2021). Under N enrichment, the optimal allocation theory would suggest that below-ground plant allocation should decline when belowground resources increase and plants become more limited by aboveground resources (e.g., light) (Gleeson and Tilman, 1992). Accordingly, root-to-shoot ratios generally decline across ecosystems with N amendments (Cleland et al., 2019; Song et al., 2019; Feng et al., 2023). However, while BNPP represents an important part of NPP in aridlands, BNPP has been suggested to be insensitive to N additions in aridlands (Cleland et al., 2019; Song et al., 2019). Though, as greater proportions of roots are found at deeper depths in aridlands, with 50- 95% deeper rooting depths than more humid ecosystems (Schenek and Jackson, 2002), it is possible that BNPP in aridlands is under-sampled (Cleland et al., 2019).

While data are limited relative to ANPP, aridland N addition studies show N inputs can have negative (Zeng et al., 2010), positive (Luo et al., 2017), no effect (e.g., Fisher et al., 1988; Ladwig et al., 2012), and even differential effects on BNPP, which result in both positive and negative effects across years (e.g., in Vourlitis, et al., 2021a). BNPP dynamics in aridlands are strongly linked with water availability (Bryne et al., 2013). Thus, conflicting BNPP response to N may be linked to the highly variable nature of water availability in aridlands. However, the scarcity of BNPP measurements in aridlands also further limits inferences of BNPP response to N in these ecosystems.

ECOSYSTEM PROCESSES: SOIL CARBON DYNAMICS

Understanding how N deposition impacts soil C pools is critical in developing accurate model predictions of C dynamics and feedbacks (Reay et al., 2008). Soil C dynamics can be influenced by various factors, such as plant growth and litter inputs, litter quality, soil C losses via soil respiration, and the export of dissolved organic C. N inputs can influence soil C in multiple ways. For instance, N inputs may enhance aboveground plant litter inputs and labile soil C inputs (Liu and Greaver, 2010), which can lead to greater soil C losses via heterotrophic respiration during decomposition. In contrast, N enrichment also often inhibits soil microbial biomass and heterotrophic respiration (LeBauer and Treseder, 2008; Ramirez et al., 2012; Xu et al., 2021), which may lead to greater soil C sequestration. The effects of N enrichment on soil C can vary due to climates, ecosystem types, as well as experimental duration (Liu and Greaver, 2010; Zhou et al., 2014; Yue et al., 2016). Additionally, while dryland soils collectively store an estimated 44% of global organic matter C pool in surface soils (top 30 cm) (Plaza et al., 2018; Hanan et al., 2021), dryland soil C response to N inputs is relatively underrepresented in global assessments (Xu et al., 2021)

Although some evidence suggests N deposition can reduce soil organic C (SOC) content in aridlands (extant N deposition gradient study Ochoa-Hueso et al., 2013), overall, experimental aridland N addition studies suggest SOC is insensitive to N inputs (e.g., Zeglin et al., 2007; Li et al., 2010; Hall et al., 2011; Wang et al., 2014; Sinsabaugh et al., 2014; Osborne et al., 2022a). Additionally, while soil microbial community composition and activities strongly influence soil C dynamics (Schimel et al., 2012), aridland N addition studies show no significant effects on soil C pools even with changes to soil microbial community structure (e.g., Ling et al., 2017), microbial biomass declines (e.g., Li et al., 2010; Bi et al., 2012; Wang et al., 2014; Su et al., 2016), declines in C acquisition enzyme activities (e.g., Sinsabaugh et al., 2015; Wang et al., 2020; Vourlitis et al., 2021b), or changes to microbial C utilization potentials (e.g., Bi et al., 2012; Sinsabaugh et al., 2015). Respiration in aridlands has also shown to be insensitive to N inputs (e.g., Schaeffer et al., 2003; Schaeffer and Evans, 2005; Li et al., 2010; Su et al., 2016; Choi et al., 2022).

At the global scale, the effect of N deposition on C storage is widely debated, with studies suggesting N deposition decreases, enhances, or does not impact C storage (Ramirez et al., 2012; Liu and Greaver, 2010; Yue et al., 2016). In aridlands, the overall lack of change to soil C may be due to the possibility of multiple resource limitations on soil C-cycling processes and differential effects on different SOC fractions. For instance, relative to N, C cycling activities have been shown to be more limited by water and C in some aridland ecosystems (Schaeffer and Evans, 2005; Choi et al., 2022). Different fractions of SOC, i.e., mineral-associated organic C (MAOC) and particulate organic C (POC), may also respond differently to N. For example, in a long-term N addition study conducted in three semi-arid Mediterranean ecosystems, N inputs did not alter POC but did reduce MAOC, with MAOC declines linked to acidification-induced calcium (Ca) losses (Püspök et al., 2023). Thus, overall, while N enrichment is not likely to increase aridland SOC storage, N inputs that result in soil acidification may lead to the destabilization of MAOC via N-induced acidification Ca losses (Püspök et al., 2023), though data are limited.

ECOSYSTEM PROCESSES: SOIL NITROGEN TRANSFORMATIONS AND LOSSES

N enrichment can alter critical N transformation processes, such as N_2 fixation, N mineralization, nitrification, and denitrification. For example, N inputs may inhibit N-cycling enzymes because of lowered N requirements (Craine et al., 2007) or through shifts in soil microbial communities (Ramirez et al., 2012). In contrast, N inputs may also increase N-cycling processes by enhancing substrate quality and quantity (Treseder, 2010).

In aridland N addition studies, N-cycling activities are often affected by N amendments in relatively consistent ways. For instance, aridland N addition studies report depressed N-fixation rates (Ochoa-Hueso et al., 2014), declines in N acquisition enzyme activities (e.g., Stursova et al., 2006; Wang et al., 2020; Vourlitis et al., 2021b), reduced ammonification rates (Ochoa-Hueso et al., 2013; Sinsabaugh et al., 2015), and enhanced nitrification rates (e.g., Vourlitis et al., 2009; Li et al., 2010; Hall et al., 2011; Ochoa-Hueso et al., 2013). Additionally, while N inputs can enhance net N mineralization rates (e.g., Sinsabuagh et al., 2015), increased nitrification rates do not necessarily always translate to changes in net N mineralization (e.g., Li et al., 2010; Hall et al., 2011; Ochoa-Hueso et al., 2013). In some cases, N-cycling responses to N amendments are conflicting among aridland N addition studies. For instance, in contrast to the studies mentioned above, some aridland N addition studies demonstrate no (Osborne et al., 2022a) or even positive effects on N acquisition activities (Sinsabaugh et al., 2015). N-cycling responses to N inputs can also vary spatially (e.g., bulk soil vs. biocrust in Sinsabaugh et al., 2015; interspace soil vs. under canopy soil in Hall et al., 2011).

The effects of N enrichment on aridland N-cycling activities have important implications on N balance in these already low N ecosystems. In particular, increased N availability may stimulate biotic (e.g., nitrification and denitrification) and abiotic processes (e.g., NH_3 volatilization and chemodentification), which emit gaseous N (e.g., NO, N_2O). N gas emissions are considered a dominant pathway of N loss in drylands (Peterjohn and Schlesinger, 1990; Homyak et al., 2016). And a growing number of aridland N addition studies report enhanced gaseous N efflux, most notably NO and NH_3, following N amendments (e.g., Peterjohn and Schlesinger, 1990; Hartley and Schlesinger, 2000; Schaeffer and Evans, 2005; McHugh et al., 2017; Krichels et al., 2022; Chapter 4).

Rapid gaseous N emissions may particularly be important in aridland N addition studies that report no change with added N. For instance, on Colorado Plateau, bacterial, fungal, and plant communities exhibited few responses to simulated N deposition (McHugh et al., 2017; Philips et al., 2021). However, in the same study ecosystem, N inputs enhanced NO emissions, and while inorganic N concentrations were elevated immediately following fertilization, treatment differences diminished one month after fertilization (McHugh et al., 2017). However, some aridland N addition studies also report no effect of N on gaseous N emissions (e.g., Stursova et al., 2006; Osborne et al., 2022a). Though, N gas efflux activities can highly depend on the timing of measurement (e.g., N_2O efflux can be highly ephemeral, Schaeffer et al., 2003; Krichels et al., 2022) and can vary due to differences in site and soil-microsite factors, e.g., soil texture, cover type, moisture, temperature, pH, and substrate supply (Schlesinger and Peterjohn, 1991; Schaeffer et al., 2003; Homyak et al., 2016; Eberwin et al., 2020). In addition to gaseous soil N losses, N leaching may also represent an important mechanism of N loss in aridlands (Reichmann et al., 2013; McHugh et al., 2017; Osborne et al., 2022a), with gaseous N emissions and N leaching having important negative implications on air and water quality (Smith et al., 2013).

CONCLUSIONS AND FUTURE RESEARCH DIRECTIONS

Overall, aridlands demonstrate a wide range of responses to experimental N inputs (Table 2.1). In general, N enrichment can drive considerable change to aridland structure and function, e.g., by reducing soil microbial and plant diversity and altering microbial, biocrust, and plant community composition and function. Aridland N enrichment can also accelerate soil N transformation processes, e.g., by enhancing N cycling activities that emit nitrogenous gases. However, the magnitude and often the direction of the effects of N inputs on most processes vary due to multiple factors, such as differences in organismal functional groups (e.g., bacteria vs. fungi, early vs. late successional biocrust, shrubs vs. grasses), environmental factors most notably water availability, particularly during periods of drought and above average precipitation, as well as spatial heterogeneity (e.g., microsite soil interspace vs. biocrust or canopy). Experimental differences, most notably N dose, which is likely linked to the presence or absence of soil acidification, also plays a considerable role in aridland ecosystem response to N enrichment.

Table 2.1. List of general soil to ecosystem-scale responses to N inputs in aridlands: (0) and grey = no effect, (+) and blue = positive effect, (-) and red = negative effect, (+/-/0) and orange = mixed effects, summary of mediating factors, and key references.

Response category	Response variable	General N effect (direction)	Important mediating factors	Relevant literature
Communities: Microbes	Fungal to Bacterial ratio	–	• Declines often linked to declines in soil pH • Bacteria sometimes appear more sensitive than fungi • Few effects at low N doses (<20 kg N ha^{-1} year^{-1}, <2 x per year),	Bi et al., 2012; Wei et al., 2013; Chen et al., 2015; Sinsabaugh et al., 2015; Su et al., 2016; Yang et al., 2017; McHugh et al., 2017; Wang et al., 2020; Porras-Alfaro et al., 2011; Muller et al., 2015; Su et al., 2016
	Diversity	–	• Declines linked to soil acidification and heightened competition between copiotrophic groups and oligotrophic groups. • Bacteria often appear more sensitive than fungi • Few effects at low N doses (<20 kg N ha^{-1} year^{-1}, <2 x per year)	Muller et al., 2015; Sinsabaugh et al., 2015; McHugh et al., 2017; Eberwein et al., 2020
	Biomass	–	• Declines linked to soil acidification, usually at higher N doses (>20 kg N ha^{-1} year^{-1}). • Positive effects were sometimes observed at lower N doses (<20 kg N ha^{-1} year^{-1}), suggesting N limitation. • No effects observed at lower N doses (<20 kg N ha^{-1} year^{-1})	Zhang et al., 2014; Zhu et al., 2016; Wang et al., 2020; Cui et al., 2021; Osborne et al., 2022a; Püspök et al., 2023 Schaeffer et al., 2003; Schaeffer and Evans, 2005; Choi et al., 2022
Communities: Biocrust	Community composition	–	• Changes can vary due to differences in soil properties (e.g., soil pH and soil organic C), biocrust successional stage and species, and study duration.	Wang et al., 2015; Rong et al., 2022
	Cover	–	• Responses can vary due to differences in soil properties (particularly soil pH and soil organic C), biocrust successional stage, species, aboveground plant cover, precipitation, and study duration.	Ochoa-Hueso et al., 2016; Benvenutto-Vargas and Ochoa- Hueso, 2020; Philips et al., 2021; She et al., 2022
Communities: Plants	Diversity	–	• Declines linked to increased abundance of grasses, annual, or alien species. • Effects often depend on water availability. • Most studies reporting significant effects apply N at high doses (>50 kg N ha^{-1} year^{-1}). • No effects at lower N doses (<20 kg N ha^{-1} year^{-1}).	Mun and Whitford, 1989; Ludwig et al., 1989; Carpenter et al., 1991; Brooks, 2003; Allen et al., 2009; Zeng et al., 2010; Philips et al., 2019;Wheeler et al., 2021
	Functional group (perennial forbs and legumes)	–	• Declines linked to increased abundance of grasses, annual, or alien species. • Effects often depend on water availability. • Most studies observing effects apply N at high doses (>50 kg N ha^{-1} year^{-1}). • No effects at lower N doses (<20 kg N ha^{-1} year^{-1}).	Brooks, 2003; Baez et al., 2007; Zeng et al., 2010; Whitford and Steinberger, 2011; Ladwig et al., 2012; Philips et al., 2019; Wheeler et al., 2021
	Functional group (grasses and annuals)	+	• Effects often depend on water availability. • Most studies observing effects apply N at high doses (>50 kg N ha^{-1} year^{-1}). • No effects at lower N doses (<20 kg N ha^{-1} year^{-1}).	Baez et al., 2007; Zeng et al., 2010; Whitford and Steinberger, 2011; Ladwig et al., 2012; Philips et al., 2019; Wheeler et al., 2021
	Non-native species	+	• Effects often depend on water availability. • Most studies observing effects apply N at high doses (>50 kg N ha^{-1} year^{-1})	Brooks, 2003; Schwinning et al., 2003; Allen et al., 2009; Rao and Allen, 2010; Vourlitis, 2017
	Foliar N	+	• Can vary seasonally and between functional groups (e.g., grasses can be more sensitive than shrubs) • Plant N uptake increases with increasing water availability • Does not increase at low N doses (<20 kg N ha^{-1} year^{-1}).	Schwinning et al., 2005; Zeng et al., 2010; Hall et al., 2011; Reichmann et al., 2013; Yahdijan et al., 2014; Sinsabaugh et al., 2015; Cui et al., 2021; Osborne et al., 2022a

Table 2.1. Continued. List of general soil to ecosystem-scale responses to N inputs in aridlands: (0) and grey = no effect, (+) and blue = positive effect, (-) and red = negative effect, (+/-/0) and orange = mixed effects, summary of mediating factors, and key references.

Response category	Response variable	General N effect (direction)	Important mediating factors	Relevant literature
Ecosystem Processes: Primary Production	ANPP	+	• Effects often depend on water availability. • Responses may differ due to climatic differences, plant functional types, and soil type. • Most studies apply N at high doses (>50 kg N ha^{-1} year^{-1}).	Ettershank et al. 1978; Romney et al., 1978; Lightfoot and Whitford, 1987; Fisher et al., 1988; Sharifi et al., 1988; Lajtha and Whitford, 1989; Barker et al., 2006; Baez et al., 2007; Allen et al., 2009; Zeng et al., 2010; Hall et al., 2011; Luo et al., 2011; Whitford and Steinberger, 2011; Ladwig et al., 2012; Yahdjian et al., 2014; Niu et al., 2018; Zhou et al., 2018; Ma et al., 2020
	BNPP	+/ – /0	• Differential responses of BNPP to N amendments in aridlands, as well as the scarcity of BNPP measurements overall, may complicate BNPP response predictions to N in these ecosystems.	Fisher et al., 1989; Zeng et al., 2010; Ladwig et al., 2012; Verburg et al., 2013; Luo et al., 2017; Vourlitis, et al., 2021a
Ecosystem Processes: Soil C Stocks and Cycling	SOC	0	• SOC is overall insensitive to N amendments, regardless of changes in pH, soil microbial biomass and function, N dose, water availability, and study duration (though few studies over +10 years). • Few studies demonstrated positive effects on SOC • There is limited evidence suggesting reductions in mineral-associated OC, linked to acidification linked calcium losses	Li et al., 2010; Zeng et al., 2010; Bi et al., 2012; Wang et al., 2014; Sinsabaugh et al., 2015; Wang et al., 2020; Vourlitis et al., 2021b; Osborne et al., 2022a; Püspök et al., 2023
	EOC	0	• There were few positive effects on EOC • Most studies showed insensitivity of EOC to N inputs regardless of changes in pH and microbial biomass and function, N dose, and water availability, and study duration (though few studies over +10 years).	Li et al., 2010; Wei et al., 2013; Wang et al., 2014; Su et al., 2016; Osborne et al., 2022a
	C- cycling enzymes	+/ – /0	• Evidence of enhanced, depressed, or no effects of N on hydrolytic C- cycling activities. • Changes linked to soil acidification and microbial biomass declines • Oxidative enzyme activities were generally insensitive to N inputs	Stursova et al., 2006; Zeglin et al., 2007; Ochoa-Hueso et al., 2014; Wang et al., 2014; Wang et al., 2020; Osborne et al., 2021
	Respiration	0	• Respiration was generally insensitive to N inputs • Some studies demonstrate serial water and C limitation	Schaeffer et al., 2003; Schaeffer and Evans, 2005; Li et al., 2010; Su et al., 2016; Zhu et al., 2016; Choi et al., 2022
Ecosystem Processes: Soil N Transformations and Losses	N- fixation	–	• Down-regulation is likely due to N availability that exceeds demand.	Ochoa- Hueso et al., 2014
	N-mineralization	0	• Changes to ammonification or nitrification rates generally do not translate to changes in net N mineralization. • N inputs can enhance net N mineralization rates, but the magnitude can vary by N dose (greater sensitivity with greater N dose) and soil depth (insensitivity at deeper depths).	Li et al., 2010; Hall et al., 2011; Ochoa-Hueso et al., 2013
	Ammonification	–	• In general, ammonification declines but some evidence for enhanced ammonification rates. • Linked to soil pH and inorganic N concentrations.	Ochoa-Hueso et al., 2013; Sinsabaugh et al., 2015
	Nitrification	+	• In general, nitrification increases, but some evidence for depressed nitrification rates • Linked to soil pH and inorganic N concentrations.	Vourlitis et al., 2009; Li et al., 2010; Hall et al., 2011; Ochoa-Hueso et al., 2013
	N-cycling enzymes	–	• N acquisition enzyme activities generally decline with N • Sensitivity can vary spatially (e.g., bulk soil vs. biocrust) • Low N dose studies report insensitivity of N-cycling activities at lower N doses (<20 kg N ha^{-1} year^{-1}).	Stursova et al., 2006; Wang et al., 2020; Vourlitis et al., 2021b; Osborne et al., 2022a
	N gas emissions	+	• N gases (particularly nitric oxide and ammonia) emissions increase following N fertilization. • N gas efflux can highly depend on the timing of measurement and can vary due to differences in site and soil-microsite factors, e.g., soil texture, cover type, moisture, temperature, pH, and substrate supply	Schlesinger and Peterjohn,1991; Hartley and Schlesinger, 2000; Schaeffer and Evans, 2005; Stursova et al., 2006; McHugh et al., 2017; Osborne et al., 2022a; Krichels et al., 2022
	N Leaching	+	• Leaching increases with increasing N inputs but occurs even at low N doses (<20 kg N ha^{-1} year^{-1}). • Leaching can increase during drought, i.e., when plant N uptake is likely low.	Reichmann et al., 2013; McHugh et al., 2017; Osborne et al., 2022a

In our synthesis, large knowledge gaps represent possible directions for future research. First, water availability in aridlands is often highly spatially and temporally variable, and aridlands across the globe are expected to experience increasingly variable precipitation patterns, enhanced aridity, and more frequent, severe, and prolonged droughts (Cook et al., 2015; Bradford et al., 2020). Here, we found that the effects of N often depend on water availability (e.g., Brooks, 2003; Ludwig et al., 1989; Ladwig et al., 2012; Whitford and Steinberger, 2011; Wheeler et al., 2021). Thus, we suggest future studies to either increase the duration of their experiment, which would make it more likely to capture year-to-year variation in precipitation (e.g., long-term N addition study in Ladwig et al., 2012), or include precipitation manipulation treatments that simulate predicted changes in precipitation and temperature in their study region (e.g., extreme drought and N addition manipulation study in Zhang et al., 2023b). Second, we found that resources other than N may constrain responses to N enrichment (e.g., water and C; Schaeffer et al., 2003; Schaeffer and Evans, 2005; Choi et al., 2022). Resource availability and dynamics can provide critical insight into how biogeochemical cycles respond to global change (Reed et al., 2015). Thus, given that aridlands are notably poor in organic C, N, and P resources (Plaza et al., 2018), assessing the possibility of multiple resource limitations (beyond N) may be important when assessing aridland vulnerability to N deposition (e.g., water, C, N, and P, limitation assessment in Choi et al., 2022). Third, most aridland N addition studies in this synthesis applied N at relatively high N doses (>50 kg N ha^{-1} yr^{-1}), thus challenging our predictions on the effects at relatively lower N deposition rates. Here, we suggest more studies that explore aridland ecosystem response to low but realistic N inputs (e.g., N added according to reported N deposition estimates in Osborne et al., 2022a) or more studies which derive information from extant N deposition gradients (i.e., low to high N deposition, e.g., Ochoa-Hueso et al., 2013). Last, as N inputs can accelerate N gas loss processes (e.g., Peterjohn and Schlesinger, 1990; Hartley and Schlesinger, 2000; Schaeffer and Evans, 2005; McHugh et al., 2017; Krichels et al., 2022), we encourage future aridland N addition studies to also include N gas efflux assessments (e.g., NO, NH_3, N_2O) as they may offer critical insight into N retention and loss under elevated N deposition in these ecosystems.

ACKNOWLEDGMENTS

We want to acknowledge the critical role Anthony J. Darrouzet-Nardi and Sasha C. Reed played in the conception of this review. Funds for this research were provided by the United States Department of the Interior, the National Park Service Cooperative Agreement with the University of Texas at El Paso [Cooperative Agreement P17AC01014], and the National Science Foundation: Drylands Critical Zone Network Grant [EAR-2012475].

AUTHOR CONTRIBUTIONS

This chapter will be submitted for review in a peer-reviewed journal and is co-authored by Jennifer Holguin (JH) and Jennie R. McLaren (JRM). JH and JRM contributed to the design of the review. Material preparation and analysis were performed by JH. The first draft of the review was written by JH. JRM provided critical feedback on all previous versions of the chapter.

CHAPTER 3: N AND P LIMIT SOIL MICROBIAL BIOMASS, BUT MULTIPLE RESOURCES LIMIT SOIL MICROBIAL FUNCTION IN A NORTHERN CHIHUAHUAN DESERT SEMI-ARID GRASSLAND

ABSTRACT

Drylands represent ~41% of Earth's terrestrial surface and are expected to be particularly vulnerable to global change stressors, such as climate change and anthropogenic nitrogen (N) deposition. Resource availability may dictate how ecosystems respond to global change, e.g., N deposition. However, we have a limited understanding of the resources (beyond water) that regulate important biogeochemical processes in drylands. Here, we performed a factorial carbon (C), N, and phosphorous (P) laboratory incubation experiment to test if Chihuahuan Desert soil microbial biomass and function are individually or interactively limited by C, N, and P when given sufficient water. Overall, we found that N and P independently limited soil microbial biomass, but the effects on measured microbial activities often depended on the co-availability of multiple resources. For example, N and P immobilization was often only enhanced when two or more resources were added (e.g., C+N, N+P, C+N+P). C alone dramatically increased specific respiration (i.e., CO_2 production per unit biomass and unit time) but had no significant effect on soil microbial biomass, suggesting low C-use efficiency (CUE). CUE appeared to improve when C was added with N or P, as these resources together enhanced microbial biomass and inhibited the effect of C on specific respiration. This study highlights the importance of assessing multiple resource limitations in low-resource environments, as such resources may influence how these ecosystems respond to global change.

INTRODUCTION

Our understanding of resources regulating biogeochemical processes in terrestrial ecosystems is improving (e.g., Fay et al., 2015; Harpole et al., 2016; Ochoa-Hueso et al., 2020). However, relative to water, we have a limited understanding of the resources that govern biogeochemical processes in drylands (Austin, 2011; Osborne et al., 2022b). Drylands cover over ~41% of Earth's terrestrial surface (Millennium Ecosystem Assessment, 2005) and play a critical role in the global carbon (C) cycle (Poulter et al., 2014; Ahlström et al., 2015). Advancing our understanding of the resources that regulate dryland biogeochemical processes (beyond water) will strengthen our ability to predict environmental change effects in these ecosystems.

Water is the dominant driver of biological processes in drylands, e.g., plant primary productivity and soil microbial activity (Noy-Meir, 1973; Reynolds et al., 2004; Collins et al., 2014). However, the strong focus on water limitation in drylands has constrained our understanding of other resources that influence important ecological processes, e.g., C and nutrient cycling (Austin, 2011). In particular, as aridity drives low levels of organic C, nitrogen (N), and phosphorus (P) in dryland soils (Plaza et al., 2018), these critical biogenic elements may also be limiting for dryland biota. Human-driven perturbations, such as climate change and N deposition, can also drive changes in resource availability (e.g., N to P limitation, Yuan and Chen, 2015; Dong et al., 2019), which may further alter or constrain biological activities in drylands.

Beyond water, N is considered a vital limiting resource in drylands (Hooper and Johnson, 1999; Yahdjian et al., 2011). However, across N addition experiments globally, the magnitude and often the direction of N addition effects are influenced by experimental N dose and study duration, e.g., in plants (Midolo et al., 2019; Song et al., 2019) and soil microbes (Jian et al., 2016; Zhou et al., 2017; Jia et al., 2020). For instance, at the global scale, N inputs at rates less than 100 kg N ha^{-1} yr^{-1} generally enhance microbial biomass, whereas rates greater than 100 kg N ha^{-1} yr^{-1} reduce microbial biomass (meta-analysis by Zhou et al., 2017). A similar pattern also emerges across aridland (arid and semi-arid ecosystems) N addition studies, where N doses of <70 kg ha^{-1} yr^{-1} and N loads <120 kg ha^{-1} exert positive effects on microbial biomass and metabolic rates, respectively, with negative effects at greater doses and loads (meta-analysis by Sinsabaugh et al., 2015). However, some aridland N addition studies also suggest that some aridlands are insensitive to N inputs (e.g., McHugh et al., 2017; Philips et al., 2021; Osborne et al., 2022a).

Growing evidence suggests that resources in addition to or other than N can be limiting in aridlands, e.g., water and C (e.g., Schaeffer et al., 2003; Schaeffer and Evans, 2005; Choi et al., 2022), and P (Cui et al., 2018). Resource limitation may be particularly important as it may dictate how ecosystems respond to global change (Reed et al., 2015). Drylands worldwide face several environmental stressors, e.g., climate change and N deposition (Fenn et al., 2003; Hoover et al., 2020), which have considerable implications for global C and N cycling (Peterjohn and Schlesinger, 1990; Delgado-Baquerizo et al., 2013). Thus, it is critical that we improve our understanding of resources capable of regulating important dryland ecosystem processes beyond water limitation.

Here, we performed a factorial C, N, and P addition laboratory incubation experiment using soils collected from a semi-arid Chihuahuan Desert grassland experiencing elevated levels of anthropogenic N deposition. N deposition has important implications for ecosystem structure and function (Bobbnik et al., 2010). However, given that some studies suggest aridlands are limited by resources in addition to or beyond N (e.g., Schaeffer et al., 2003; Schaeffer and Evans, 2005; Cui et al., 2018; Choi et al., 2022), we were also interested in exploring how C, N, and P may individually or interactively influence soil microbial biomass and function in our study ecosystem. We based experimental N inputs on a rate similar to modeled N deposition in our study ecosystem (4 kg N ha^{-1} yr^{-1}). Therefore, as microbial biomass and metabolic rates generally increase at lower N addition rates (e.g., <70 kg ha^{-1} yr^{-1} for microbial biomass in Sinsabaugh et al., 2015), we hypothesized that our N inputs would enhance soil microbial biomass and activities. However, given that dryland soils are also notably poor in organic C and P (Plaza et al., 2018), we also hypothesized that N added with C or P would surpass the effects of N alone by further enhancing soil microbial biomass and activities.

METHODS

Site Description

We collected soils from a Chihuahuan Desert semi-arid grassland on a stream terrace at Carlsbad Caverns National Park (CAVE, 32°10′31″N 104°26′38″W) N.M., USA. CAVE is modeled to be experiencing elevated levels of N deposition at a rate ranging from 3.5 to 4.5 kg N ha^{-1} yr^{-1} due to nearby oil and natural gas operations (Sullivan et al., 2016; Naimie et al., 2022). The region's mean annual temperature is 16.8 °C, with a mean annual precipitation of 378.7 mm, +60% of which falls during the summer monsoon (generally from July to September). Vegetation at our study site contained a mix of warm-season C_4 grasses, annuals, and forbs, including native perennial grasses, blue grama (*Bouteloua gracilis*), and black grama (*B. eriopoda*), and exotic invasive bunchgrass Lehmann lovegrass (*Eragrostis lehmanniana*). Soil parent material is characterized as gravelly alluvium derived from limestone (websoilsurvey.sc.egov.usda.gov/). Soil texture is a sandy loam mixture characterized as 71% sand, 25% silt, and 4% clay, with an average pH of 7.6 (2:1 dH_2O).

Soil Collection

We collected soils during pre-monsoon drought conditions in June 2018, at 0 to 5 cm, from open spaces between plants. Soil sample locations were free of mid to late successional biological soil crusts (e.g., darkly pigmented cyanobacterial and moss crust) but likely included early successional lightly pigmented cyanobacterial crust. Soil sample locations were collected at a minimum distance of 2 m from each sample location and large N-fixing plants (e.g., mesquite). Soils were combined, homogenized, passed through a 2-mm sieve, and stored at 4 °C for approximately one day before the start of the incubation.

Incubation Experiment

To explore the influence of C, N, and P availability on soil microbial biomass and activities, we performed a 24-hour and 16-day C, N, and P addition laboratory incubation experiment using soils collected from the field. For the incubation, we weighed 50 g of soil (dry-equivalent) into 355 mL glass mason jars. Treatments included a control (CT, deionized water only) and factorial additions of C, N, and P (eight treatments total). N was added in an amount similar to modeled N deposition at CAVE (4 kg N ha^{-1} yr^{-1}). Proportions of C and P additions were based on our N addition dose and globally estimated microbial C:N:P stoichiometry (60:7:1, Cleveland and Liptzin, 2007), with the assumption that all three resources were added in excess. The specific amount of C, N, and P added was based on the 50 g of soil within each jar, which was calculated to be the equivalent to the top 3 cm of soil from 0.00054 m^2, with a field bulk density of 1.41 g/cm^3. N was added as ammonium nitrate (0.83 g/m^2; NH_4NO_3), C was added as sucrose (6.66 g/m^2; $C_{12}H_{22}O_{11}$), and P as triple superphosphate (0.25 g/m^2; Ca $(H_2PO_4)_2$ *H_2O). Additionally, as we wanted to specifically test for C, N, and P limitation (i.e., not water limitation), all soils received deionized water, which brought soils to 60% water holding capacity (14.7 mL). Treatments were replicated 12 times to allow for two incubation periods, which included two destructive soil samplings (i.e., at 24 hours and day 16). Soils were incubated at 30 °C (comparable to monsoon season temperatures in this region), with jar lids loosely fitted to reduce evaporation (Melle et al., 2015). Incubated soils were maintained at 60% field capacity by supplementing deionized water every two days (Allen and Schlesinger, 2004).

Soil Extractable Pools and Microbial Biomass

Extractable organic carbon (EOC), extractable total nitrogen (ETN), available soil phosphate (PO_4^{-3}-P), microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), and microbial biomass phosphorus (MBP) were measured at 24 hours, and on the 16th day of the incubation. EOC, ETN, and available phosphate (PO_4^{-3}-P) extracts were obtained by shaking 5 g of fresh soil in 0.5 M K_2SO_4 for two hours and filtering through glass filter paper. EOC and ETN concentrations were determined using a Shimadzu analyzer (TOC-VCPN; Shimadzu Scientific Instruments Inc., Columbia, MD, USA). Available soil PO_4^{-3} was assessed colorimetrically (BioTEK Synergy HT microplate reader) using a malachite green assay (D'Angelo, 2001). MBC, MBN, and MBP estimates were derived using a modified chloroform fumigation-extraction technique (Brooks et al., 1985). We incubated 5 g of fresh soil in 2 mL of ethanol-free chloroform for 24 hours at room temperature. Then we extracted fumigated soils and analyzed them for EOC, ETN, and PO_4^{3-} (as described above). MBC, MBN, and MBP were calculated as the difference between non-fumigated

EOC, ETN, and PO_4^{3-} concentrations and fumigated EOC, ETN, and PO_4^{3-} concentrations, respectively.

Respiration and Specific Respiration

Respiration was measured immediately before destructively harvesting soils for microbial biomass and extractable soil pools at 24 hours and again on the 16th day. To prep the soils for respiration measurements, we uncovered the incubation jars and fanned them to ensure that ambient air was mixed within the jars. Following fanning, we tightly sealed the jars and incubated them at room temperature (20 °C) for 1 hour. Following the 1-hour incubation period, we drew air samples from a rubber septum fitted at the top of each jar using a 5 mL needle syringe. Sample air was fed into an infrared gas analyzer (LI-820, LI-COR Biosciences, Lincoln, Nebraska, USA). Ambient carbon dioxide (CO_2) concentrations were measured using three empty acid-washed jars treated as controls during respiration measurements (Melle et al., 2015). We determined respiration rates as the net accumulation of CO_2 over time. Specific respiration, which represents CO_2 production per unit biomass and unit time (called metabolic quotient of CO_2 in Anderson and Domsch, 1985), was calculated as CO_2 produced per μg of microbial biomass C using MBC as determined above.

Statistical Analyses

All measures were analyzed using a three-way ANOVA for each incubation period (24h and 16 days). C, N, and P factors were treated as binary dummy variables, such that control = (0,0,0), C addition = (1,0,0), N addition = (0,1,0), P addition = (0,0,1), C+N addition = (1,1,0), C+P addition = (1,0,1), N+P addition = (0,1,1), and C+N+P addition = (1,1,1). We assessed models for normality (Shapiro-Wilks) and heteroscedasticity and log-transformed when the data did not meet these assumptions (respiration data only). All statistical analyses were performed using JMP 10.0 (2010, Cary, NC, USA).

RESULTS

Microbial Biomass

Relative to control conditions, MBC responded to N and P additions independently, while MBN and MBP generally only responded when we added resources in combination. Microbial biomass responses were also more evident on day 16 than after 24 hours (Table 3.1). Relative to the control treatment, MBC marginally increased with added C after 24 hours (Fig. 3.1a). By day 16, MBC increased with N and P, but not C additions (Fig. 3.1b). MBN was not affected by any of our resource additions after 24 hours (Fig. 3.1c). However, there was a three-way interaction between C, N, and P additions on day 16 (Table 3.1), with MBN increasing more when any two resources added together relative to when a single or all three resources were added simultaneously (Fig. 3.1d). MBP showed a marginal three-way interaction between resource additions after 24 hours, with MBP increasing with N addition unless added with C or P (Fig. 3.1e, Table 3.1). On day 16, a two-way interaction between N and P revealed that N and P added together increased MBP but had no significant effect when N or P were added alone (Fig. 3.1f, Table 3.1)

Table 3.1. Summary of 3-way ANOVA with the main factors of carbon (C), nitrogen (N), and phosphorus (P) for microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), and microbial biomass phosphorus (MBP) in a fully factorial Chihuahuan Desert soil resource addition laboratory incubation experiment after 24 hours and 16 days. Bold values are significant at $p<0.05$, and italicized values indicate marginal significance at $0.05<p<0.1$. df for all responses is 1, 40.

	MBC - 24h		**MBC - Day 16**		**MBN - 24h**		**MBN - Day 16**		**MBP - 24h**		**MBP - Day 16**	
	F	p	F	p	F	p	F	p	F	p	F	p
C	2.95	*0.094*	2.51	0.121	1.54	0.222	4.54	**0.039**	5.43	**0.025**	3.97	**0.053**
N	0.14	0.708	18.79	**<0.001**	0.10	0.753	10.10	**0.003**	1.25	0.269	32.59	**<0.001**
P	1.19	0.282	17.21	**<0.001**	0.14	0.714	14.62	**<0.001**	5.43	**0.025**	2.12	0.153
C x N	0.00	0.998	1.89	0.177	0.65	0.426	0.13	0.718	1.36	0.251	1.01	0.320
C x P	0.68	0.415	2.39	0.130	1.66	0.205	2.46	0.125	1.25	0.269	1.20	0.279
N x P	0.41	0.525	0.90	0.349	0.36	0.552	0.20	0.658	0.01	0.929	10.54	**0.002**
C x N x P	1.85	0.181	1.47	0.232	1.19	0.283	12.25	**0.001**	3.05	*0.088*	0.55	0.464

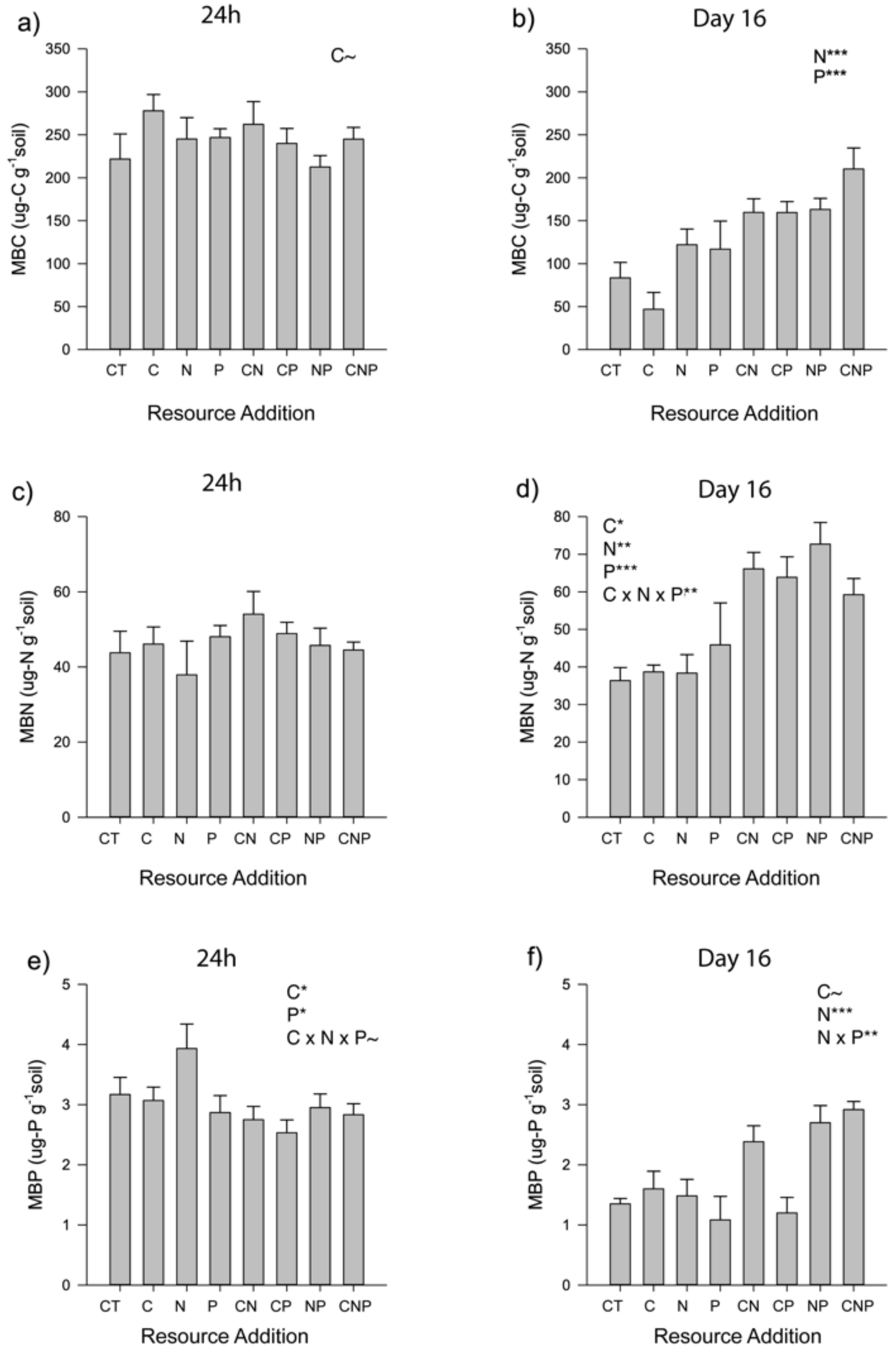

Fig. 3.1. Mean (± SE) microbial biomass carbon (MBC; a, b), microbial biomass nitrogen (MBN; c, d), and microbial biomass phosphorus (MBP; e,f) in a fully factorial Chihuahuan Desert soil resource addition laboratory incubation experiment (CT= control, C = carbon, N = nitrogen, and P = phosphorus) after 24 hours and 16 days. Levels of significance for a 3-way ANOVA with the main factors of C, N, and P are shown. *$p<0.05$ **$p<0.01$ ***$p<0.001$, and ~ indicates marginal significance at $0.05<p<0.1$.

Soil Extractable Pools

Resource addition effects on EOC, ETN, and available soil PO_4^{3-} were observed during incubation periods, but effects often contained interactive effects between two or more resources (Table 3.2). EOC had a complex three-way interaction between resource additions after 24 hours, with the lowest EOC concentrations when all three resources were added together (Fig. 3.2a, Table 2). On day 16, C added alone had little effect on EOC, but EOC declined when C was added with N (Fig. 3.2b). ETN increased with N but declined with C inputs after 24 hours (Fig. 3.2c, Table 3.2). On day 16, ETN had a three-way interaction, where ETN increased with N alone but declined to its lowest concentrations when N was added with C or when C was added with P (Fig. 3.2d). After 24 hours, available soil PO_4^{3-} increased P inputs, and two-way interaction revealed that C or N added alone had little effect on PO_4^{3-}but increased when C and N were added together (Fig. 3.2e). Soil PO_4^{3-} had a three-way interaction on day 16 (Table 3.2), where PO_4^{3-} was lowest when we added C and N together, but this effect did not occur when C and N were added with P (Fig. 3.2f).

Table 3.2. Summary of 3-way ANOVA with the main factors of carbon (C), nitrogen (N), and phosphorus (P) for extractable organic carbon (EOC), extractable total nitrogen (ETN), and available phosphate (PO_4^{3-}) in a fully factorial Chihuahuan Desert soil resource addition laboratory incubation experiment after 24 hours and 16 days. Bold values are significant at $p<0.05$, and italicized values indicate marginal significance at $0.05<p<0.1$. df for all responses is 1, 40.

	EOC - 24h		**EOC - Day 16**		**ETN - 24h**		**ETN - Day 16**		**PO_4^{3-} - 24h**		**PO_4^{3-} - Day 16**	
	F	p	F	p	F	p	F	p	F	p	F	p
C	0.66	0.421	2.50	0.122	24.14	**<0.001**	20.85	**<0.001**	0.40	0.531	9.73	**0.003**
N	1.56	0.219	3.56	*0.066*	99.64	**<0.001**	3.29	*0.077*	0.53	0.472	5.88	**0.020**
P	1.19	0.283	3.30	*0.077*	0.06	0.811	1.60	0.213	65.26	**<0.001**	9.73	**0.003**
C x N	1.60	0.214	0.53	0.471	1.87	0.179	2.41	0.129	4.54	**0.039**	1.08	0.305
C x P	1.87	0.179	2.98	*0.092*	1.07	0.307	4.98	**0.031**	0.29	0.593	5.88	**0.020**
N x P	1.81	0.186	14.69	**<0.001**	0.36	0.552	1.21	0.278	0.67	0.417	0.02	0.883
C x N x P	7.41	**0.010**	5.27	**0.027**	0.39	0.533	8.24	**0.007**	0.04	0.834	5.88	**0.020**

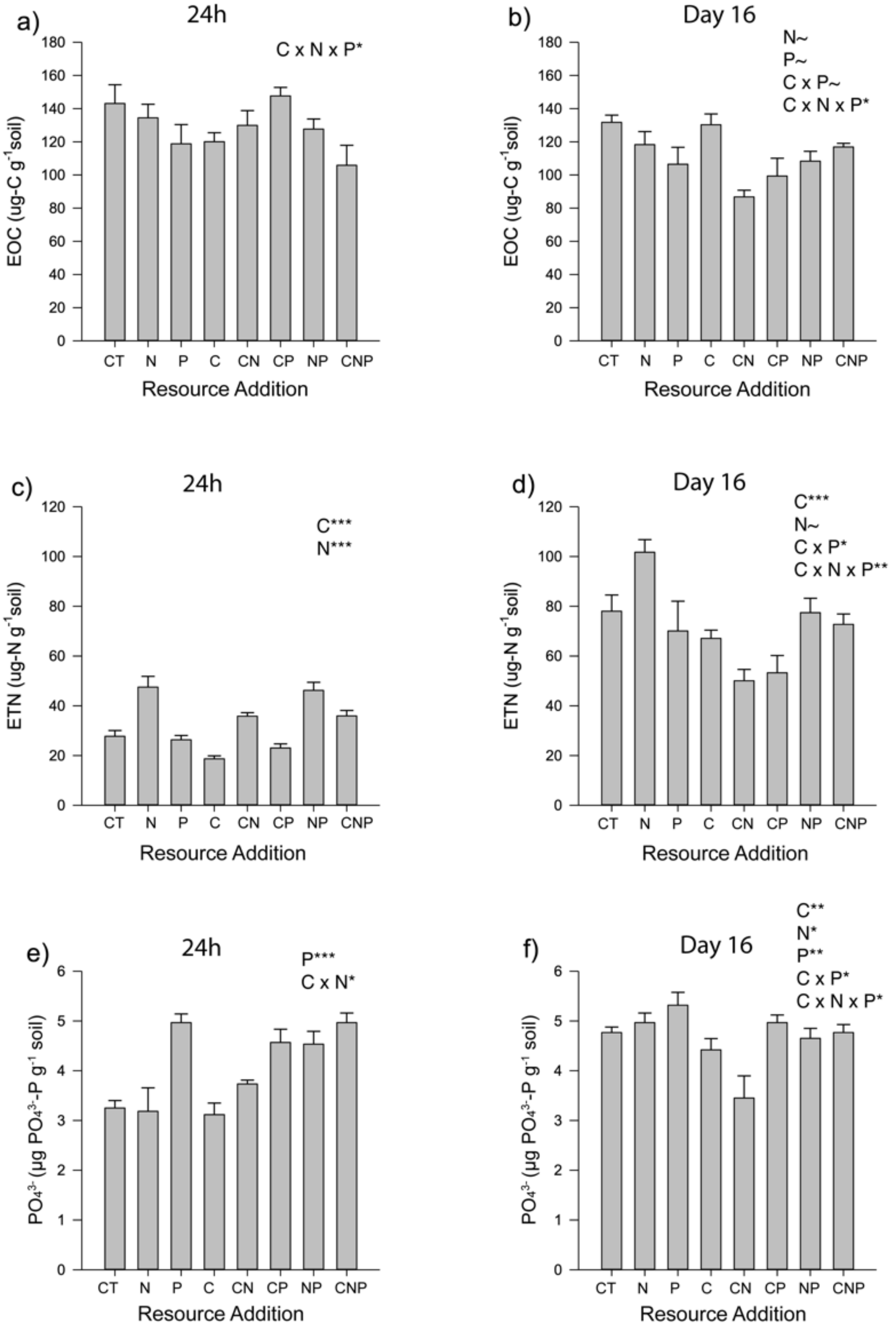

Fig. 3.2. Mean (± SE) extractable organic carbon (EOC; a, b), extractable total nitrogen (ETN; c, d), and available soil phosphate (PO_4^{3-}; e,f) in a fully factorial Chihuahuan Desert soil resource addition experiment (CT= control, C = carbon, N = nitrogen, and P = phosphorus) after 24 hours and 16 days. Levels of significance for a 3-way ANOVA with the main factors of C, N, and P are shown. *$p<0.05$ **$p<0.01$ ***$p<0.001$, and ~ indicates marginal significance at $0.05<p<0.1$.

Respiration and Specific Respiration

Overall, respiration and specific respiration increased with the addition of C, with some interaction effects with N and P (Table 3.3). In both incubation periods, C addition increased respiration and had a marginal interaction between N and P, which showed that respiration slightly increased when N and P were added together (Fig. 3.3a, b, Table 3.3). Specific respiration responses to resource additions differed between each measurement period (Table 3.3). At 24 hours, specific respiration had a three-way interaction which showed that N and P added alone had a slightly negative effect on specific respiration, but when resources were added together, specific respiration increased (Fig. 3.3c). On day 16, a marginal two-way interaction between C and N and a significant two-way interaction between C and P showed that when C added alone, specific respiration increased dramatically, but this positive effect was inhibited when C was added with N or P (Fig. 3.3d, Table 3.3).

Table 3.3. Summary of 3-way ANOVA with the main factors of carbon (C), nitrogen (N), and phosphorus (P) for soil respiration rate (Respiration) and specific respiration rate (Sp. Resp) in a fully factorial Chihuahuan Desert soil resource addition laboratory incubation experiment after 24 hours and 16 days. Bold values are significant at p<0.05, and italicized values indicate marginal significance at 0.05<p<0.1. df for all responses is 1, 40.

	Respiration - 24h		**Respiration - Day 16**		**Sp. Respiration - 24h**		**Sp. Respiration - Day 16**	
	F	p	F	p	F	p	F	p
C	7.80	**0.008**	8.64	**0.005**	0.38	0.541	4.39	**0.043**
N	0.80	0.377	0.07	0.791	0.75	0.391	9.43	**0.004**
P	2.12	0.153	0.36	0.554	2.41	0.128	3.77	*0.059*
C x N	2.70	0.108	1.91	0.174	1.22	0.276	3.49	*0.069*
C x P	0.01	0.933	1.20	0.280	0.37	0.546	6.28	**0.016**
N x P	3.63	*0.064*	2.93	*0.094*	3.46	*0.070*	0.06	0.812
C x N x P	1.68	0.202	0.32	0.577	4.21	**0.047**	1.56	0.219

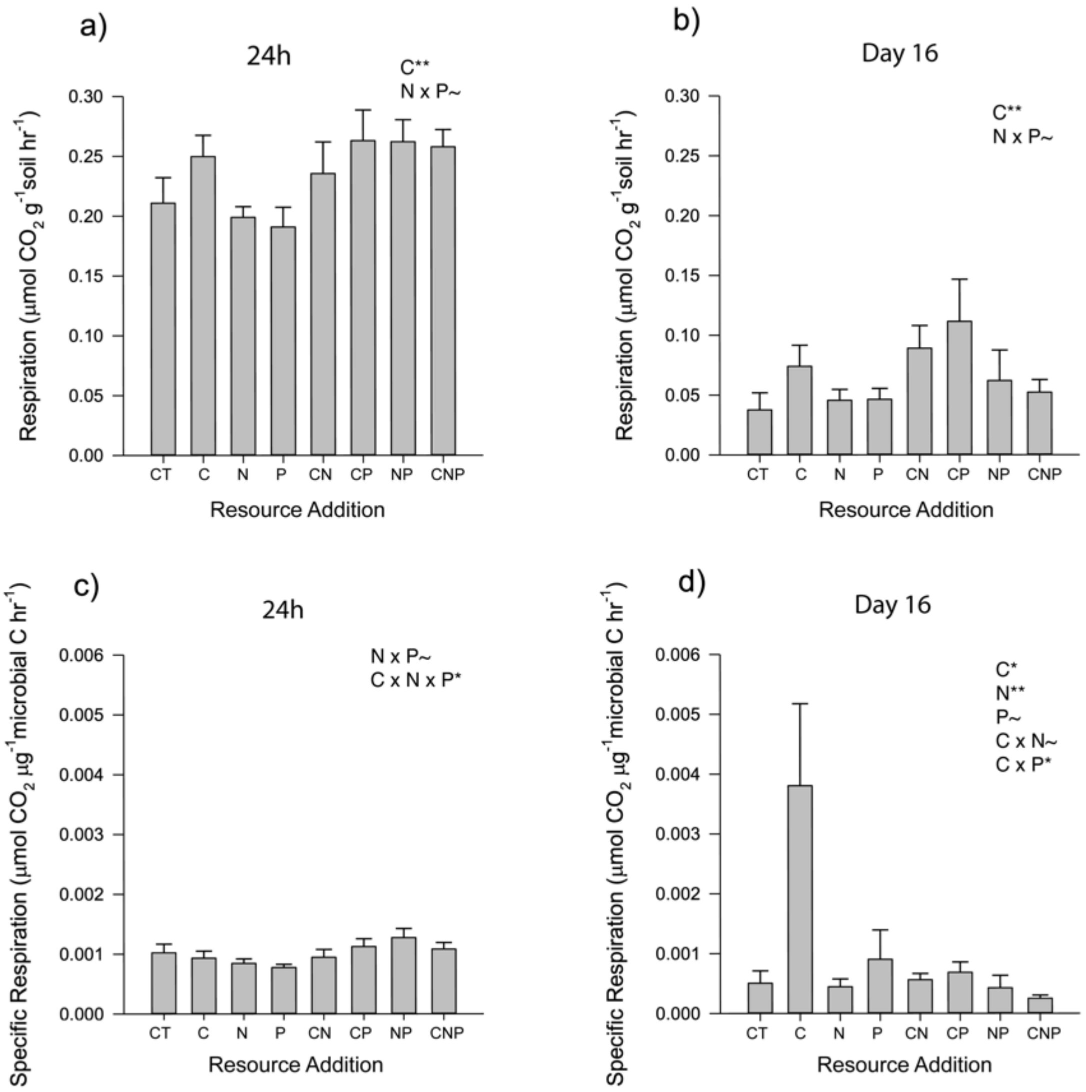

Fig. 3.3. Mean (± SE) soil respiration rate (a, b) and specific respiration rate (c, d) in a fully factorial Chihuahuan Desert soil resource addition experiment (CT= control, C = carbon, N = nitrogen, and P = phosphorus) after 24 hours and 16 days. Levels of significance for a 3-way ANOVA with the main factors of C, N, and P are shown. $*p<0.05$ $**p<0.01$ $***p<0.001$, and ~ indicates marginal significance at $0.05<p<0.1$.

DISCUSSION

Our study adds to the growing evidence demonstrating how aridland ecosystem processes can be limited or co-limited by multiple resources beyond water. Overall, our first hypothesis was only partially supported as N added alone only enhanced soil microbial biomass but did not significantly affect our microbial function measures. Our second hypothesis, which predicted that the effects of

N would be further enhanced when N was added with C or P, was also only partially supported as we observed synergistic effects with N and other resources. However, in the absence of N addition, we also found that microbial biomass and activity measures were independently influenced by P alone or by combinations of C and P.

In our study, N added alone increased soil microbial biomass, indicating N limitation. As we added N at a dose similar to modeled N deposition occurring at our collection site (i.e., 4 kg N ha^{-1}), our findings are consistent with a recent meta-analysis of aridland N addition studies that reported positive effects on soil microbial biomass at N application doses <70 kg ha^{-1} yr^{-1} (Sinsabaugh et al., 2015). However, despite the positive effects of N on biomass, N alone did not affect any of our measured soil microbial function responses (i.e., N and P immobilization, respiration, and specific respiration). Thus, the lack of effect with N inputs alone on our soil microbial function measures contrasts aridland meta-analysis by Sinsabaugh et al. (2015), which suggests positive effects on microbial metabolic activities at N loads <120 kg N ha^{-1}, as well as other later studies that report changes in soil microbial activities with N additions, e.g., enhanced heterotrophic respiration (Fang et al., 2018; Yue et al., 2018) and N immobilization (Choi et al., 2022). Though, it is possible that respiration did not increase with N inputs due to shifts in soil microbial communities (e.g., from oligotrophic to copiotrophic organisms), which exhibit lower microbial respiration rates due to reduced decomposition of recalcitrant organic matter (Ramirez et al., 2012; Mori et al., 2018).

Unlike N amendments alone, changes to our soil microbial function measures generally occurred with the addition of two or more resources (e.g., N+P, C+P, C+N+P). For instance, N immobilization (i.e., MBN) increased significantly with additions of N with C or P, but not with

N alone. Additionally, despite not receiving supplemental N, C, and P added together, increased microbial N uptake (i.e., indicated by lower ETN and greater MBN with C+P additions). Microbial N transformation and uptake processes are mediated by enzymatic processes that require not only N but energy as well (as reviewed by Geisseler et al., 2010). Thus, soil microbes may have been only able to use or mine for N after other limiting resources (e.g., C or P) were available in sufficient quantities. In a similar aridland C, N, and P resource addition soil incubation study using soils collected on the Colorado Plateau, N utilization was serially limited by water and C (Choi et al., 2022). Greater P availability has also shown to accelerate N-cycling processes such as gross N mineralization (meta-analysis by Wang et al., 2022).

P independently limited soil microbial biomass in our study ecosystem, which adds to the growing number of aridland studies showing P to be equally or even more limiting than N (e.g., James et al., 2005; Ma et al., 2009; Cui et al., 2018; Yang et al., 2021). While total P soil content can be high in dryland soils relative to more mesic systems (Plaza et al., 2018), arid soils also contain lower concentrations of organic P and exhibit lower P availability than in more humid regions due to alkaline soils and a greater abundance of compounds that bind to P (Vitousek et al., 2010; de-Bashan et al., 2022). However, other aridland P addition studies also indicate that soil microbes are not P limited (e.g., Li et al., 2010; Wang et al., 2020; Choi et al., 2022). Conflicting findings between aridland P amendment studies may be associated with differences in P availability, which can vary due to differences in geological history, pedogenesis, climatological and soil physiochemical factors, as well as contributions from aeolian dust (as reviewed by de-Bashan et al., 2022). P availability may also differ due to biological soil crust (biocrust) cover differences. Biocrust are a consortium of photosynthetic and heterotrophic organisms such as cyanobacteria, lichen, and mosses that occur in all drylands globally (Weber et al., 2022). Biocrust successional stage (Young et al., 2022) and P acquisition strategies (Crain et al., 2018) can strongly influence P availability in aridlands. While we did not directly measure biocrust cover, soils were collected from plant interspaces that were not covered mid to late successional biocrust but likely included early successional light cyanobacterial crust, which are expected to have the least extractable PO_4^{3-} content (Young et al., 2022).

P has been shown to widely influence C-cycling processes (Elser et al., 2007; Reed et al., 2011; Xiao et al., 2018). However, P alone had few effects on our soil microbial function measures. Instead, like N, soil microbial function effects were most apparent when P was added with another resource (e.g., N+P and C+P). For example, P added alone significantly increased microbial biomass but exerted little effect on N immobilization. However, when P was added with C or N, N immobilization (as well as microbial biomass) significantly increased. P is a critical component of P-rich ribosomal RNA and biochemical energy (as adenosine triphosphate, ATP) (Sterner and Elser, 2003). However, given that C and N are notably low in dryland soils (Plaza et al., 2018) and play a critical role in energy and enzyme production (Sterner and Elser, 2003; Marklein and Houlton, 2012), P may have been further utilized when C and N were available in sufficient concentrations.

In contrast to N and P alone and together, our labile C addition treatment was the only treatment that significantly increased heterotrophic and specific respiration. However, despite this increase, C amendments did not significantly affect microbial biomass. Our findings only partially agree with other aridland studies that report greater respiration with C and water additions than water alone (Schaeffer et al., 2003; Schaeffer and Evans, 2005; Choi et al., 2022). However, these studies also reported enhanced microbial biomass (Choi et al., 2022) and increased N immobilization (Schaeffer and Evans, 2005). The lack of effect on soil microbial biomass with enhanced respiration rates with C amendments suggests low (CUE), i.e., a smaller relative proportion of C assimilated into microbial biomass versus the C lost as CO_2 (as reviewed by Schimel and Schaeffer, 2012). Under our C addition treatment, overflow respiration may have resulted from a strong imbalance of resources which has been suggested to occur when nutrients (e.g., N or P) are limited (as reviewed by Manzoni et al., 2012). For instance, when C was added with N or P, microbial biomass increased, and the net positive effect of C on specific respiration was inhibited, thus, indicating relatively higher CUE than when C was added alone. High CUE signals efficient growth and relatively low release of CO_2, which may increase the stability of C soils, whereas low CUE suggests less conversion of C to biomass and large C losses through respiration and exudation, which may reduce the potential for long-term C sequestration (as reviewed by Manzoni et al., 2012).

CONCLUSIONS

In conclusion, we found that when given sufficient water, Chihuahuan Desert soil microbes were individually and interactively limited by multiple resources. Our findings found supporting and conflicting evidence from other aridland resource addition experiments (field and laboratory incubation experiments). Aridlands landscapes are highly spatially heterogeneous, and it can be challenging to generalize responses between and even within aridland regions (Sinsabaugh et al., 2015; Osborne et al., 2022b). Thus, as resources may dictate how aridlands respond to global change (e.g., N deposition), we emphasize the importance of assessing multiple interacting limiting resources across a diverse number of aridland landscapes. Drylands are expanding on all continents except for South America and Europe (Prăvălie et al., 2019) and face several anthropogenic-related stressors, including changing nutrient inputs (Fenn et al., 2003) and climate change (Huang et al., 2016, 2017; Hoover et al., 2020). Improving our understanding of the resources which regulate dryland ecosystem processes, with resource scarcity and possible spatial heterogeneity in mind, will help to advance our ability to forecast the effects of global change stressors in these ecosystems.

ACKNOWLEDGMENTS

We would like to give special thanks to Ellen Trautner, Alejandro Benhumea, Anthony J. Darrouzet-Nardi, and Sasha C. Reed for their logistical assistance. We would also like to thank Carlsbad Caverns National Park personnel and the Chihuahuan Desert Biodiversity REU program, directed by Michael Moody. Funds for this research were provided by the United States Department of the Interior, the National Park Service Cooperative Agreement with the University of Texas at El Paso [Cooperative Agreement P17AC01014], and the Chihuahuan Desert Biodiversity REU award [NSF-DBI- 1950810].

AUTHOR CONTRIBUTIONS

This Chapter has been submitted for review in a peer-reviewed journal (Biogeochemistry) and is co-authored by Jennifer Holguin (JH), Rebecca Dunkleberger (RD), Violeta Mendoza-Martinez (VM), and Jennie R. McLaren (JRM). JH, RD, and JRM contributed to the study's conception and design. Data collection was performed by JH, RD, and VM. Analysis was performed by JH, RD, and JRM. The first draft of the manuscript was written by JH. All authors commented on previous versions of this manuscript. All authors read and approved the final manuscript.

DATA ACCESSIBILITY

All data will be made publicly available when the manuscript is published.

CHAPTER 4: LOW BUT REALISTIC LEVELS OF SIMULATED N DEPOSITION HAVE LITTLE EFFECT ON PLANTS, ECOSYSTEM PROPERTIES, AND BIOGEOCHEMICAL PROCESSES BUT ENHANCE NITRIC OXIDE EMISSIONS IN THE NORTHERN CHIHUAHUAN DESERT

ABSTRACT

Anthropogenic nitrogen (N) deposition can considerably alter terrestrial ecosystem structure and function. However, the effect of N inputs in aridlands (arid and semi-arid ecosystems) can be complicated due to conflicting experimental results linked to experimental N dose or water availability. Here, we conducted a four-year N fertilization and water addition experiment in three northern Chihuahuan Desert grassland sites. We were interested in determining: $^{1.}$ if low but realistic N inputs (i.e., based on modeled N deposition at our study site; 0, 2, and 4 kg N ha^{-1} yr^{-1}) impact aridland plant communities, soil ecosystem properties, and biogeochemical dynamics, and $^{2.}$ if effects of N depend on periods of above-average water availability. Field N additions had few transient effects on our plant community, soil ecosystem, and biogeochemical measures. Water limitation may have complicated responses (even with supplemental water) during the first three years of this experiment, as summer rainfall was 50%, 54%, and 71% below the 30-year normal. However, N addition effects were still not evident during the final year, when summer precipitation was 25% above the long-term average. After four years of N inputs, we performed a complementary water and N addition (4 kg N ha^{-1}) laboratory experiment using soils collected from N field addition plots, which revealed elevated levels of nitric oxide emissions in N-treated soils. While periods of drought and above-average precipitation may have complicated responses to N, our study suggests that aridlands may be resistant to low levels of N deposition. Aridland resistance to N inputs may be due to their low capacity to retain N, which may be attributed to significant gaseous N losses.

INTRODUCTION

Anthropogenic nitrogen (N) deposition, primarily linked to fossil fuel and intensive agriculture operations, is one of the most prominent factors driving global change (Phoenix et al., 2006; Bobbink et al., 2010). Across the globe, N inputs have driven dramatic changes to terrestrial ecosystem structure and function, such as reduced plant diversity (Payne et al. 2017; Midolo et al. 2019) and changes to soil microbial community structure and activities involved in carbon (C) and nutrient cycling (Ramirez et al., 2012; Chen et al., 2018; Jia et al., 2020). However, across aridland (arid and semi-arid) N addition studies, conflicting responses associated with the possible insensitivity to N added at low doses (e.g., McHugh et al., 2017; Philips et al., 2021; Osborne et al., 2022a) and variable water availability (e.g., Brooks, 2003; Hall et al., 2011; Ladwig et al., 2012) challenges our ability to predict the effects of N deposition in these ecosystems. Aridlands cover a third of Earth's terrestrial surface (Plaza et al., 2018), play an important role in the interannual variability of the global C-cycle (Poulter et al., 2014; Ahlström et al., 2015), and are home to 18.5% of the world's population (Millennium Ecosystem Assessment, 2005). Improving our understanding of the effects of N deposition in aridlands is critical in assessing their vulnerability and developing effective mitigation strategies in response to anthropogenic N deposition in these ecosystems.

N is an important limiting resource across various ecosystems (LeBauer and Treseder, 2008; Song et al., 2019; Li et al., 2022). However, ecosystem response to N inputs can vary due to factors such as differences in climate, edaphic properties, experimental duration, and N dose (e.g., Midolo et al., 2019; Zhou et al., 2017; Borer and Stevens, 2022). In particular, N addition dose often appears to strongly influence the magnitude and sometimes the direction of change (e.g., Zhou et al., 2017; Song et al., 2019). However, most simulated N deposition experiments add N at high rates (Zhou et al., 2017; Song et al., 2019), thus limiting our understanding of the effects of low and possibly more realistic levels of N deposition.

Relative to more mesic systems, aridlands have notably low soil N stocks (Plaza et al., 2018) and are expected to be sensitive to even low levels of N inputs (Pardo et al., 2011; Sinsabaugh et al., 2015). However, some aridland N addition studies suggest aridlands are insensitive to N at low doses. For instance, many studies reporting significant changes to aridland ecosystem structure and function apply N at relatively high rates ≥50 kg N ha^{-1} yr^{-1}, e.g., declines in plant diversity, shifts in plant community composition (Zeng et al., 2010; Vourlitis, 2017; Wheeler et al., 2021), and changes to soil microbial communities and function (Zhang et al., 2013; Yang et al., 2017; Püspök et al., 2023). In contrast, aridland studies simulating N at elevated but relatively low levels of N deposition report little to no change in plant communities or soil microbial community structure and function (2, 5, and 8 kg N ha^{-1} yr^{-1} in McHugh et al., 2017, Philips et al., 2021, and Osborne et al., 2022a).

Beyond N addition dose, aridland response to N inputs often depends on water availability (Hooper and Johnson, 1999; Yahdjian et al., 2011). In aridlands, rainfall is highly spatially and temporally heterogenous and is the primary driver of biological activities (Noy-Meir, 1973; Austin et al., 2004; Collins et al., 2008). In many aridland N addition studies, water availability, particularly periods of above-average rainfall, has been shown to strongly influence aridland response to N (e.g., Whitford and Steinberger, 2011; Ladwig et al., 2012; Wheeler et al., 2021). For instance, in the Mojave Desert, 32 kg N ha^{-1} yr^{-1} added for two years reduced species richness and biomass of native species and increased the biomass of alien plant species, but only during the second year, which experienced above average precipitation (Brooks, 2003). In the Chihuahuan

Desert, a long-term N addition study revealed that N added at a rate of 100 kg N ha^{-1} yr^{-1} only became limiting to above-net primary production following periods of above-average rainfall (Ladwig et al., 2012). Thus, contrasting responses between relatively wet and dry periods may complicate predictions of the effects of N deposition in these often water-limited ecosystems.

To enhance our understanding of the effects of relatively low but elevated levels of N deposition in aridlands and to assess whether N addition effects depend on periods of high-water availability, we conducted a four-year field experiment that simulated low N deposition and enhanced water availability in three grassland sites within Carlsbad Caverns National Park (CAVE), in the northern Chihuauhan Desert. CAVE is in the Permian Basin, adjacent to oil and natural gas operations (Sullivan et al., 2016; Dix et al., 2020; Naimie et al., 2022). When designing this experiment, CAVE was modeled to be experiencing elevated levels of N deposition occurring in a gradient at a rate of 3.5 to 4.5 kg N ha^{-1} yr^{-1}, and all three sites were established in the lower end of the modeled N deposition gradient (Fig. 1.2). During the first two years of this experiment, annual and monsoon season precipitation was well below average, with little effects of N addition treatments on plant and soil biogeochemical processes (Table S1.1; Fig. S1.1). Therefore, to assess whether N addition effects depend on water availability, we supplemented water (+55 mm) at one site during the third year of this experiment.

We were interested in determining [1.] if low but realistic simulated N deposition impacts plant community structure, soil ecosystem properties, and biogeochemical processes and [2.] if N addition effects depend on periods of above-average water availability. For our first hypothesis, we predicted that our N amendments would follow effects observed across most aridland N addition studies (see Chapter 2) by significantly altering plant community structure, soil ecosystem properties, and biogeochemical processes. In particular, we first hypothesized that N inputs would reduce plant diversity by enhancing dominant grass cover, including invasive perennial grass *Eragrostis lehmanniana* (which dominated one of the three sites). We also predicted that N additions would acidify soils and depress soil microbial biomass and extracellular enzyme activities, particularly activities involved in N cycling. For our second hypothesis, we expected N responses to be only evident during periods of above-average water availability.

METHODS

Site Description

This study was conducted in three geographically adjacent (<5km apart) Chihuahuan Desert grasslands at Carlsbad Caverns National Park (CAVE), New Mexico, U.S.A. (32°10′31″N 104°26′38″W). The mean annual temperature (MAT) in this region is typically 16.8° C, with a mean annual precipitation (MAP) of 378.7 mm, >60% of which falls during the summer monsoon (typically from July to September). Dominant grass species differed between sites, with one site dominated by an invasive C_4 perennial bunchgrass *E. lehmanniana* (site hereafter referred to as Invasive Lovegrass), C_4 native perennial grass *Muhlenbergia setifolia* (site hereafter referred to as Native Muhly), and C_4 native perennial grass *Bouteloua gracilis* (site hereafter referred to as Native Grama site). While there were few plant species shared between sites, some shared species included C_4 perennial grasses, *B. gracilis, B. eriopoda,* and forbs such as *Thymophylla pentachaeta, Ditaxis neomexicana, Talinum spp.,* and *Croton spp*. All sites were situated on alluvial floodplains and shared similar soil parent material characterized as gravelly alluvium derived from limestone. (websoilsurvey.sc.egov.usda.gov/). Soils in all sites are characterized as sandy-loam soils with average site pH ranging from 7.6 –7.8 (measured before the start of the N fertilization experiment) (Table S4.1).

MAP and summer precipitation varied between years, with the first three years receiving considerably less summer rainfall than the 30-year normal from June to September (Table S1.1; Fig. S1.1). In 2018, 2019, and 2020 summer rainfall was 50%, 54%, and 71% less than the 30-year normal for June- September (252 mm), respectively. The final year (i.e., 2021) received 25% more summer rainfall than the 30-year normal (Fig. S1.1).

Field N Addition Experiment

We established a field N addition experiment at each site before the start of the monsoon season in 2018 (3rd week of July). We based N addition treatments on modeled CAVE N deposition (~4 kg N ha^{-1} yr^{-1}; Fig. 1.2). N addition treatments included a background N deposition treatment (Ambient; ~4 kg N ha^{-1} yr^{-1}), +N Low (+2 kg N ha^{-1} yr^{-1}), +N High (+4 kg N ha^{-1} yr^{-1}), and a labile C addition treatment, i.e., N reduction treatment (+C; +6 g of sucrose, $C_{12}H_{22}O_{11}$) which was intended to assess N saturation by stimulating microbial N immobilization (Steers et al., 2011). N was applied as ammonium nitrate (NH_4NO_3), and C was applied as sucrose ($C_{12}H_{22}O_{11}$). N addition treatments were dissolved in 2.25 L of water (equating to a 1 mm rainfall event) and sprayed evenly in all plots using a broadcast hand sprayer. Treatments were replicated five times in 1.5 m^2 plots at each site (60 plots total). We ensured that plots were separated at a minimum distance of 1.5 m from other plots and large N-fixing plant species (e.g., *Mimosa aculeaticarpa*). We applied treatments once annually for four years (2018- 2021) before the start of the monsoon season (mid-July). In 2021, the monsoon season began earlier than usual (early June). Therefore, N addition treatments in 2021 were applied during the monsoon season.

Field N and Water Addition Experiment

MAP and summer precipitation was well below average during the first two years of this experiment (2018 and 2019; Table S1.1; Fig. S1.1). Therefore, to assess whether N addition effects depend on water availability, we began adding supplemental water at one site (Native Grama) during the monsoon season in 2020. The amount of water added to each plot was intended to increase monsoon season rainfall by 30% based on a 75-year mean rainfall from July- September (182.9 mm; 1930-2005; National Weather Service Cooperative Network; Station ID #: 291480-7). For eight weeks (August to September), we added a total of 55 mm of water to each plot at the Native Grama site. Each watering event occurred once weekly, at the break of dawn, and equated to a 7 mm rainfall event (15.44 L per plot) using modified handheld 20 L planter watering cans. However, in 2020, the monsoon season was drier than in previous years (Table. S1.1; Fig. S1.1). Thus, even with water amendments, rainfall during the 2020 monsoon season was still well below the 30-year normal. We also planned to repeat the water addition treatment in 2021. However, a heavier and earlier-than-normal monsoon season resulted in flooding and road closures at the park, preventing us from accessing the water addition site by vehicle. However, summer rainfall in 2021 was 25% higher than the 30-year normal (Fig. S1.1).

Vegetation Sampling

We measured plant cover annually during peak plant biomass (the first week of October in 2018-2020 and the first week of September in 2021). To avoid edge effects, plant cover measurements excluded spaces within 10 cm of the edge of each plot. Plants were identified at the species level and were grouped into five functional groups: C_4 perennial grasses (includes native and invasive grasses), perennial forbs, annuals (annual forbs and grasses), shrubs, and cacti. Relative percent plant cover was calculated by summing the total cover of all plants within a plot, then dividing the specific species (i.e., *E. lehmanniana*) or functional group by the plot's total cover.

Plant Tissue Collection and Analysis

During the final monsoon season of 2021 (i.e., September), we sampled green, fully mature leaves free of herbivore damage from plant species present across most plots within a site. To avoid edge effects, plant tissue collection excluded spaces within 10 cm of the edge of each plot For the Invasive Lovegrass site, we sampled tissue from the dominant invasive C_4 grass *E. Lehmanniana,* native perennial grass *Lycurus setosus* and perennial forb *Sida abutilifolia.* At the Native Muhly site, we sampled from the dominant native perennial grass *M. setifolia* and native forb *Croton lindheimerianus tharpii.* At the Native Grama site, we sampled from dominant native perennial grass *B. gracilis* and native forb *C. pottsii.* Leaf samples were dried at 50 °C, ground, and analyzed for foliar C (foliar % C) and foliar N (foliar % N) using a dry combustion C and N analyzer (ECS 4010; Costech Analytical Technologies, Valencia, California, USA).

Soil Sampling

Each year (except in 2018), we collected soils at three time points: pre-monsoon (early June), monsoon (early September), and winter (early February). Soils were randomly collected from each plot (omitting spaces within 10 cm of the edge of each plot) using a 1.9 cm diameter soil corer at a depth of 5 cm. Soil moisture was also measured at a depth of 12 cm during each soil collection period using a soil moisture probe (HydroSense II Soil Moisture Measurement System, Campbell Scientific, Thuringowa Central, QLD, Australia). Following collection, we homogenized the soil samples and passed them through a 2 mm sieve. Soil samples were maintained at 4 °C until further processing (stored for a maximum of 72 hours). Within 24 hours, we froze a subsample at -80 °C for later analysis of soil microbial extracellular enzyme potentials.

Soil Ecosystem Properties

Soil pH was measured from soils sampled during the monsoon sampling season of each year using a 2:1 slurry (dH_2O: soil). Available soil nutrients nitrate (NO_3^-), ammonium (NH_4^+), phosphate (PO_4^{3-}), extractable organic carbon (EOC), and extractable total nitrogen (ETN) were measured during all seasons and years. We obtained nutrient, EOC, and ETN extracts by shaking 5 g of fresh soil in 0.5 M K_2SO_4 for two hours and filtering through glass filter paper. All nutrients were assessed using colorimetric microplate assays (BioTEK SynergH.T.HT, Winooski VT, USA). We analyzed available soil NO_3^- -N using a modified Griess reaction (Doane and Horwáth, 2003), soil NH_4^+-N using the Berlethot reaction protocol (Rhine et al., 1998), and PO_4^{3-} -P using a malachite green assay (D'Angelo et al., 2001). We measured EOC and ETN using a Shimadzu analyzer (TOC-VCPN; Shimadzu Scientific Instruments Inc., Columbia, MD, USA). Soil % organic C (soil %OC) and soil % N (soil %N) were measured once annually from soils collected during the monsoon sampling period. Soil %OC and soil %N samples were dried at 50 °C, ground, and treated for carbonates using an HCl fumigation protocol (Harris et al., 2001) and then analyzed using a dry combustion C and N analyzer (ElementarPyroCube®)

Soil Microbial Biomass

Microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), and microbial biomass phosphorous (MBP) were assessed using a modification of the chloroform fumigation-extraction technique (Brookes, 1985). We incubated 5 g of fresh soil in 2 mL of ethanol-free chloroform for 24 hours at room temperature. Then we extracted fumigated soils and analyzed them for EOC, ETN, and PO_4^{3-}-P (as described above). MBC, MBN, and MBP were calculated as the difference between non-fumigated EOC, ETN, and PO_4^{3-}-P concentrations and fumigated EOC, ETN, and PO_4^{3-}-P concentrations, respectively.

Soil Microbial Extracellular Enzymes

Soils collected during the monsoon season of each year were examined for hydrolytic and oxidative enzyme potential activities using standard high-throughput microplate protocols (Saiya-Cork et al., 2002; McLaren et al., 2017). Hydrolytic extracellular enzyme potentials included enzymes involved in C-cycling: α-Glucosidase (α-gluc), β-glucosidase (β-gluc), cellobiohydrolase (Cello), and β-xylosidase (Xylo), N-cycling: leucyl aminopeptidase (LAP) and NAG, and P-cycling: acid phosphatase (Phos) and phosphodiesterase (phos-D). We also measured oxidative enzymes peroxidase (Perox) and phenol oxidase (Phenol), which aid in the decomposition of recalcitrant organic matter. Immediately before each measurement, we thawed 1g of frozen (stored at -80 °C) soil samples at room temperature. Samples were then blended with 125 mL of modified universal buffer at a pH ranging from 7.7 to 7.9 (depending on pH for a given year). Soil slurries were pipetted into 96-well plates containing eight analytical replicates per sample. Fluorescing, 4-methylum-belliferone (MUB) tagged substrate (β -D-glucoside, β -D-cellobioside, N-acetyl- β -D-glucosaminide and phosphatase) or 7- amino-4-methylcoumarin (MC) tagged substrate (Leucine amino peptidase) were added to each hydrolytic enzyme assay. Hydrolytic enzyme assays were incubated at room temperature (20 °C) for 4 hours and 30 minutes, with measurements taken every 45 minutes to ensure a linear rate of reaction. Background fluorescence was measured for each soil, substrate, and quenching of MUB or MC (LAP only) by soils, and we used MUB/MC standard curves to calculate the rate of substrate hydrolyzed. Oxidative enzyme analysis was performed using L-3,4 dihydroxyphenylalanine (L-DOPA) as substrate and incubated in the dark for 25 hours. Sample fluorescence of hydrolytic enzymes and oxidative enzyme color absorbance was measured at 360 nm excitation and 460 nm emission, respectively, using a BioTek Synergy HT microplate reader (BioTek Instruments Inc., Winooski, VT, USA).

N-Mineralization, Nitrification, and Ammonification Laboratory Incubation Experiment

During the monsoon of 2019 (September), we collected soils from all experimental plots to perform a 30-day laboratory incubation experiment to assess potential N mineralization, nitrification, and ammonification. Following collection, we passed the soils through a 2mm sieve and established the experiment within 24 hours of collection. For the incubation, we weighed 10 g of dry-equivalent soil into 50-mL centrifuge tubes and added deionized water to bring soils to 60% water holding capacity (WHC). We incubated the soils at room temperature (20 °C) with centrifuge tube lids loosely fitted to reduce soil moisture evaporation losses for 30 days. Throughout the 30-day incubation, we maintained soils at 60 % WHC by supplementing deionized water every two days (Allen and Schlesinger, 2004). We determined initial (at the time of collection) and final inorganic N concentrations following the extraction and nutrient analysis methods for NO_3^- and NH_4^+ (as described above). Net nitrification and ammonification were calculated as the difference between final and initial NO_3^--N or NH_4^+-N concentrations (i.e., final

– initial NO_3^- or NH_4^+ concentrations) (Finzi et al., 2006). Net N mineralization was calculated as the sum of nitrification and ammonification.

NO_x Potential Laboratory Incubation Experiment

In the final year of the study, we performed a laboratory incubation experiment using soils collected from the N fertilization field experiment to measure NO_x (NO_x= NO + NO_2) potential soil gas emissions. We collected soils from plant interspaces in October 2021 (4 years after fertilization treatments began) from Ambient, +N low, and +N High treatment plots at all sites. At the time of collection, plants were fully senesced, and soils were fully dry. Following collection, soil samples were homogenized, passed through a 2 mm sieve, and stored at room temperature until the start of the incubation experiment. Less than 24 hours before the start of the incubation experiment, we measured initial concentrations of available soil NO_3^-, NH_4^+, and PO_4^- (as described above). We weighed 300 g of soil into pint-sized acid-washed glass mason jars for incubation. In the laboratory, we treated soils with water (DI) or N at levels equating to our +N high N field treatment (+N high; 4 kg N ha^{-1} yr^{-1}). Both treatments added water, which brought soils to 20% WHC (i.e., adding either 33 mL of DI or 33 mL of DI + NH_4NO_3). Immediately after adding each treatment, we incubated the soils at room temperature (20 °C) in the dark for 1 and 24 hours, with lids loosely fitted. At 1 and 24 hours, we measured NO and NO_2 fluxes using a chemiluminescent NO_x analyzer (Model T200UP, Teledyne API, USA) dynamic flow-through system. Compressed air containing ambient air flowed through a sealed glass jar containing the treated soils. We recorded the difference between the NO and NO_2 concentrations (nmol mol^{-1}) at steady state, which was typically reached within 1-2 minutes, and the background NO and NO_2 from the air source (typically ~0 nmol mol^{-1} for both NO and NO_2) (Moyes et al. 2010; Hall et al., 2018). To calculate NO flux, NO concentration differences between the steady-state airstream going through the jar and the background concentrations were multiplied by the measured gas flow rate (~0.9 L/min at STP) and divided by the molar density of air (22.41 mol/L). We converted these fluxes to area-specific units using the bulk density of the soil (1.41 g cm^3) and the depth of the soil core (5 cm).

Statistical Analyses

We conducted all statistical analyses using R statistical software version 4.1.2 (R. Development Core Team, 2021) and R studio (RStudio Team, 2021). Data figures were constructed using the ggplot2 package (Wickham, 2016).

The sampling scheme for all response variables is summarized in Table S4.2. To investigate the effects of N addition on responses that were repeatedly measured over time, we performed mixed-effects models (LMM) or generalized linear mixed-effects models (GLMM) for non-Gaussian distributions using the glmmTMB R package (Brooks et al., 2017). We performed data exploration by following the protocol described in Zuur, Ieno, and Elphick (2010). All mixed-effects model analyses included a random effect to account for repeated measures of each plot (Barr et al., 2013). Mixed effects model evaluation was based on an information-theoretic approach using Akaike's Information Criterion corrected for small sample size (AICc, Akaike, 1978; Burnham and Anderson, 2002). Beginning with the maximal model, we used backward step-wise model selection (REML = FALSE for Gaussian models) to eliminate variables that resulted in less than 5 AICc change (Pinheiro and Bates, 2000; Zuur et al., 2009). The best fitting, simplest model was selected based on a 5 AICc improvement. Model validation for LMMs (i.e., normality and homogeneity of residuals) was conducted using the ‘Diagnostics for HierArchical Regression Models’ (DHARMa) package (Hartig, 2021). For GLMMs, model validation involved plotting Pearson residuals against fitted values for all covariates used and not used in the model (Zuur et al., 2009). We report maximal models, final models, and respective model distribution links, e.g., Gaussian (link= identity), beta (link= log), and Gamma (link =log), in supplementary tables (Table S4.3 to S4.9). We used the car package and ANOVA function to compare glmmTMB model fixed effects using the Wald $\chi 2$ statistic (Type II) and the emmeans function from the R package EMMEANS (Lenth, 2020) to conduct Tukey post hoc comparisons. For responses that were measured during the monsoon, pre-monsoon, and winter seasons of each year (except for the first year, where these variables were only measured during the monsoon season; see Table S4.2), we tested the effects of N addition treatments, site, season, and year. For responses only measured once per year, we tested N addition treatments, site, and year (see Table S4.2). For the NOx incubation experiment, we tested the effects of laboratory N addition treatments, field N addition treatments, site, and incubation period (1 or 24 hrs.). We calculated Shannon's diversity (H) and species richness (SR) using the VEGAN R package (Oksanen et al., 2022). The severe drought in 2020 resulted in near-zero values for plant cover across most plots and sites. Therefore, we omitted all 2020 plant data from analyses. Cacti and shrub functional groups were not consistently present across plots and sites and thus were not analyzed. Annual plant species cover was also not consistently present across plots and sites during the first three years of this experiment. However, annual species cover increased dramatically across all sites during the final year. Therefore, annual species response measures were only analyzed during 2021 using a two-way ANOVA with the main factors of N addition and site.

For response variables with a single sampling period (see Table S4.2), we used a two-way ANOVA with the main factors of N addition and site. We assessed two-way ANOVA model residuals for normality using quantile-quantile (Q-Q) plots (Kozak and Piepho, 2018). Data that did not meet ANOVA model assumptions were log-transformed, and interaction terms were removed if they were not significant. ANOVA pair-wise comparisons were performed using Tukey HSD post hoc tests (package EMMEANS). We determined significance at $\alpha = 0.05$ for all data.

RESULTS

Plant Community

Few vegetation cover responses were significantly affected by our N addition treatments and mainly differed by site and year (Table 4.1; Fig. 4.1). Total vegetation cover had a treatment-by-year interaction with lower cover under +N low treatment (relative to Ambient), but only during the first year (Table S4.3; Fig. 4.1a). C_4 perennial grass cover was not significantly affected by any of our N addition treatments but varied by site and year (Table S4.3; Fig. 4.1b). In general, C_4 perennial grass cover was greatest during the first two years of this experiment across all sites and lowest following the severe 2020 summer drought but only in the Native Muhly and Invasive Lovegrass site (Table S4.3; Fig. 4.1c). Perennial forb cover had a treatment-by-site and a site-by-year interaction, with greater cover under the +C addition treatment compared to the ambient but only in the Invasive Lovegrass site (Table S4.3; Figure 4.1b). Annual species cover was low across all plots and sites during the first three years of this experiment and thus was only analyzed during the final year when annual species cover increased dramatically (Table S4.3). There were no significant differences in annual species cover between N addition treatments, but annual species cover differed by site (Table S4.3; Fig. 4.1b,c). Relative cover for *E. lehmanniana* was only assessed in the Invasive Lovegrass site, which was the only site where it was present. *E. lehmanniana* was not significantly affected by N addition treatments (Fig. 4.1d) but differed by year, with significantly lower cover following the 2020 drought, relative to 2018 and 2019 (Table S4.3; Figure S4.1b).

Table 4.1. Summary of responses to a 4-year (2018- 2021) field N simulation experiment in the northern Chihuahuan Desert. (+) indicates a significant positive effect, and (-) indicates a significant negative effect ($p< 0.05$; see supplementary tables S4.3- S4.8 for statistical details).

Response category	**Response variable**	**Significant factors**	**+C**	**+N low**	**+N high**
Relative % Cover	Total	**Treatment: Year,** Site: Year		- (2018)	
	C_4 grasses (all)	Site: Year			
	Perennial forbs	**Treatment: Site**, Site: Year	+ (Invasive lovegrass)		
	Annuals	Site			
	E. lehmanniana	Year			
H	All	Site: Year			
	C_4 grasses (all)	Site: Year			
	Perennial forbs	Site: Year			
	Annuals	Site			
SR		Site: Year			
Foliar	%C	Site: Functional Group			
	%N	**Treatment: Site: Functional Group**		+ (Forbs Native Grama)	
Soil ecosystem properties	pH	Site: Year			
	NO^{3-}	Season, **Treatment: Year**, Site: Year		- (2019)	
	NH_4^+	Site: Season, Site: Year			
	PO_4^{3-}	Site: Season, Site: Year			
	EOC	Site: Season, Site: Year			
	ETN	Site: Season, Site: Year			
	%OC	Site: Year			
	%TN	Site: Year			
Microbial biomass	MBC	Site: Season, Site: Year			
	MBN	Season, Site: Year			
	MBP	Year, Site: Season			
Extracellular enzymes	β-cello	Site: Year			
	β-gluc	Site: Year			
	α-gluc	Site: Year			
	β-xylo	Site: Year			
	NAG	Site: Year			
	LAP	Site: Year			
	Phos	Site: Year			
	Phos-D	Site: Year			
	Phenol	Site: Year			
	Perox	Site: Year			

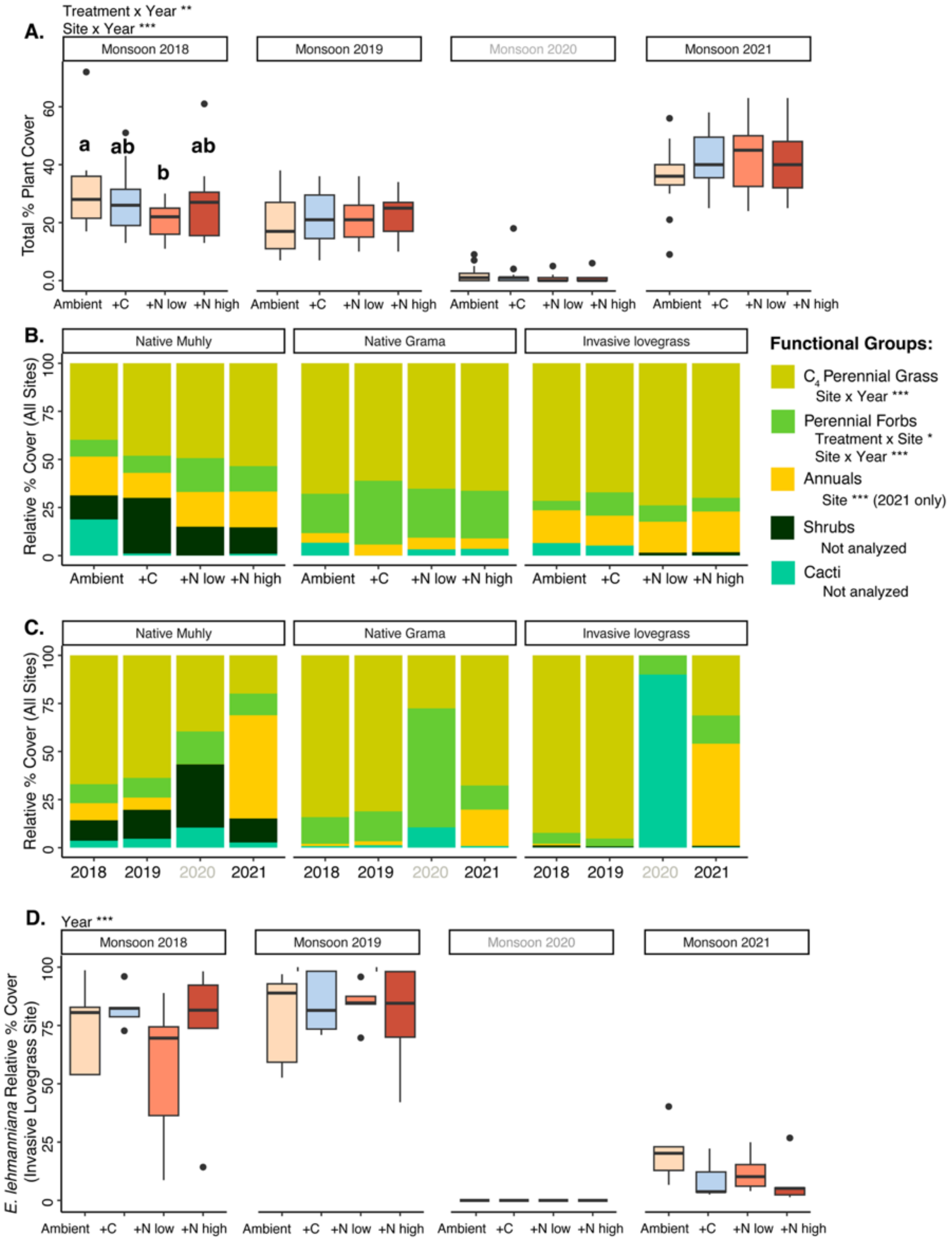

Fig. 4.1. Boxplots of total % plant cover (A), relative % cover of plant functional groups separated by site and N addition treatment (B), relative % cover of plant functional groups separated by site and year (C), and relative % cover of Eragrostis lehmanniana (D) in a 4-year field N addition experiment conducted in the Chihuahuan Desert N.M. USA. Statistical analyses did not include the Monsoon 2020 sampling period. Shrub and cacti functional groups were not analyzed as they were not consistently present in plots. Annual species were also not

consistently present across plots from 2018 and 2019, thus, was only analyzed in 2021. Exotic C4 grass E. lehmanniana was only present and analyzed in the Invasive Lovegrass site. Bold lowercase letters indicate significant treatment effects (estimated marginal means, $p<0.05$). See the top left or legend for significant main and interaction effects (significance codes: *** < 0.001, ** $p<0.01$, * $p< 0.05$, . $p<0.1$.).

None of our measured plant diversity metrics were affected by N addition treatments and mainly varied by site and year (Table 4.1; Fig. S4.2). Shannon's diversity (H) had a site-by-year interaction, with the greatest H observed in 2021 but only in the Native Muhly and Invasive Lovegrass sites (Table S4.4; Fig. S4.2a). H for C_4 perennial grasses and perennial forb functional groups were not significantly affected by N addition treatments (Fig. S4.2c,d,e) but varied by site and year with greater H for C_4 perennial grasses in 2021 (Table S4.4: Fig. S4.3c) and greater H for perennial forbs in 2021 but only in Native Grama site (Table S4.4; Fig. S4.3d). H for annual species was only analyzed in 2021 but also did not differ between N addition treatments and only differed by site (Table S4.4; Fig. S4.3e). SR did not differ between N addition treatments (Table S4.4; Fig S4.2b) but showed a significant year-by-site interaction, with the greatest SR observed in 2021 across all sites and the lowest SR in 2019 but in the Invasive Lovegrass site only (Table S4.4; Fig. S4.3b). Foliar %C did not differ between treatments (Table S4.4; Fig. S4.2f). Foliar %N had a three-way interaction between treatment, site, and functional group, showing greater foliar %N content (relative to the +C treatment) in forbs but only at the Native Grama site (Table S4.4; Fig. S4.2g).

Soil Ecosystem Properties

There were very few transient effects of N addition treatments on soil ecosystem property measures (Table 4.1; Fig. 4.2). The only treatment effect was lower soil NO_3^- in the +N low plots relative to ambient, but only in 2019 (Table S4.5; Figure 4.2b). All other soil ecosystem response differences depended on site, season, and year (Table S4.5; Fig. S4.4. For instance, soil pH was generally greatest during the year with the lowest rainfall (i.e., 2020; Table S4.5; Fig. S4.4a), and soil nutrients, EOC, and ETN concentrations were greatest during the lowest (2020) and highest (2021) rainfall years (Table S4.5; Fig. S4.4c,d,e,f).

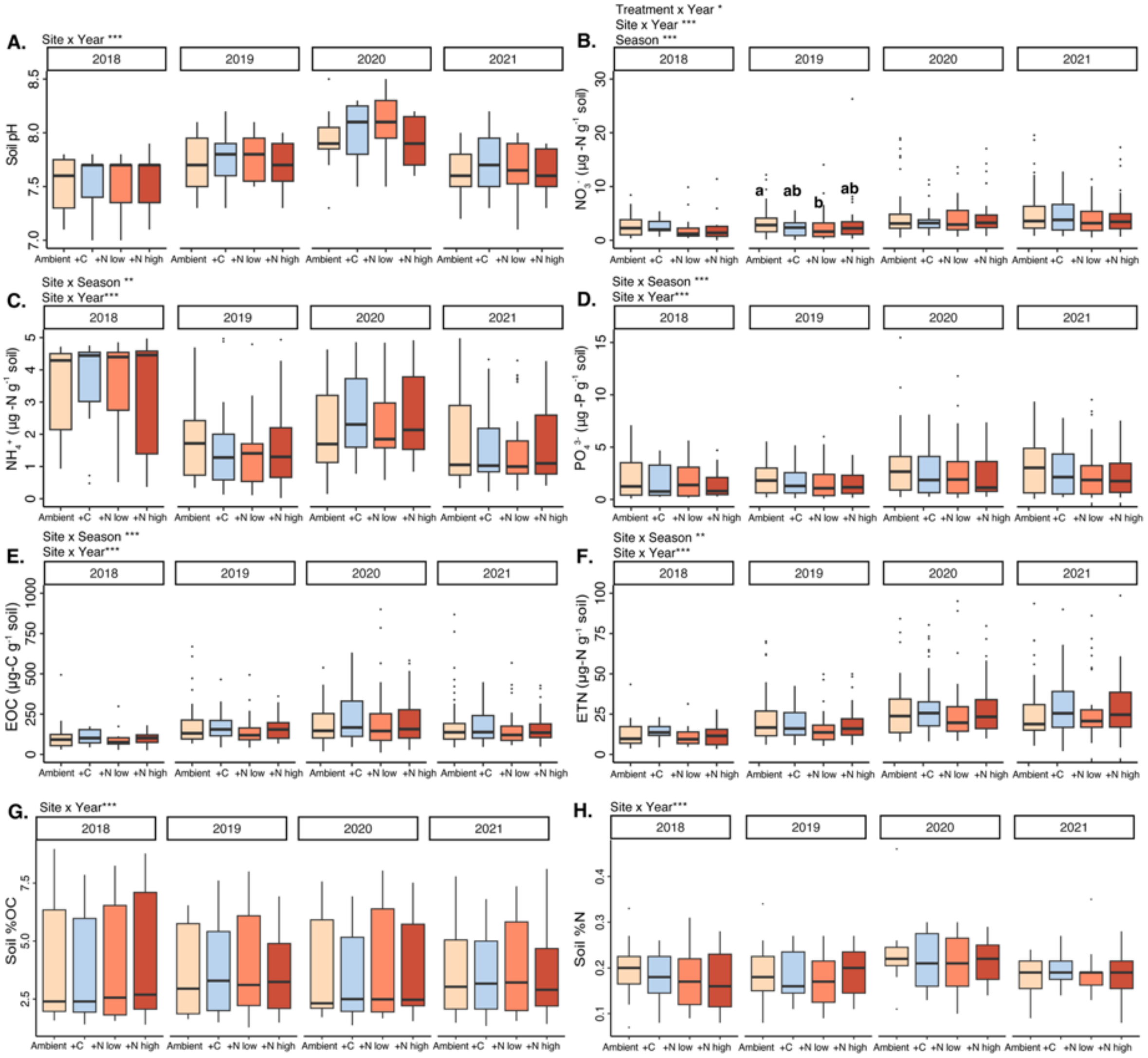

Fig. 4.2. Boxplots of soil ecosystem properties: soil pH (A) available soil nitrate (NO$_3^-$, B), ammonium (NH$_4^+$, C), phosphate (PO$_4^{3-}$, D), extractable organic carbon (EOC, E), extractable total nitrogen (ETN, F), soil %OC (G), and soil %N (H) in a 4-year N addition experiment conducted three adjacent Chihuahuan Desert grasslands, N.M. USA. Bold lowercase letters indicate significant treatment effects (estimated marginal means, $p<0.05$). See the top left or legend for significant main and interaction effects (significance codes: *** < 0.001, ** $p<0.01$, * $p< 0.05$, . $p<0.1$).

Soil Microbial Responses

N addition treatments did not significantly affect soil microbial biomass or extracellular enzyme activities, with differences best explained by site, season, and year (Table 4.1; Table S4.6; Table S4.7; Fig. 4.3). In general, microbial biomass (MBC, MBN, and MBP) was greatest during the highest rainfall year (Table S4.6; Fig. S4.5). Hydrolytic enzyme activities involved in C, N, and P cycling were elevated in 2019 and 2021, with greater oxidative enzyme potentials during 2019 and 2020 (Table S4.7; Fig. S4.7).

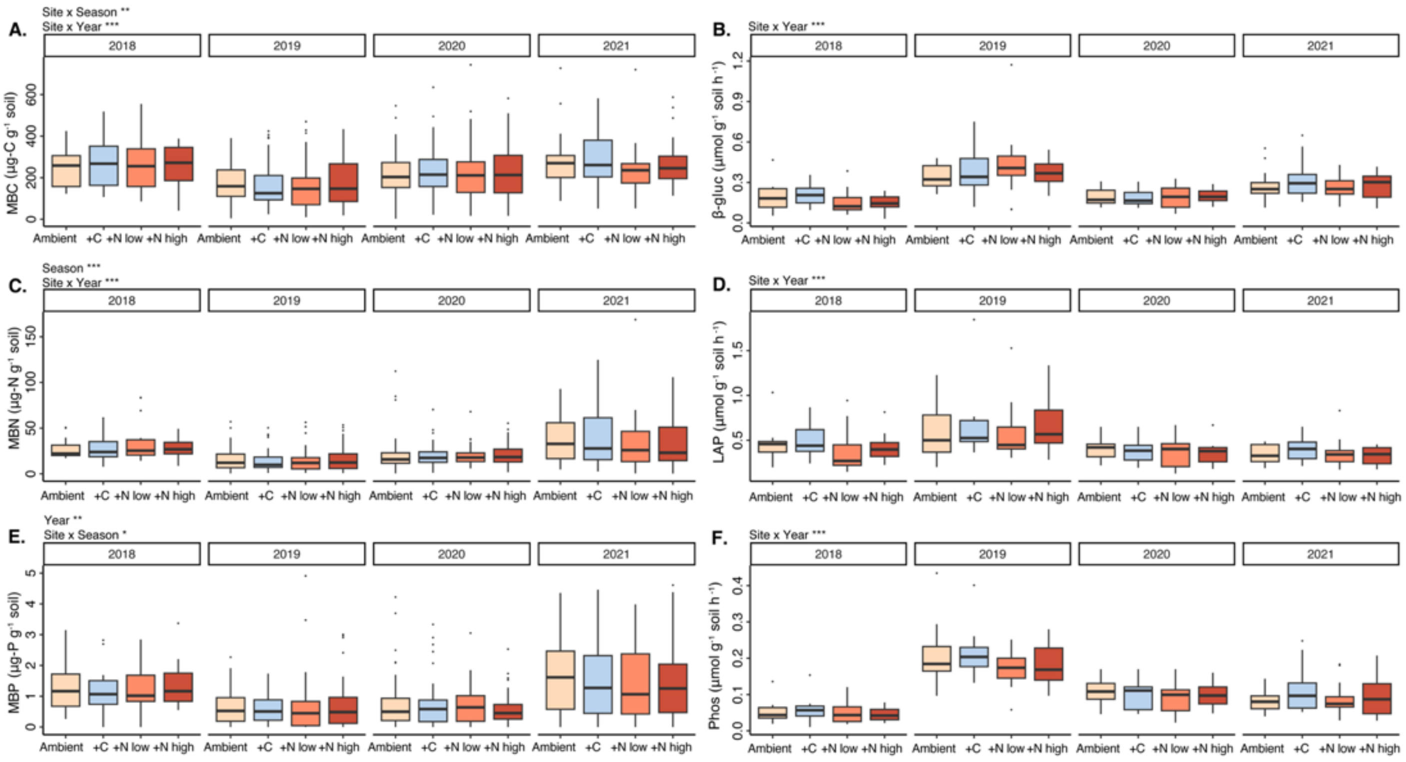

Fig. 4.3. Boxplots of soil microbial biomass carbon (MBC, A), soil microbial biomass nitrogen (MBN, C), microbial biomass phosphorus (MBP, D), and select extracellular enzyme activities involved in C (β-glucosidase, β-gluc, B), N (leucine aminopeptidase, LAP, D), and P-cycling (phosphatase, Phos, F) in a 4-year N addition experiment conducted three adjacent Chihuahuan Desert grasslands, N.M. USA. See the top left of each response for significant main and interaction effects (significance codes: *** < 0.001, ** p<0.01, * p< 0.05, . p<0.1). For the full suite of enzyme activity responses to N additions, see Fig. S4.6.

N-Mineralization, Nitrification, and Ammonification (Incubation Experiment)

At the start of the incubation, initial inorganic N concentrations (NO_3^- and NH_4^+) did not differ between N addition treatments (Table S4.8; Fig. S4.8), but there were some field N addition treatment differences for net N mineralization, nitrification, and ammonification (Table S4.8; Fig. 4.4). Relative to field Ambient soils, net N mineralization and nitrification were lower in +N low field soils (Table S4.8; Fig.4.4b,c). Net ammonification had a treatment-by-site interaction that showed greater ammonification in +N low treatment relative to the ambient and +C treatments, but only in the Invasive Lovegrass site (Table S4.8; Fig. 4.4c).

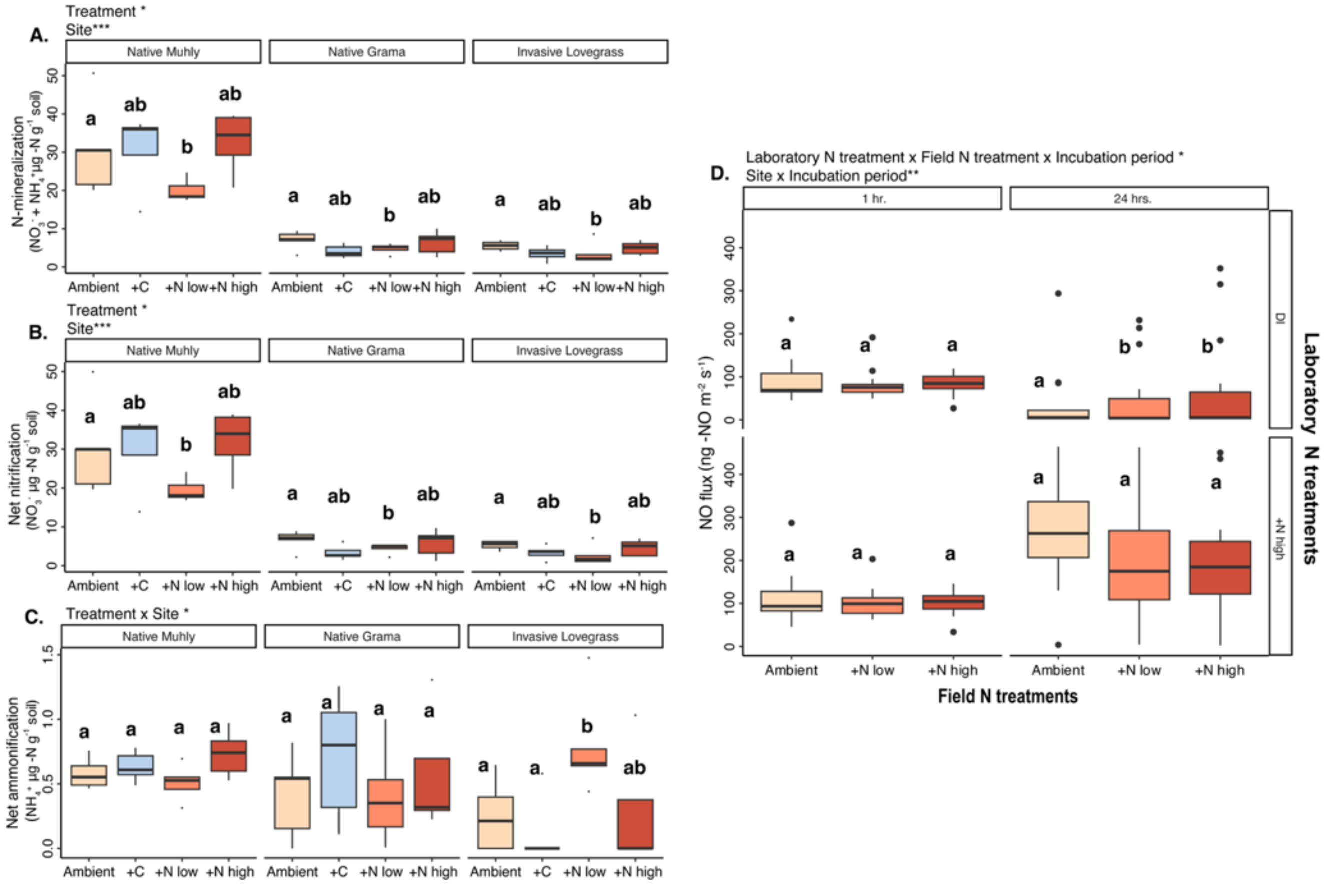

Fig. 4.4. Boxplots of N-mineralization (A), net nitrification (B), and net ammonification (C) in a 30-day laboratory incubation experiment, and nitric oxide (NO) efflux (D) in a 1 and 24-hour laboratory incubation experiment. Soils in the 30-day laboratory incubation experiment were treated in the field with N addition treatments (Ambient, +C, +N low, and +N high) for 2 years and incubated at room temperature at 60% WHC. Soils used in the 1 and 24-hour incubation experiment assessed NO efflux field Ambient, +N low, and +N high soils after 4 years of treatment. In the laboratory, soils were treated with deionized water (DI) or N (+N high), incubated at 20% WHC, and NO efflux was measured at 1 and 24 hours. Bold lowercase letters indicate significant treatment effects (estimated marginal means, p<0.05). See the top left for significant main and interaction effects (significance codes: *** < 0.001, ** p<0.01, * p< 0.05, . p<0.1).

NO_x Incubation Experiment

Initial NO_3^- concentrations differed by site and field N treatments, with lower NO_3^- in both +N low and +N high field treatments relative to the ambient field treatment (Table S4.9; Fig. 4.9a). Initial NH_4^+ and PO_4^{3-} did not differ between field N treatments but did differ by site (Table S4.9; Fig. S4.9b,c). Most NO_2 measurements were below detectable levels for both 1hr and 24hr incubation periods and thus were not analyzed, but NO efflux differed between both field N and laboratory N addition treatments (Table 4.1; Table S4.9). NO efflux had a three-way interaction between laboratory N treatment (i.e., DI or +N high), field N treatments (i.e., Ambient, +N low, and +N high), and laboratory incubation period (i.e., 1 hour and 24 hours) (Table S4.9). This three-way interaction showed greater NO emissions from field N addition treatment soils (+N low and +N high) relative to Ambient field soils, but only at 24 hours and under the laboratory DI addition treatment (Fig. 4.4d).

DISCUSSION

This study explored the effects of simulated low-dose N deposition (0, 2, 4 kg N ha^{-1} yr^{-1}) on plant communities, soil ecosystem properties, and biogeochemical processes in three semi-arid Chihuahuan Desert grassland sites and whether N effects depended on periods of above-average water availability. Overall, periods of drought and above-average precipitation may have complicated responses to N. Nevertheless, contrary to our first hypothesis, experimental N inputs were rarely an important factor driving differences in plant community structure, soil ecosystem properties, and soil microbial biomass and activities. Our study also did not support our second hypothesis, as we did not observe any interactive effects between N additions, supplemental water additions, and years that naturally experienced above-average rainfall. During the final year of our field experiment, we performed a complementary laboratory water and N addition incubation experiment using soils from our Ambient, +N low, and +N high field plots. This incubation experiment revealed that N inputs might be readily lost as NO emissions in our study ecosystem.

Simulated N inputs had little to no effect on plant communities, soil ecosystem properties, and biogeochemical processes

Overall, N addition treatments had few transient effects on our plant community measures. The overall lack of effect on plant communities contrasts other aridland N addition studies that report significant changes to vegetation with N inputs, such as enhanced foliar N content (Hall et al., 2011; Reichmann et al., 2013; Yahdjian et al., 2014), diversity declines (Brooks, 2003; Nui et al., 2018; Wheeler et al., 2021), and enhanced growth of dominant annual and grass species (Mun and Whitford, 1989; Nui et al., 2018; Wheeler et al., 2021), including alien plant species (e.g., Brooks, 2003; Schwinning et al., 2005; Rao and Allen, 2010; Vourlitis, 2017). The lack of N addition effects on our plant community measures could be because of the relatively low amount of N we added. For instance, N-induced soil acidification can result in the loss of soil base cation and increased availability of toxic soil metals (Bobbink et al., 2010; Tian and Nui, 2015) and is considered a prominent driver of change in plant communities (Pardo et al., 2011; Simkin et al., 2016), However, across N addition studies globally, soil pH often decreases linearly with N addition rates, with significant acidification occurring at N inputs rates greater than 50 kg N ha^{-1} yr^{-1} (Tian and Niu, 2015). While aridland N addition studies applying N at relatively low rates are limited relative to those adding N at high rates, our findings coincide with other low-dose aridland N addition studies that report no significant changes to aridland plant communities (e.g., 2, 5, and 8 kg N ha^{-1} yr^{-1} in Philips et al., 2021). However, as we only measured vegetation cover during the monsoon season, seasonal changes to plants with N inputs may have been overlooked. For instance, in other Chihuauhan Desert N addition experiments, N reduced diversity and increased the growth of winter or spring annuals (Gutierrez et al., 1988; Ludwig et al., 1989; Mun and Whitford, 1989). These studies, however, applied N at much higher rates than in the present study (100 kg N ha^{-1} yr^{-1} compared with our 2 and 4 kg N ha^{-1} yr^{-1}, in Gutierrez et al., 1988, Ludwig et al., 1989, and Mun and Whitford, 1989).

Field N additions also had few and often transient effects on our measured soil ecosystem properties, soil microbial biomass, and extracellular enzyme activities. The overall lack of soil responses from our field study contrasts other aridland N addition studies that report changes to soil ecosystem properties and soil microbial biomass and function, such as enhanced soil inorganic N, declines in soil microbial biomass (e.g., Li et al., 2010; Bi et al., 2012; Wang et al., 2020), as well as changes to extracellular enzyme activities such as enhanced C and P cycling enzymes (Stursova et al., 2006; Zeglin et al., 2007) and depressed N- cycling enzyme activities (Stursova et al., 2006; Wang et al., 2020). Additionally, while microbes may be C-limited, our +C addition treatment did not stimulate microbial N immobilization as observed in other aridland studies (Gallardo and Schlesinger, 1995; Schaeffer and Evans, 2005; Choi et al., 2022).

The only effect observed in our field study was lower soil NO_3^- under the +N low treatment, but only during the second year of this experiment (i.e., 2019). Here, lower soil NO_3^- may be due to multiple reasons, such as enhanced plant N uptake (e.g., Hall et al., 2011; Reichmann et al., 2013), increased soil microbial N immobilization (e.g., Choi et al., 2022) or N losses via leaching or gaseous N losses (e.g., McHugh et al., 2017; Osborne et al., 2022a; Krichels et al., 2022). However, while we only measured foliar N during the final year of this experiment, we found no effects on foliar N content with N addition treatments (relative to control). There were also no significant differences in MBN (i.e., N immobilization) with our N amendments. N leaching losses may have also been unlikely, as there was no indication of N accumulation even during the year which experienced severe summer drought (i.e., -71% of the 30-year normal in 2020), which contrasts other aridland studies reporting significant inorganic N accumulation during dry periods (Vourlitis, 2017) even at relatively low simulated N deposition levels (e.g.,10 kg N ha^{-1} yr^{-1} in Stursova et al., 2006).

N enrichment can influence N transformation processes, such as N_2 fixation, N mineralization, nitrification, and denitrification (Li et al., 2010; Ochoa-Hueso et al., 2014), which can have important implications for soil N balance. In our 2019 complementary 30-day laboratory incubation experiment, we found that the +N low treatment resulted in lower net N mineralization, with overall declines linked to lower nitrification (a process that converts NH_4^+ to NO_3^-). N inputs in a Mediterranean semi-arid ecosystem also negatively affected N mineralization and nitrification rates (Ochoa-Hueso et al., 2014). However, unlike Ochoa-Hueso et al. (2014), which linked N mineralization and nitrification declines to soil NO_3^- concentrations, initial soil NO_3^- in our incubation experiment did not significantly differ between treatments. Nonetheless, given that effects in the field were rare and transient, ecosystem properties and soil microbial processes appear insensitive to N inputs.

Like our plant measures, the overall lack of effect on our soil ecosystem properties and biogeochemical measures may have been primarily due to our experimentally low N addition doses. For instance, many aridland studies reporting significant changes to soil chemical properties and microbial processes add N at relatively high rates, with responses often linked to significant declines in soil pH (e.g., >50 kg N ha^{-1} yr^{-1} in Li et al., 2010; Zhang et al., 2013; Wang et al., 2020). In contrast, our findings were similar to other aridland N addition studies conducted in the Colorado Plateau, which added N at relatively low rates and reported little to no effect on a suite soil ecosystem property and soil microbial measures (e.g., 2, 5, and 8 kg N ha^{-1} yr^{-1} in McHugh et al., 2017 and Osborne et al., 2022a). In the Chihuahuan Desert, applications of 10 kg N ha^{-1} yr^{-1} strongly influenced extracellular enzyme activities; however, this effect was linked to aridity and soil properties which allowed N to accumulate during periods of scarce precipitation (Stursova et al., 2006).

Enhanced water availability did not promote responses to simulated low-dose N deposition, but multi-year drought and extremely low to above-average summer rainfall may have complicated responses to N

Many aridland N addition studies show responses to N during above-average rainfall (Chapter 2). Our water addition treatment aimed to increase summer monsoon precipitation by 30% (+55mm). However, as summer rainfall during the year we added water was 70% below the 30-year normal, combined rainfall and supplemental water additions were still well below the long-term average (~50% rainfall deficit relative to the 30-year normal). However, the following year (i.e., 2021), above-average summer rainfall (+25% of the 30-year normal) still did not induce an N addition effect.

In aridlands, altered precipitation regimes, including shifts in precipitation seasonality and dry and wet extremes, have considerable implications on ecosystem structure and function (Nielsen and Ball, 2015; Griffin-Nolan et al., 2019; Holguin et al., 2022). In our study, multi-year drought (2018- 2020), as well as extreme low (2020) and above-average (2021) summer rainfall, may have complicated responses to N addition treatments. For instance, while the first two years also experienced drought, a severe drought in 2020 resulted in near-zero live plant cover. The following year, above-average summer rainfall and earlier than typical monsoon season rainfall (~1.5 months earlier) may have also driven significant changes in our study ecosystem, including the shift from the dominance of perennial grasses in 2018 and 2019 to high annual plant species cover in 2021. As aridland dynamics are first governed by water availability (Noy-Meir, 1973; Austin et al., 2004; Collins et al., 2014), it is likely that the effects due to other global change stressors, e.g., N deposition, may be masked by chronic water limitation and extreme climate events in these ecosystems.

N inputs may be lost via gaseous N loss

Enhanced N availability can stimulate N cycling activities that emit gaseous N, e.g., biotic processes such as nitrification and denitrification and abiotic processes such as ammonia (NH_3) volatilization and chemodentification. In our complimentary NO_x laboratory incubation experiment, N addition field soils released greater NO emissions than field ambient soils. Rapid gaseous N loss may help to explain why we did not detect differences in soil N pools (e.g., soil inorganic N, ETN, MBN, foliar N, which were tested 1 to 2 months following fertilization), as N inputs may have been readily lost from the system. Enhanced gaseous N emissions with N amendments have been observed in other aridland N addition studies (e.g., Hartley and Schlesinger, 2000; McHugh et al., 2017; Eberwein et al., 2020; Krichels et al., 2022). N gas emissions are considered a dominant pathway of N loss in drylands (Peterjohn and Schlesinger, 1990; Homyak et al., 2016) and may explain aridland resistance to N amendments, particularly at lower N doses.

CONCLUSIONS

Our findings suggest that Chihuahuan Desert grassland plant communities, soil ecosystem properties, and soil microbial activities are insensitive to low-dose N inputs even during years experiencing above-average precipitation. However, given the importance of water availability in aridlands, drought and extreme climate events throughout the field study may have complicated responses to N. Aridlands worldwide are expected to experience dramatic shifts in climate regimes (e.g., Seager et al., 2007; Pascale et al., 2017; Bradford et al., 2020), which may make it exceedingly difficult to assess the vulnerability of these ecosystems to other global change stressors. Nevertheless, the overall lack of response to N in our field experiment and findings from our NO_x laboratory incubation experiment suggests that this ecosystem is overall resistant to low levels of N deposition, as N inputs may be readily lost via gaseous N emissions. Heightened gaseous N loss has important implications for air quality in aridland ecosystems experiencing elevated levels of N deposition (e.g., in Carlsbad Caverns National Park; Naimie et al., 2022) and may also offer insight into why some aridland ecosystems appear insensitive to low N inputs.

ACKNOWLEDGMENTS

We thank Michael D. Bell, Emmi Felker-Quinn, Sasha C. Reed, and Marguerite Mauritz-Tozer for their invaluable insight. We would also like to thank Carlsbad Caverns National Park personnel, especially Ellen Trautner and Valerie Morgan, for all their logistical assistance. Special thank you to the army of undergraduates and volunteers who supported field and laboratory operations throughout this study, especially Violeta Mendoza-Martinez, Carina

Sanchez, Anna Maxwell, Mariana Lafon, Michael Bush, Diana Marquez, Cynthia Rae-Moreno, Kathleen Schaeffer, Daniela Aguirre, Catherine Cort, Sydne Spinella, Austin Roy, and Miguel A. Bocanegra. Funds for this research were provided by the United States Department of the Interior, the National Park Service Cooperative Agreement with the University of Texas at El Paso [Cooperative Agreement P17AC01014].

AUTHOR CONTRIBUTIONS

This chapter will be submitted for review in a peer-reviewed journal (*Journal of Ecology*) and is co-authored by Jennifer Holguin (JH), Anthony J. Darrouzet-Nardi (AJD), and Jennie R. McLaren (JRM). JH, AJD, and JRM contributed to the study's conception and design. Data collection was led by JH. Analysis was performed by JH. The first draft of the manuscript was written by JH. JRM provided critical feedback on all versions of the chapter.

DATA ACCESSIBILITY

All data will be made publicly available when the manuscript is published.

CHAPTER 5: THE INTERACTIVE EFFECTS OF *ERAGROSTIS LEHMANNIANA* REMOVALS AND SIMULATED NITROGEN DEPOSITION IN THE NORTHERN CHIHUAHUAN DESERT

ABSTRACT

Exotic plant invasions can drive considerable changes in terrestrial ecosystems by reducing plant diversity and altering soil microbial community structure and function. Non-native plant invasions have been attributed to factors such as changes to competitive interactions between invasive and native plant species as well as environmental change stressors, such as nitrogen (N) deposition. However, management and predictions regarding the spread of non-native plants in drylands are challenged due to the need for more in-depth information on the interaction between changing environmental conditions and plant invasions. Here we performed a four-year exotic grass (*Eragrostis lehmanniana)* removal, recovery, and N addition field study in a Chihuahuan Desert grassland dominated by *E. lehmanniana*. We were interested in determining: [1.] How the presence and removal of *E. lehmanniana* impact plant community structure, soil ecosystem properties, and biogeochemical processes, and if effects persist when removals cease. [2.] If N deposition intensifies the effects of *E. lehmanniana* or impedes *E. lehmanniana* removal efforts. We found that removing *E. lehmanniana* enhanced C_4 native grass cover, perennial forb cover, and diversity but exerted little to no effect on a suite of soil ecosystem properties and biogeochemical processes. Additionally, most positive plant community responses only occurred during years when *E. lehmanniana* was actively removed. However, plant community responses to removals were likely complicated by severe drought and above-average summer precipitation that occurred during years when *E. lehmanniana* removals ceased. We also found no interactive effects between *E. lehmanniana* removal and N addition treatments, with very few main effects of N addition. Beyond *E. lehmanniana* removal and N input effects, the most significant control over *E. lehmanniana* cover appeared to be climate, where *E. lehmanniana* cover significantly declined following the

most severe summer drought and did not rebound despite above-average summer rainfall occurring the following year. Our findings suggest that while *E. lehmanniana* exerts strong effects on native plant communities, but its presence and removal have little effect on soil ecosystem properties and processes, at least in the short term. Additionally, our study suggests that the current extent and future spread of *E. lehmanniana* in the Chihuahuan Desert may not be driven by low levels of N deposition, though it is uncertain if *E. lehmanniana* will benefit from higher N deposition levels. Lastly, we encourage land managers and future research to consider how extreme climate conditions may impede or facilitate *E. lehmanniana* expansion.

INTRODUCTION

Non-native plant invasions threaten biodiversity, ecosystem functioning, and ecosystem services worldwide (Vila et al., 2011; Torres et al., 2021; Xu et al., 2022). Drylands have been experiencing non-native plant invasions at alarming rates (Williams and Baruch, 2000; Hoover et al., 2020; Burruss et al., 2022). Non-native plant invasion and spread can be attributed to several factors, such as shifts in competitive interactions between non-native and native plant species (Vila et al., 2011; Oduor, 2013), as well as environmental change stressors, such as climate change and nitrogen (N) deposition (Williams and Baruch, 2000; Fenn et al., 2003; Burruss et al., 2022). However, in drylands, management and estimations of the current and future extent of non-native plant species are challenged due to the need for more in-depth information on environmental factors (e.g., climate, soils, and disturbance) and often-species-specific mechanisms that may constrain or promote expansion (Mau-Crimmins et al., 2004; Burruss et al., 2022). Improving our understanding of the drivers and environmental conditions that facilitate invasion may help develop effective control strategies and improve predictions regarding the spread of non-native plant species in these ecosystems (Wolfe and Kilironomos, 2005; Inderjit and van der Putten, 2010).

In aridlands (arid and semi-arid ecosystems), the often intentional and accidental introduction of non-native species has dramatically altered ecosystem structure and function. For instance, across the southwestern U.S., the introduction and subsequent spread of alien plant species originating from southern Europe, southern Asia, and South Africa have led to the displacement of native species, diversity losses, and changes to fire regimes (e.g., Simonin, 2001; Brooks et al., 2004; Marshall et al., 2012). Exotic plant invasions in aridlands can also modify soil microbial community composition and function (Kuske et al., 2002; Gornish et al., 2020; Rodriguez- Caballero et al., 2020), which may further facilitate invasion and has important implications on soil carbon (C) and nutrient fluxes and stocks (Inderjit and van der Putten, 2010; Elsheikh et al., 2021).

In the southwestern U.S., one important exotic invasive species of concern is the South African C_4 grass *Eragrostis lehmanniana* Nees. In the 1930s, *E. lehmanniana* was introduced to restore degraded rangelands in Arizona, New Mexico, Texas, and northern Mexico (Cox et al., 1984). The deliberate and accidental spread of *E. lehmanniana* has led to dramatic changes in southwestern aridlands, such as monospecific stands, displacement of native species, diversity losses (Geiger, 2006; Bock et al., 2007), and increases in the intensity and severity of wildfire (McDonald and McPherson, 2011). Since its introduction, *E. lehmanniana* has spread across 1,470,000 acres of southwestern rangelands (Gori and Enquist, 2003) and is expected to continue to spread under current management practices and changing climate regimes (Schussman et al., 2006; Burruss et al., 2022).

The successful spread of *E. lehmanniana* has been primarily attributed to superior competitive abilities relative to native species, e.g., greater water use and drought avoidance strategies (Abbott et al., 1993; Frasier and Cox, 1994), abundant seed production (Anabel et al., 1992), and resilience to fire and grazing (Cox et al., 1990; McClaran and Anabel, 1992; McGlone and Huenneke, 2005). However, limited data also suggest that *E. lehmanniana* may benefit under N-enriched conditions (Billy et al., 1973; Fernandez-Giménez and Smith, 2004). Across the western U.S., while vast portions of land are exposed to low levels of atmospheric N deposition, hotspots of N deposition also occur downwind of large metropolitan centers and adjacent to agricultural and oil and gas operations (Fenn et al., 2003; Cook et al., 2018; Dix et al., 2020). N deposition can have significant impacts on terrestrial ecosystem structure and function (Pardo et al., 2011; Bobbnik et al., 2010), including by enhancing the growth of non-native plants (e.g., Brooks, 2003; Schwinning et al., 2005; Rao and Allen, 2010) and hindering exotic plant removal efforts (Allen et al., 2009; Lyons et al., 2013).

To improve predictions regarding the future extent and management of non-native plant species in drylands, we performed a four-year exotic grass (*E. lehmanniana)* removal, recovery, and N addition field experiment in a semi-arid Chihuauhan Desert grassland dominated by *E. lehmanniana*. In particular, we were interested in determining: $^{1.}$ How does the presence and removal of *E. lehmanniana* impact plant community structure, soil ecosystem properties, and biogeochemical processes? Do these effects persist when removals cease? $^{2.}$ Does N deposition amplify the effects of *E. lehmanniana* on plant communities, soil ecosystem properties, and biogeochemical processes? Do N inputs impede *E. lehmanniana* removal efforts?

For our first hypothesis, we expected *E. lehmanniana* removals would reduce the competition between *E. lehmanniana* and native plants for resources and, thus, would enhance native plant species cover and diversity. We also predicted *E. lehmanniana* removals to impact soil resources, microbial biomass, and activities by reducing the competition for resources (e.g., nutrients) or reducing plant inputs (e.g., root exudates and litter). We also expected the effects of *E. lehmanniana* removals to persist in years immediately following the cessation of *E. lehmanniana* removal treatment (i.e., 1 -2 years post removal). Second, as non-native plants, including *E. lehmanniana*, have been demonstrated to benefit from N enrichment in aridlands, we hypothesized that N inputs would intensify the effects of *E. lehmanniana* on plant communities, soil ecosystem properties, and microbial biomass and activities. We also predicted that N inputs would hinder *E. lehmanniana* removal efforts by enhancing *E. lehmanniana* recovery.

METHODS

Site Description

This study was conducted in a Chihuahuan Desert grassland dominated by exotic C_4 bunchgrass *E. lehmanniana* located on a stream terrace at Carlsbad Caverns National Park (CAVE; 32°10′31″N 104°26′38″W) New Mexico, U.S.A. While dominated by *E. lehmanniana,* the site also contained native perennial grasses such as *Bouteloua gracilis, B. eriopoda,* and *Enneapogon desvauxii*, and forbs such as *Sida abutilifolia, Thymophylla pentachaeta, Ditaxis neomexicana, Talinum spp.,* and *Croton spp*. Soil parent material at the site is characterized as gravelly alluvium derived from limestone (websoilsurvey.sc.egov.usda.gov/). Soil texture is a loamy sand mixture characterized as 71% sand, 25% silt, and 4% clay, with an average pH of 7.6.

The mean annual temperature (MAT) in this region is typically 16.8° C, with a mean annual precipitation (MAP) of 378.7 mm, +60% of which falls during the summer monsoon (typically from July to September). However, MAP and seasonal (summer monsoon) precipitation varied between years (Fig.S1.1; Table S1.1). The first three years received substantially less summer monsoon rainfall than the 30-year normal from June- September (252mm; 1981-2010), with 2018, 2019, and 2020 receiving 50%, 54%, and 71% less rainfall, respectively (Fig. S1.1; Table S1.1).

The final year (2021) received 25% more rainfall than the June- September 30- year normal rainfall (Fig.S1.1; Table S1.1).

Eragrostis lehmanniana Removal, Recovery, and N Addition Field Experiment

We established a factorial *E. lehmanniana* removal, recovery, and N addition field experiment in July of 2018 (prior to the start of the monsoon season). We performed *E. lehmanniana* removals (G= *E. lehmanniana* present and GR= *E. lehmanniana* removed) in 2018 and again in 2019. We removed *E. lehmanniana* by hand by applying herbicide (Round-up™ glyphosate, a non-selective herbicide) using a small paintbrush, followed by clipping and biomass removal, thus having minimal non-target effects on neighbors (McLaren and Turkington, 2010). To assess *E. lehmanniana* recovery and possible legacy effects, we did not perform removals in 2020 and 2021. N addition, treatments were factorially crossed with *E. lehmanniana* removal treatments. N addition treatments were based on modeled CAVE N deposition (~4 kg N ha^{-1} year^{-1}). N addition treatments included a background N deposition treatment (Ambient; ~4 kg N ha^{-1} year^{-1}), +N low (+2 kg N ha^{-1} year^{-1}), +N high (+ 4 kg N ha^{-1} year^{-1}), and a labile C addition treatment, i.e., N reduction treatment (+C; +6 g of sucrose, $C_{12}H_{22}O_{11}$) which was intended to assess N saturation by stimulating microbial N immobilization (Steers et al., 2011). N was applied as ammonium nitrate (NH_4NO_3), and C was applied as sucrose ($C_{12}H_{22}O_{11}$). N addition treatments were dissolved in 2.25 L of water (equating to a 1 mm rainfall event) and sprayed evenly in all plots using a broadcast hand sprayer. Treatments were replicated five times in 1.5 m^2 plots at each site (40 plots total). Plots were separated at a minimum distance of 1.5 m from other plots and large N-fixing plant species (e.g., *Mimosa aculeaticarpa*). N addition treatments were applied annually, before the start of the monsoon season (mid-July) from 2018- 2021 and when performed, following *E.*

lehmanniana glyphosate application and biomass clipping. We applied treatments once annually for four years (2018- 2021) before the start of the monsoon season (mid-July). In 2021, the monsoon season began earlier than usual (early June). Therefore, N addition treatments in 2021 were technically applied during the monsoon season.

Vegetation Sampling

We measured plant cover annually during peak plant biomass (the first week of October for 2018-2020 and the first week of September in 2021). To avoid plot-level edge effects, plant cover measurements excluded spaces within 10 cm of the edge of each plot. Plants were identified at the species level and were grouped into five functional groups: *E. lehmanniana*, native C_4 perennial grasses, perennial forbs, annuals (annual forbs and grasses), shrubs, and cacti. Relative % plant cover was calculated by summing the total cover of all plants within a plot, then dividing the cover of specific species or functional group by the plot's total cover.

Plant Tissue Collection and Analysis

During the final monsoon season of 2021 (i.e., September), we sampled green, fully mature leaves that were free of herbivore damage from plant species that were present across most plots within a site. To avoid plot-level edge effects, plant tissue collection excluded spaces within 10 cm of the edge of each plot. We sampled from grasses *E. lehmanniana* and *E. desvauxii* and perennial forb species *S. abutilifolia*.Leaf samples were dried at 50 °C, ground, and analyzed for foliar C (foliar %C) and foliar N (foliar %N) using a dry combustion C and N analyzer (ECS 4010; Costech Analytical Technologies, Valencia, California, USA).

Soil Sampling

Each year (with the exception of 2018), we collected soils at three time points: pre-monsoon (early June), monsoon (early September), and winter (early February). Soils were randomly collected from each plot (omitting spaces within 10 cm of the edge of each plot) using a 1.9 cm diameter soil corer at a depth of 5 cm. Soil moisture was also measured at a depth of 12 cm during each soil collection period using a soil moisture probe (HydroSense II Soil Moisture Measurement System, Campbell Scientific, Thuringowa Central, QLD, Australia). Following collection, we homogenized the soil samples and passed them through a 2 mm sieve. Soil samples were maintained at 4 °C until further processing (stored for a maximum of 72 hours). Within 24 hours, we froze a subsample at -80 °C for later analysis of soil microbial extracellular enzyme potentials.

Soil Ecosystem Properties

Soil pH was measured from soils sampled during the monsoon sampling season of each year using a 2:1 slurry (dH_2O: soil). Available soil nutrients nitrate (NO_3^-), ammonium (NH_4^+), phosphate (PO_4^{3-}-P), extractable organic carbon (EOC), and extractable total nitrogen (ETN) were measured during all seasons and years. We obtained nutrient, EOC, and ETN extracts by shaking 5 g of fresh soil in 0.5 M K_2SO_4 for two hours and filtering through glass filter paper. All nutrients were assessed using colorimetric microplate assays (BioTEK SynergH.T.HT, Winooski VT, USA). We analyzed available soil NO_3^- -N using a modified Griess reaction (Doane and Horwáth, 2003), soil NH_4^+-N using the Berlethot reaction protocol (Rhine et al., 1998), and PO_4^{3-} -P using a malachite green assay (D'Angelo et al., 2001). We measured EOC and ETN using a Shimadzu analyzer (TOC-VCPN; Shimadzu Scientific Instruments Inc., Columbia, MD, USA). Soil % organic C (soil %OC) and % N (soil %N) was measured once annually from soils collected during the monsoon sampling period. Soil %OC and %N samples were dried at 50 °C, ground, and treated for carbonates using an HCl fumigation protocol (Harris et al., 2001) and then analyzed using a dry combustion C and N analyzer (ElementarPyroCube®)

Soil Microbial Biomass

We analyzed microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), and microbial biomass phosphorous (MBP) using a modification of the chloroform fumigation-extraction technique (Brookes, 1985). We incubated 5 g of fresh soil in 2 mL of ethanol-free chloroform for 24 hours at room temperature. We then extracted fumigated soils and analyzed them for EOC, ETN, and PO_4^{3-}-P (as described above). MBC, MBN, and MBP were calculated as the difference between non-fumigated EOC, ETN, and PO_4^{3-}-P concentrations and fumigated EOC, ETN, and PO_4^{3-}-P concentrations.

Soil Extracellular Enzyme Potentials

Soils collected during the monsoon season of each year were examined for hydrolytic and oxidative enzyme potential activities using standard high-throughput microplate protocols (Saiya-Cork et al., 2002; McLaren et al., 2017). Hydrolytic extracellular enzyme potentials included enzymes involved in C-cycling: α-Glucosidase (α-gluc), β-glucosidase (β-gluc), cellobiohydrolase (Cello), and β-xylosidase (Xylo), N-cycling: leucyl aminopeptidase (LAP) and NAG, and P-cycling: acid phosphatase (Phos) and phosphodiesterase (phos-D). We also measured oxidative enzymes peroxidase (Perox) and phenol oxidase (Phenol), which aid in the decomposition of recalcitrant organic matter. Immediately before each measurement, we thawed 1g of frozen (stored at -80 °C) soil samples at room temperature. Samples were then blended with 125 mL of modified universal buffer at a pH ranging from 7.7 to 7.9 (depending on pH for a given year). Soil slurries were pipetted into 96-well plates, which contained eight analytical replicates per sample. Fluorescing, 4-methylum-belliferone (MUB) tagged substrate (β -D-glucoside, β -D-cellobioside, N-acetyl- β -D-glucosaminide and phosphatase) or 7- amino-4-methylcoumarin (MC) tagged substrate (Leucine amino peptidase) were added to each hydrolytic enzyme assay. Hydrolytic enzyme assays were incubated at room temperature (20 °C) for 4 hours and 30 minutes, with measurements taken every 45 minutes to ensure a linear rate of reaction. Background fluorescence was measured for each soil, substrate, and quenching of MUB or MC (LAP only) by soils, and we used MUB/MC standard curves to calculate the rate of substrate hydrolyzed. Oxidative enzyme analysis was performed using L-3,4 dihydroxyphenylalanine (L-DOPA) as substrate and incubated in the dark for 25 hours. Sample fluorescence of hydrolytic enzymes and oxidative enzyme color absorbance was measured at 360 nm excitation and 460 nm emission, respectively, using a BioTek Synergy HT microplate reader (BioTek Instruments Inc., Winooski, VT, USA).

Statistical Analyses

The sampling scheme for all response variables is summarized in Table S5.1. All statistical analyses were performed using R statistical software version 4.1.2 (R Development Core Team, 2021) and R studio (RStudio Team, 2021). Data figures were constructed using the ggplot2 package (Wickham, 2016).

To investigate the effects of *E. lehmanniana* removal and N addition treatments across seasons and time (i.e., year), we performed mixed effects models (LMM) or generalized linear mixed-effects models (GLMM) for non-Gaussian distributions, using the glmmTMB function of the glmmTMB R package (Brooks et al., 2017). Data exploration was carried out following the protocol described in Zuur, Ieno, and Elphick (2010). LMM and GLMM analyses included a random effect to account for repeated measures of each plot (Barr et al., 2013). Model evaluation was based on an information-theoretic approach using Akaike's Information Criterion correction for small sample sizes (AICc, Akaike, 1978; Burnham and Anderson, 2002). Beginning with the maximal model, we used backward step-wise model selection to eliminate variables that resulted in less than 5 AICc change (Pinheiro and Bates, 2000; Zuur et al., 2009). The best fitting, simplest model was selected based on a 5 AICc improvement. For LMMs, model validation (i.e., normality and homogeneity of residuals) was conducted using the 'Diagnostics for HierArchical Regression Models' (DHARMa) package (Hartig, 2021). For GLMMs, model validation involved plotting Pearson residuals against fitted values for all covariates used and not used in the model (Zuur et al., 2009). Following model selection and validation, we performed parametric bootstrapping to obtain 95% confidence intervals (CI) by first performing a single parametric bootstrap of the original model data using the glmmTMB *refit* function, then performing multiple refits (i.e., simulations, N = 1000) using the *bootMer* function (glmmTMB bootstrapping details in Brooks et al., 2023) of the lme4 package (Bates et al., 2015). A CI spanning zero was considered nonsignificant. We report respective maximal models, final models, and distributions, e.g., Gaussian (link= identity), beta (link= log), Gamma (link =log), etc., model AICc, marginal R^2 (fixed effects), conditional R^2 (fixed and random effects) from the MuMIN package (Barton, 2016; Johnson, 2014; Nakagawa and Schielzeth, 2013), and bootstrapped CIs in result tables (supplementary materials).

For all analyses investigating treatment effects across seasons and years (Table S5.1), the non-grass removal treatment (i.e., G) and Ambient N treatment during the pre-monsoon season of

2018 were set as the reference level and are represented by the intercept. Because we only collected data during the monsoon season in 2018, we were unable to conduct a fully factorial (i.e., treatment by season by year) analysis. All analyses investigating treatment effects over time (i.e., year; Table S5.1), non-grass removal treatment (i.e., G), the Ambient N treatment, and the year 2018 were set as the reference level and represented by the intercept. Shannon's diversity (H) and species richness (SR) were calculated using the VEGAN R package (Oksanen et al., 2022). However, due to the extreme drought in 2020, most relative percent plant cover values in 2020 were zero, resulting in non-convergence of models, even with zero-inflated GLMM methods. Thus, all 2020 plant % cover data were omitted from analyses. Cacti and shrub functional groups were not consistently present across plots and thus were not analyzed. For response variables with a single sampling period (Table S5.1), we performed a two-way ANOVA (with the main factors of N addition and grass removal treatments. Interaction terms were removed when not significant (significance at α= 0.05). H for the annual plant species functional group was low from 2018 to 2020, resulting in model non-convergence. Thus, annual species H was only analyzed during 2021. ANOVA model residuals were assessed for normality using quantile-quantile (Q-Q) plots (Kozak and Piepho, 2018). ANOVA post hoc comparisons were performed using Tukey HSD post hoc tests.

RESULTS

Plant Community Responses

Overall, vegetation cover differences were best explained by *E. lehmanniana* removal treatments and year, with no significant differences between N addition treatments (Table S5.2; Fig. 5.1). In particular, vegetation cover changes were dependent on years when *E. lehmanniana* was actively removed (i.e., 2018 and 2019), as well as year-to-year differences that were likely associated with years with well-below (-71% summer rainfall relative to the 30-year normal in 2020) and well-above (+25% of 30-year normal average summer rainfall in 2021) summer precipitation. In 2018 and 2019 (when *E. lehmanniana* removals were performed), total relative vegetation cover and *E. lehmanniana* cover were lower under the GR treatment relative to the G treatment (Table S5.2; Fig. 5.1a,b). However, during the year with the wettest summer (i.e., 2021), which was the year following the year with the driest summer (i.e., 2020 data not analyzed), total vegetation cover was well above the cover observed in 2018 and 2019, but *E. lehmanniana* cover was significantly lower than in 2018 and 2019, with diminished differences between removal treatments (Table S5.2; Fig. 5.1a,b). Native C_4 perennial grass cover was also altered by removal treatments in 2018 and 2019, with greater relative native C_4 perennial grass under the GR treatment, but by 2021, significant differences between removal treatments diminished (Table S5.2; Fig. 5.1c). Unlike perennial grasses, perennial forb cover was greater under the GR relative to G across all years (i.e., 2018, 2019, and 2021, 2020 data not analyzed; Table S5.2; Fig. 5.1d). Annual plant cover was relatively low in cover from 2018 to 2020 and did not differ between removal treatments but did increase substantially in 2021 (Table S5.2; Fig; 5.1e). Foliar %C and %N varied among species but did not significantly differ between removal or N addition treatments (Table S5.2; Fig. 5.1f,g).

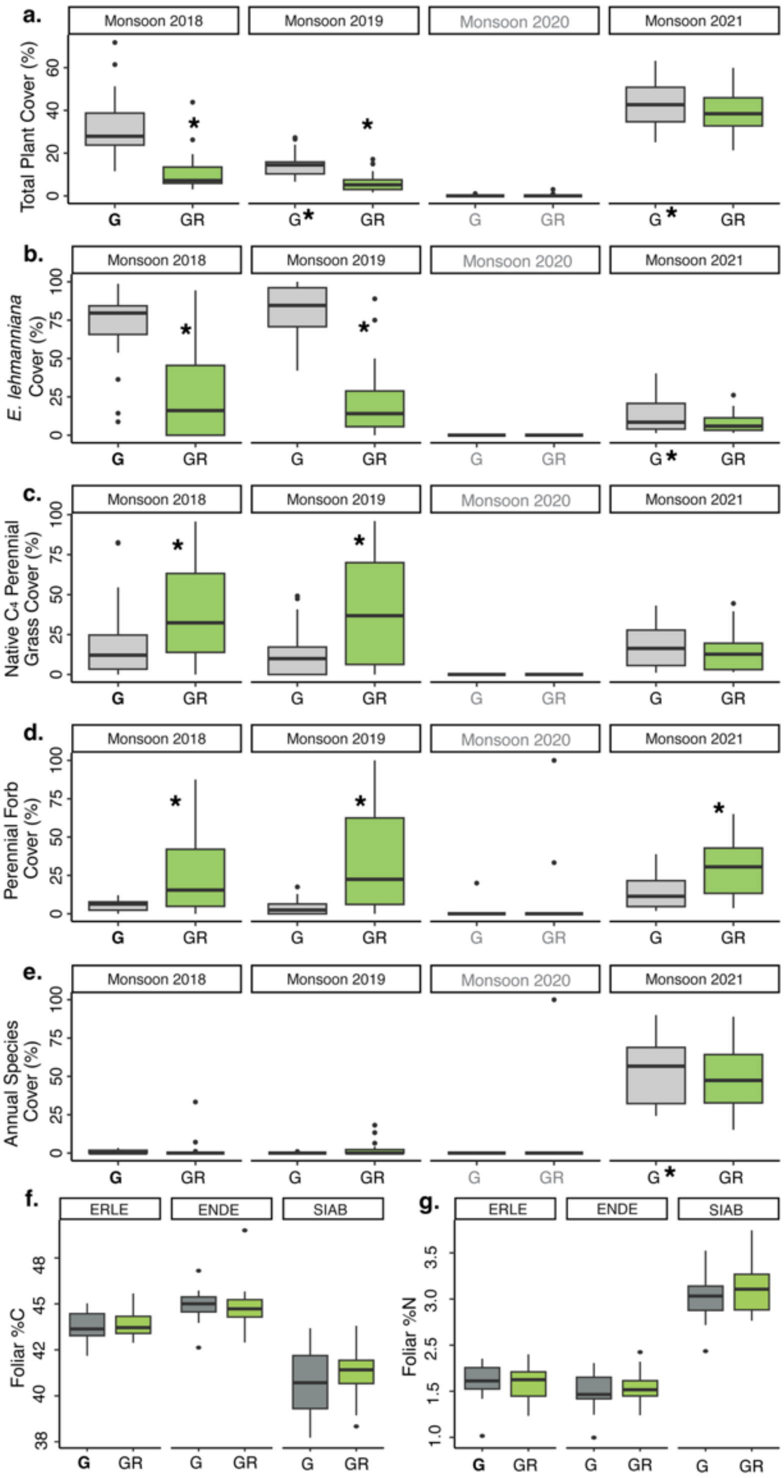

Fig. 5.1. Boxplots of relative % total plant cover (a), *Eragrostis lehmanniana* cover (b), native C$_4$ perennial grass cover (c), perennial forb cover (d), annual plant species cover (e), foliar %C (f), and foliar %N (g) in a 4-year invasive grass removal (2018 and 2019), recovery (2020 and 2021), and N addition manipulation experiment. Statistical analysis for plant cover did not include the Monsoon 2020 sampling period. Foliar measurements were only assessed during the final year of this study and included exotic C$_4$ grass *E. lehmanniana* (ERLE), native C$_4$ grass *Enneapogon desvauxii* (ENDE), and forb *Sida abutilifolia* (SIAB). Removal treatments include *E. lehmanniana* present (G), and *E. lehmanniana* removed (GR). Asterixis above GR boxplots indicate significant

differences between treatments relative to control (first G on the x-axis in bold). Asterix next to G (x-axis) indicates a significant change in control relative to G in 2018.

Species richness (SR) was not affected by removal treatments or N addition treatments but significantly declined in 2019 and increased in 2021 (Table S5.3; Fig. 5.2a). Shannon's diversity (H) differed between *E. lehmanniana* removal treatments and year, but N-addition treatments were not a significant factor in H differences (Table S5.3; Fig 5.2b). Overall, H was greater under the GR treatment in 2018 and 2019, but these differences diminished by 2021 after removals had stopped (Fig. 5.2a). Additionally, relative to 2018, H in non-removal plots significantly declined in 2019 and increased significantly in 2021 (Table S5.3; Fig. 5.2a). H for different functional groups did not differ between removal treatments (Table S5.3; Fig 5.2c,d,e).

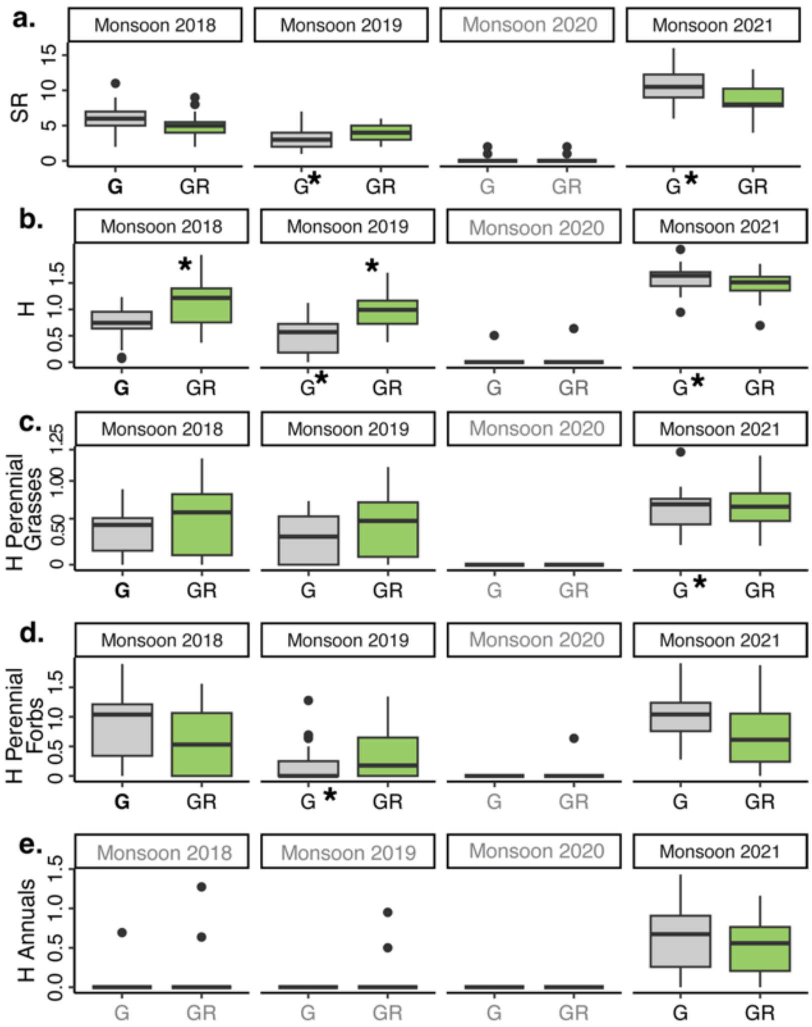

Fig. 5.2. Boxplots species richness (SR, a), Shannon's diversity (H, b), H for perennial grass functional group (c), H for perennial forb functional group (d), and H for annual species functional group (e) in a 4-year invasive grass removal (2018 and 2019), recovery (2020 and 2021), and N addition manipulation experiment. Statistical analysis for a-d did not include the Monsoon 2020 sampling period, and e did not include Monsoon 2018 to 2020 sampling periods. Removal treatments include *E. lehmanniana* present (G), and *E. lehmanniana* removed (GR). Asterix above GR boxplots indicate significant differences between treatments relative to control in 2018 (first G on the x-axis in bold). Asterix next to G (x-axis) indicates a significant change in control relative to G in 2018.

Soil Ecosystem Responses

Despite changes in plant community cover and diversity, most soil ecosystem properties were not significantly affected by *E. lehmanniana* removal nor by N addition treatments; instead, differences were primarily associated with year and season (Table S5.4; Fig. 5.3). Soil pH varied across years and was lowest in 2018 and highest in 2020 (Table S5.4; Fig. 5.3a). Available soil

NO_3^- had a significant interaction between *E. lehmanniana* removal treatments and years 2020 and 2021 (Table S5.4). Here, relative to the G treatment in 2018, soil NO_3^- was higher in both removal treatments in 2020 and 2021, with similar NO_3^- concentrations between removal treatments within each year (Fig. 5.3b). Available soil $NH4^+$ and PO_4^{3-} mainly varied by year with higher concentrations in 2020 and 2021 (Fig. 5.3c,d). EOC and ETN concentrations also showed year-to-year differences, with greater EOC and ETN concentrations in 2019, 2020, and 2021 relative to 2018 (Fig. 5.3e,f). Like soil NO_3^-, ETN also had a significant removal-by-year interaction during 2020 and 2021; however, ETN also increased in both G and GR treatments in 2020 and 2021 relative to G 2018, but there were no post-hoc differences detected between G and GR treatments (Table S5; Fig. 5.3e). Soil %OC and %N varied over time but did not significantly differ between removal or N addition treatments (Table S5.4; Fig. 5.3g,h).

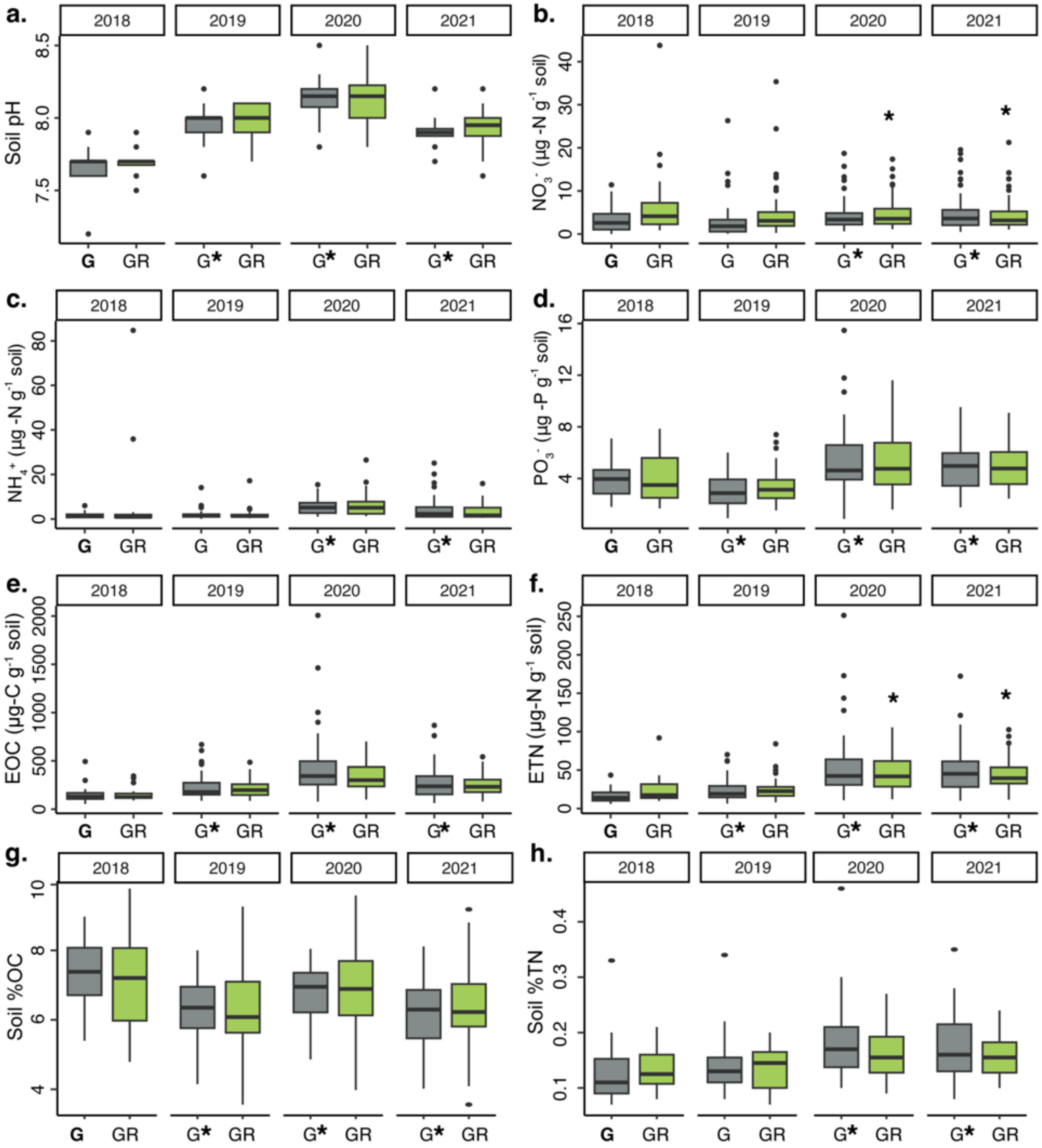

Fig. 5.3 Boxplots of soil ecosystem properties: pH (a), available soil nitrate (NO$_3^-$, b), available soil ammonium (NH$_4^+$, c), available soil phosphate (PO$_4^{3-}$, d), extractable organic carbon (EOC, e), extractable total nitrogen (ETN, f), soil % organic C (%OC, g), soil % N (%N, h) in a 4-year invasive grass removal (2018 and 2019), recovery (2020 and 2021), and N addition manipulation experiment. Removal treatments include *E. lehmanniana* present (G), and *E. lehmanniana* removed (GR). Asterix above GR boxplots indicate significant differences between treatments relative to control in 2018 (first G on the x-axis in bold). Asterix next to G (x-axis) indicates a significant change in control relative to G in 2018.

Soil Microbial Biomass and Extracellular Enzyme Activities

MBC, MBN, and MBP were not significantly affected by *E. lehmanniana* removal nor N addition treatments but did often differ by season and year (Table S5.5; Fig. 5.4). Relative to 2018, MBC declined in 2019 and 2020 (Fig. 5.4a) MBN declined in 2019 (Fig. 5.4c), and MBP declined in 2019 but also increased in 2021 (Fig. 5.4e). Most extracellular enzyme activity potentials also varied by year, with generally increasing and decreasing hydrolytic enzyme activity potentials in 2019 and 2020, respectively, and greater oxidative enzyme activities in 2019 and 2020 (Table S5.6; Fig 5.4b,d,f and S5.1). *E. lehmanniana* removal and N addition treatments showed few but transient effects on extracellular enzyme activity potentials (Table S5.6; Fig. 4 and S5.1). Most *E. lehmanniana* removal treatment effects on extracellular enzyme activities occurred in 2019, with generally lower C, N, and P cycling hydrolytic enzymes (Fig. 4b,f and Fig. S5.1). N addition treatments affected only NAG activity, but treatment effects were only evident in some years (Table S5.6). Relative to the Ambient N treatment in 2018, the +N low and +N high treatments had greater NAG potential activity in 2018 and 2019, respectively, with no significant differences in 2020 and 2021 (Fig. S5.2).

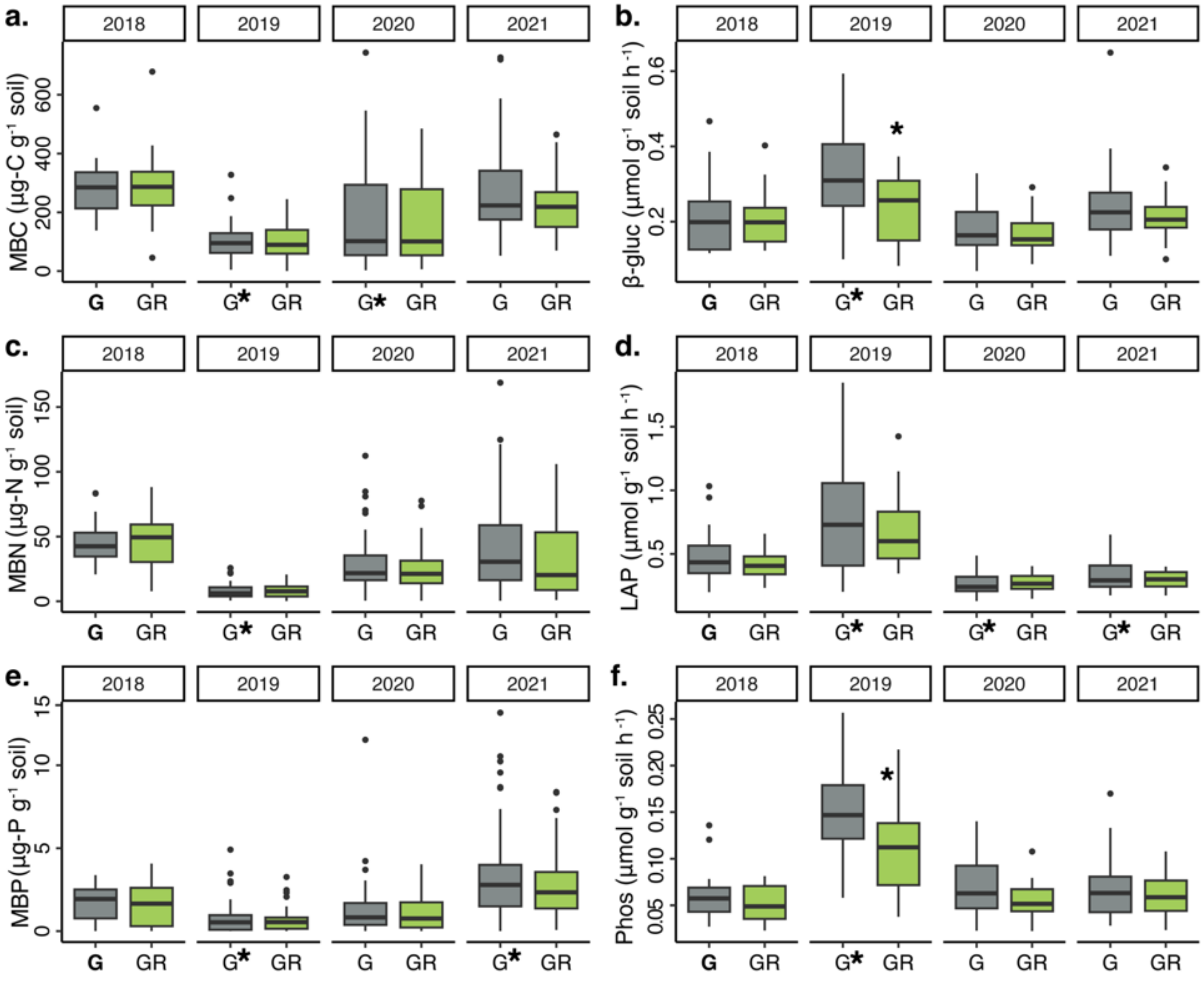

Fig. 5.4. Boxplots of soil microbial biomass carbon (MBC, a), soil microbial biomass nitrogen (MBN, c), soil microbial biomass phosphorus (MBP,e), extracellular hydrolytic enzymes β-glucosidase (β-gluc, b), leucyl aminopeptidase (LAP, d), and acid phosphatase (Phos, f) in a 4-year invasive grass removal (2018 and 2019), recovery (2020 and 2021), and N addition manipulation experiment. Removal treatments include *E. lehmanniana* present (G), and *E. lehmanniana* removed (GR). Asterix above GR boxplots indicate significant differences between treatments relative to control in 2018 (first G on the x-axis in bold). Asterix next to G (x-axis) indicates a significant change in control relative to G in 2018.

DISCUSSION

In an *E. lehmanniana* dominated Chihuauhan Desert grassland, we examined how 1. the presence and removal of *E. lehmanniana* impact plant community structure, soil ecosystem properties, and biogeochemical processes, and whether these effects persist when removals cease. 2. If N deposition augments the effects of *E. lehmanniana* on plant communities, soil ecosystem properties, and biogeochemical processes, and whether N inputs impede *E. lehmanniana* removal efforts. Our findings only partially supported our first hypothesis, which showed greater native plant cover, diversity, and species richness when *E. lehmanniana* was removed. However, while most plant community effects associated with the removal treatment diminished during the years after the removal treatment ceased, responses may have been complicated by well below (-71% of the 30-year normal in 2020) to well above (+25% of the 30-year normal in 2021) average summer precipitation. Additionally, despite *E. lehmanniana* removal effects on aboveground plant communities, we found little and often transient effects of *E. lehmanniana* removal treatments on soil ecosystem properties and biogeochemical measures. We found no support for our second hypothesis, as the effects of removal on plant community structure, soil ecosystem properties, biogeochemical measures, and *E. lehmanniana* recovery did not interact with our N addition treatments.

Plant community responses were associated with *E. lehmanniana* removal treatments and study year. While the positive removal effect on perennial forbs was maintained throughout this study, the positive effect of removal on native C_4 grass cover and diversity only occurred during years when *E. lehmanniana* was actively removed (i.e., 2018 and 2019). In general, our findings agree with other *E. lehmanniana* removal studies, which demonstrate an enhanced abundance and diversity of native species when *E. lehmanniana* is removed (Buerdsell and Lehnhoff, 2023; Crimmins and McPherson, 2008). Other studies also suggest that *E. lehmanniana* may need to be repeatedly removed to deplete *E. lehmanniana* seed reserves (Crimmins and McPherson, 2008). However, the diminished removal effect on native C_4 grass and diversity after removals ceased was likely complicated by the subsequent extreme summer climate conditions. For instance, in

2020, the most severe summer drought of the study occurred and resulted in near-zero live plant cover. In the year following 2020, above-average summer rainfall resulted in a dramatic shift in plant community composition, from one dominated by *E. lehmanniana* to one dominated by annual plant species regardless of removal treatment. Thus, extreme summer drought and high rainfall may complicate the effectiveness of *E. lehmanniana* removals on aridland plant community dynamics.

E. lehmanniana is considered a highly drought-tolerant species (Williams and Baruch, 2000), capable of producing up to four times more biomass forage than native grasses during dry summers (Cox et al., 1990). In our study, the greatest *E. lehmanniana* cover was observed in 2018 and 2019, which received 50% and 54% less summer rainfall than the 30-year summer normal, respectively. However, in 2020, all grass cover, including *E. lehmanniana*, was zero. Our study agrees with other Chihuahuan Desert studies that report significant declines in *E. lehmanniana* and native grasses during extreme dry summer conditions (Buerdsell et al., 2022). Additionally, while above-average summer precipitation has been demonstrated to significantly increase *E. lehmanniana* production relative to below or equal summer long-term average precipitation (Cox et al., 1990), above-average summer rainfall in 2021 still resulted in low *E. lehmanniana* cover relative to previous years (i.e., 2018 and 2019) after having cover significantly reduced in 2020. Due to climate differences, the Chihuauhan Desert is considered relatively more resistant to E. lehmanniana invasion than the Sonoran Desert (Burruss et al., 2021). However, shifts in climate namely increases in temperature and precipitation, are expected to enhance *E. lehmanniana* recruitment in the Chihuahuan Desert (Burruss et al., 2021). Though, it may also be critical to consider how extreme climate conditions may impede or facilitate *E. lehmanniana* expansion.

Enhanced diversity and cover of annual species following severe drought may help to support ecosystem stability and functioning during future droughts (Griffin-Nolan et al., 2019). Despite near-zero plant cover during the 2020 monsoon season, above-average summer rainfall in 2021 resulted in the highest total vegetation cover, diversity, and species richness of all study years. Dry years (as seen during the first two years of this study) often result in low functional diversity due to the dominance of drought-tolerant grasses, with less common species employing drought avoidance and escape strategies, e.g., slower growth or early flowering (Gherardi and Sala 2015). In contrast, wet years (such as that seen in 2021) showed an increased abundance of drought avoiders, such as fast-growing annual species and deep-rooted forbs, which can readily utilize large rainfall events that permeate deeper soil profiles (Gherardi and Sala, 2015). In addition to the severe summer drought in 2020 which drastically reduced all plant cover, drought avoidance and adaptations may explain the shift in the dominance of *E. lehmanniana* cover to annual plant cover from 2018 and 2019 to 2021 and the persistence of higher perennial forb cover (unlike native C_4 grass cover) in *E. lehmanniana* removal plots in 2021.

Overall, removing *E. lehmanniana* had little and often transient effects on measured soil ecosystem properties and, perhaps consequently, soil microbial responses. Our findings contrast other studies which suggest that non-native plant invasions, including that by E. Lehmanniana, modify their invaded soil environment by altering soil nutrient availability and microbial community composition and function (Torres et al., 2021; Xu et al., 2022). However, it is possible that four years of treatment may not be enough time to detect differences in soil microbial function following grassland restoration practices (Potthof et al., 2006; Carey et al., 2015). Additionally, due to the nature of our measurements (i.e., focus on microbial function rather than composition), changes to soil microbial community composition may have occurred, as demonstrated in other aridland exotic plant removal experiments (Kuske et al., 2002; Gornish et al., 2020; Williams et al., 2021).

Beyond the effects of non-native plants on soil ecosystem properties and microbial function, the mass ratio hypothesis suggests that highly abundant species can substantially affect ecological processes, such as energy flow and biogeochemical cycling, due to their high biomass and distribution (Grime, 1998). However, despite being the dominant form of biomass, the few and primarily transient (i.e., only occurred in one year) effects of *E. lehmanniana* removals also contrast studies that report dominant species removals to significantly impact ecosystem properties and function, e.g., soil nutrient availability (e.g., Bret-Harte et al., 2004; Wardle and Zackrisson, 2005; McLaren and Turkington, 2010), microbial biomass (Chen et al., 2016), and enzyme activities (Melendez-Gonzalez et al., 2018). Instead, typical of water-limited ecosystems, soil ecosystem properties and microbial activity dynamics were best explained by seasonal and year-to-year differences, which likely reflect differences in water availability (Bell et al., 2009; Brown et al., 2022).

N addition treatments had no direct effects and did not interact with *E. lehmanniana* removal for effects on plant community structure, including *E. lehmanniana* recovery. Our findings contrast other aridland N addition studies that report changes to plant community composition (Baez et al., 2007; Zeng et al., 2010), declines in diversity (Mun and Whitford, 1989; Nui et al., 2018; Wheeler et al., 2021), and enhanced growth of non-native plant species (Brooks, 2003; Allen et al., 2009; Vourlitis, 2017). Dryland plant community response to N amendments can be strongly linked to water availability (e.g., Ladwig et al., 2011; Whitford and Steinberger, 2011; Wheeler et al., 2021). Thus, the lack of responses to our N amendments may have been due to the below-average rainfall during the first three years of this experiment. However, unlike aridland N addition studies that report N addition responses following above average precipitation (e.g., Brooks, 2003; Schwinning et al., 2005; Allen et al., 2009; Rao and Allen, 2010) N was still not an important factor driving change during the final year of this experiment which experienced above average summer rainfall.

N addition treatments also had no direct nor interactive effects with *E. lehmanniana* removal on soil ecosystem properties or biogeochemical measures. Overall, our findings contrast other aridlands N addition studies which report significant changes to soil chemical properties and biogeochemical processes, such as declines in soil pH, enhanced levels of inorganic N, reductions in soil microbial biomass (e.g., Li et al., 2010; Wei et al., 2013; Wang et al., 2020), and changes to soil extracellular enzyme activities (Stursova et al., 2006; Sinsabaugh et al., 2015; Vourlitis et al., 2021). However, other aridland N addition studies have reported no significant effects of N inputs on soil ecosystem properties and biogeochemical measures (Osborne et al., 2022a). Additionally, evidence from aridland exotic grass removal and N addition experiments have reported little to no significant interactive changes to soil microbial communities with removal and N inputs (Carey et al., 2015; Williams et al., 2022).

The overall lack of response to N inputs suggests that this ecosystem is resistant to the effects of N enrichment, including its effects on exotic plants. However, because we intended to apply N at a rate similar to modeled N deposition in our study region, insensitivity to N may be due to the relatively low amount of N, we added. For instance, aridland N addition studies demonstrating strong responses to N amendments, including effects on alien plant species, add N at relatively higher rates (e.g., 32 kg N ha^{-1} yr^{-1} in Brooks, 2003; 40 kg N ha^{-1} yr^{-1} in Schwinning et al., 2005; 50 kg N ha^{-1} yr^{-1} in Vourlitis, 2017). In contrast, other studies adding N at similar lower doses report no significant change to plant community structure (including invasive plant cover) and soil microbial community structure and function (Philips et al., 2021; McHugh et al., 2017; Osborne et al., 2022a). Other relatively low N addition dose studies in the Chihuahuan Desert have reported effects on soil microbial enzyme activities (Stursova et al., 2006). However, effects in Stursova et al. (2006) were linked to soil N accumulation occurring during periods of scarce precipitation. In contrast, our study found no indication of N accumulation in N addition plots, even during the most severe drought year (i.e., 2020) or during years when *E. lehmanniana* (the dominant form of biomass in this ecosystem) was removed. This overall insensitivity to N inputs, including the lack of N accumulation during periods of drought or with *E. lehmanniana* removal treatments, suggests that N inputs may have been readily lost from the system via leaching, or perhaps more likely gaseous N loss, as observed in other aridland N amendment studies (e.g., Reichmann et al., 2013; McHugh et al., 2017; Krichels et al., 2022).

CONCLUSIONS

In conclusion, we found few effects of removing a dominant invasive grass or N-additions in a 4-year experiment in a Chihuahuan Desert grassland. Removing *E. lehmanniana* increased C_4 native grass cover, perennial forb cover, and diversity but exerted little to no effect on a suite of measured soil ecosystem properties and biogeochemical processes. While most positive plant community responses only occurred during years when *E. lehmanniana* was removed, responses were likely complicated by severe drought and above-average summer precipitation that occurred during years when *E. lehmanniana* removals stopped. We also found no interactive effects between *E. lehmanniana* removal and N addition treatments and very few main effects of N addition, which we attribute to the realistically low N-addition rates used in this study (same order of magnitude as modeled N-deposition for the area). Thus, the future spread of *E. lehmanniana* in the Chihuahuan Desert may not be driven by low levels of N deposition, but it is uncertain if *E. lehmanniana* will benefit from higher N deposition levels. The most significant control over *E. lehmanniana* cover appeared to be climate, where *E. lehmanniana* cover significantly declined following the most severe summer drought and did not rebound despite above-average summer rainfall occurring the following year. In the Chihuahuan Desert, shifts in climate, namely, rising temperatures and precipitation, are predicted to enhance *E. lehmanniana* recruitment (Burruss et al., 2022). While predictions of the magnitude and direction of change to the North American Monsoon are unclear (Bukovsky et al., 2015), understanding how extreme climate conditions impede or facilitate *E. lehmanniana* expansion may be critical for future land management decisions.

ACKNOWLEDGMENTS

We thank Michael D. Bell, Emmi Felker-Quinn, and Marguerite Mauritz-Tozer for their invaluable insight. We would also like to thank Carlsbad Caverns National Park personnel, especially Ellen Trautner and Valerie Morgan, for all their logistical assistance. Special thank you to the army of undergraduates and volunteers who supported field and laboratory operations throughout this study, especially Violeta Mendoza-Martinez, Carina Sanchez, Anna Maxwell, Mariana Lafon, Michael Bush, Diana Marquez, Cynthia Rae-Moreno, Kathleen Schaeffer, Daniela Aguirre, Catherine Cort, Sydne Spinella, Austin Roy, and Miguel A. Bocanegra. Funds for this research were provided by the United States Department of the Interior, the National Park Service Cooperative Agreement with the University of Texas at El Paso [Cooperative Agreement P17AC01014].

AUTHOR CONTRIBUTIONS

This chapter will be submitted for review in a peer-reviewed journal (*Ecological Applications*) and is co-authored by Jennifer Holguin (JH) and Jennie R. McLaren (JRM). JH and JRM contributed to the study's conception and design. Data collection was led by JH. Analysis was performed by JH. The first draft of the manuscript was written by JH. JRM provided critical feedback on all versions of this chapter.

DATA ACCESSIBILITY

All data will be made publicly available when the manuscript is published.

CHAPTER 6: CONCLUSIONS

SUMMARY OF DISSERTATION FINDINGS

In this dissertation, I provide new insights into the implications of (N) deposition in aridland ecosystem structure and function. The first chapter reviews experimental N addition effects from microbial to ecosystem scales in aridlands. Chapters three to five provide findings from N simulation experiments performed in northern Chihuahuan Desert semi-arid grasslands. Findings for each chapter are summarized below.

In **Chapter 2**, I reviewed the literature examining the effects of N inputs on aridlands from microbial to ecosystem scales**.** Overall, I found that aridlands demonstrate a wide range of responses to experimental N inputs. For instance, N enrichment often drove changes to aridland ecosystem structure and function, e.g., by reducing soil microbial and plant diversity and altering microbial, biocrust, and plant community composition and function. N enrichment also accelerated soil N transformation processes, such as N cycling activities that emit nitrogenous gases. However, the magnitude and the direction of the effects of N inputs on most aridland processes often varied due to multiple factors, such as differences in organismal functional groups, environmental factors such as water availability, as well as experimental differences, most notably N dose.

In **Chapter 3**, I assessed the influence of carbon (C), N, and phosphorus (P) availability on soil microbial biomass and function in a northern Chihuahuan Desert grassland ecosystem. Here, we performed a short-term factorial C, N, and P laboratory incubation experiment to test if Chihuahuan Desert soil microbial biomass and function are individually or interactively limited by C, N, and P when given sufficient water. I found that N and P independently limited soil microbial biomass, but the effects on measured microbial activities often depended on the co-availability of multiple resources in our study ecosystem. Resource limitations may dictate how an ecosystem may respond to global change stressors such as N deposition. Thus, this study highlights the importance of assessing multiple limiting resources in low-resource ecosystems, such as aridlands.

In **Chapter 4**, I report on results from a four-year N fertilization and water addition experiment in three Chihuahuan Desert grassland sites modeled to be experiencing N deposition. This study investigated how low but realistic N inputs (i.e., based on modeled N deposition; 0, 2, and 4 kg N ha^{-1} yr^{-1}) impact aridland plant communities, soil ecosystem properties, and biogeochemical dynamics and if effects depended on periods of above-average water availability. I found that Chihuahuan Desert grassland plant communities, soil ecosystem properties, and soil microbial activities are insensitive to low-dose N inputs even during years with above-average precipitation. Additionally, a complementary laboratory water and N addition incubation experiment using soils collected after 4 years of treatment revealed that N inputs may be readily lost as nitric oxide emissions in our study ecosystem. Periods of drought and above-average precipitation throughout our field study may have complicated responses to N. Nevertheless, our study suggests that aridlands may be resistant to low levels of N deposition, with such resistance possibly linked to gaseous N losses.

Finally, in **Chapter 5**, I provide findings from a four-year exotic grass (*Eragrostis lehmanniana)* removal, recovery, and N addition field study in a Chihuauhan Desert grassland dominated by *E. lehmanniana*. I found that *E. lehmanniana* presence (i.e., removed vs. not removed) strongly controlled native plant cover and diversity but only during years when *E.*

lehmanniana was actively removed. However, responses were likely complicated by periods of severe drought and above-average summer precipitation that occurred during years when *E. lehmanniana* removals ceased. Additionally, despite being the dominant species, *E. lehmanniana* presence (i.e., removed vs. not removed) had little to no effect on a suite of soil ecosystem properties and biogeochemical processes, regardless of the removal treatment. We also found no interactive effects between *E. lehmanniana* removal and N addition treatments, with very few main effects of N addition. Beyond the effects of *E. lehmanniana* removal and N inputs, the most significant control over *E. lehmanniana* cover appeared to be climate, where *E. lehmanniana* cover significantly declined following the most severe summer drought and did not rebound despite above-average summer rainfall occurring the following year. Overall, the current extent and future spread of *E. Lehmanniana* in the Chihuahuan Desert may not be driven by low levels of N deposition, though it is uncertain if *E. Lehmanniana* will benefit from higher N deposition levels. Lastly, we encourage land managers and future research to consider how extreme climate conditions may impede or facilitate *E. lehmanniana* expansion.

CONCLUSIONS AND FUTURE RESEARCH DIRECTIONS

This dissertation reveals large knowledge gaps that may represent directions for future research. First, while water availability in aridlands is often highly spatially and temporally variable, aridlands are also expected to experience dramatic shifts in climate regimes (e.g., Seager et al., 2007; Cook et al., 2015; Bradford et al., 2020). Climate change, including extreme climate events, may make it exceedingly difficult to assess the vulnerability of these aridlands to global change stressors. In Chapter 2, I found that N input effects often depended on periods of above-average water availability and were also often confounded by periods of drought (e.g., Brooks, 2003; Stursova et al., 2006; Ladwig et al., 2012; Vourlitis, 2017; Wheeler et al., 2021). Thus, to understand how global change stressors may interact, it is imperative that future studies aim to capture possible variations in climate (normal to extreme) by increasing the duration of their experiment, which may increase the likelihood of capturing both natural year-to-year variation in precipitation and extreme events (e.g., long-term N addition study in Ladwig et al., 2012). Alternatively, global change studies may also include climate manipulation treatments that simulate predicted changes in precipitation and temperature for their study region (e.g., extreme drought and N addition manipulation study in Zhang et al., 2023b).

Second, in Chapter 3, I found that resources other than N may constrain responses to N enrichment. However, what is limiting in one aridland ecosystem is not always limiting for others. In particular, the high spatial and temporal heterogeneity across and within dryland landscapes challenge global change response predictions (Sinsabaugh et al., 2015; Duniway et al., 2022; Osborne et al., 2022b). Thus, to advance our ability to forecast the effects of global change stressors, in aridlands, it may be critical that we increase our understanding of the resources that regulate aridland biogeochemistry across a diverse number of aridland landscapes. Globally distributed networks, such as the Nutrient Network (NutNet; for an overview, see Borer and Stevens 2022), may offer important insights into the benefits of globally distributed and standardized research.

Third, most aridland N addition studies in our Chapter 2 review applied N at relatively high N doses (>50 kg N ha^{-1} $year^{-1}$). Thus, this focus on relatively high N deposition rates challenges our predictions on the effects of N at the relatively lower N deposition rates, which are commonly observed in aridland regions. Additionally, N deposition in aridlands is understudied, can be difficult to estimate, and can be greatly overestimated by regional scale N deposition models (Cook et al., 2018). Thus, while we would benefit from more aridland N addition studies that explore the effects of low but realistic N inputs in aridlands (e.g., N added according to reported N deposition estimates as in Osborne et al., 2022a; Chapter 4 and 5), I also suggest more studies to derive inferences from extant N deposition gradients (i.e., low to high N deposition, as in Ochoa-Hueso et al., 2013).

Finally, in Chapter 1 and Chapter 4, I found that N inputs often accelerated N gas loss processes. Heightened gaseous N loss with N inputs has important implications for air quality in aridland ecosystems experiencing elevated levels of N deposition (e.g., in Carlsbad Caverns National Park; Naimie et al., 2022). Additionally, N gas losses may explain why some aridland ecosystems appear insensitive to low N inputs. In drylands, N gas emissions are considered a dominant pathway of N loss (Peterjohn and Schlesinger, 1990; Homyak et al., 2016). Thus, future aridland N addition studies should also incorporate N gas effluxes (e.g., NO, NH_3, N_2O) measurements (preferably under field conditions as in Krichels et al., 2022) as these measurements may offer critical insight into N loss and retention in response to anthropogenic N deposition in these ecosystems.

REFERENCES

Abbott, L.B. and Roundy, B.A., 2003. Available water influences field germination and recruitment of seeded grasses. Rangeland Ecology & Management/Journal of Range Management Archives, 56, 56-64.

Ahlström, A., Raupach, M.R., Schurgers, G., Smith, B., Arneth, A., Jung, M., Reichstein, M., Canadell, J.G., Friedlingstein, P., Jain, A.K. and Kato, E., 2015. The dominant role of semi-arid ecosystems in the trend and variability of the land CO_2 sink. Science, 348, 895-899.

Akaike, H., 1978. On the likelihood of a time series model. Journal of the Royal Statistical Society: Series D (The Statistician), 27, 217-235.

Allen, A.S. and Schlesinger, W.H., 2004. Nutrient limitations to soil microbial biomass and activity in loblolly pine forests. Soil Biology and Biochemistry, 36, 581-589.

Allen E.B., Rao L.E., Steers R.J., Bytnerowicz A., Fenn M.E., 2009. Impacts of atmospheric nitrogen deposition on vegetation and soils in Joshua Tree National Park. In: Webb RH, Fenstermaker LF, Heaton JS, Hughson DL, McDonald EV, Miller DM (eds) The Mojave Desert: ecosystem processes and sustainability. University of Nevada, Las Vegas

Anable, M.E., McClaran, M.P. and Ruyle, G.B., 1992. Spread of introduced Lehmann lovegrass Eragrostis lehmanniana Nees. in southern Arizona, USA. Biological Conservation, 61, 181-188.

Anderson, T.H. and Domsch, K.H., 1985. Determination of ecophysiological maintenance carbon requirements of soil microorganisms in a dormant state. Biology and Fertility of soils, 1, 81-89.

Anderson, D.R. and Burnham, K.P., 2002. Avoiding pitfalls when using information-theoretic methods. The Journal of wildlife management, 66, 912-918.

Austin, A.T., Yahdjian, L., Stark, J.M., Belnap, J., Porporato, A., Norton, U., Ravetta, D.A. and Schaeffer, S.M., 2004. Water pulses and biogeochemical cycles in arid and semiarid ecosystems. Oecologia, 141, 221-235.

Austin, A.T., 2011. Has water limited our imagination for aridland biogeochemistry? Trends in Ecology & Evolution, 26, 229-235.

Báez, S., Fargione, J., Moore, D.I., Collins, S.L. and Gosz, J.R., 2007. Atmospheric nitrogen deposition in the northern Chihuahuan desert: temporal trends and potential consequences. Journal of Arid Environments, 68, 640-651.

Baldarelli, L.M., Throop, H.L., Collins, S.L. and Ward, D., 2021. Nutrient additions have direct and indirect effects on biocrust biomass in a long-term Chihuahuan Desert grassland experiment. Journal of Arid Environments, 184, p.104317.

Barger, N.N., Weber, B., Garcia-Pichel, F., Zaady, E. and Belnap, J., 2016. Patterns and controls on nitrogen cycling of biological soil crusts. Biological soil crusts: an organizing principle in drylands, 226, 257-285.

Barker, D. H., C. Vanier, E. Naumburg, T. N. Charlet, K. M. Nielsen, B. A. Newingham, and S. D. Smith . 2006. Enhanced monsoon precipitation and nitrogen deposition affect leaf traits and photosynthesis differently in spring and summer in the desert shrub Larrea tridentata. New Phytologist, 169, 799- 808.

Barr, D.J., Levy, R., Scheepers, C. and Tily, H.J., 2013. Random effects structure for confirmatory hypothesis testing: Keep it maximal. Journal of memory and language, 68, 255-278.

Bates, D., Mächler, M., Bolker, B. and Walker, S., 2014. Fitting linear mixed-effects models using lme4. Journal of Statistical Software,1-48.

Barton, K., 2016. Multi-model inference (MuMIn). R package version, 1(6).

Bettina Weber., Belnap, J., Büdel, B., Antoninka, A.J., Barger, N.N., Chaudhary, V.B., Darrouzet-Nardi, A., Eldridge, D.J., Faist, A.M., Ferrenberg, S. and Havrilla, C.A. et al., 2022. What is a biocrust? A refined, contemporary definition for a broadening research community. Biological Reviews, 97, 1768-1785.

Bell, C.W., Acosta-Martinez, V., McIntyre, N.E., Cox, S., Tissue, D.T. and Zak, J.C., 2009. Linking microbial community structure and function to seasonal differences in soil moisture and temperature in a Chihuahuan desert grassland. Microbial ecology, 58, 827-842.

Benvenutto-Vargas, V.P. and Ochoa-Hueso, R., 2020. Effects of nitrogen deposition on the spatial pattern of biocrusts and soil microbial activity in a semi-arid Mediterranean shrubland. Functional Ecology, 34, 923-937.

Bi, J., Zhang, N., Liang, Y., Yang, H., Ma, K., 2012. Interactive effects of water and nitrogen addition on soil microbial communities in a semiarid steppe. Journal of Plant Ecology, 5, 320-329.

Billy, B., Stroehlein, J.L. and Ogden, P.R., 1973. Response of Lehmann lovegrass to time of fertilizer application. Rangeland Ecology & Management/Journal of Range Management Archives, 26, 222-224.

Bobbink, R., Hicks, K., Galloway, J., Spranger, T., Alkemade, R., Ashmore, M., Bustamante, M., Cinderby, S., Davidson, E., Dentener, F. and Emmett, B., 2010. Global assessment of nitrogen deposition effects on terrestrial plant diversity: a synthesis. Ecological applications, 20, 30-59.

Bock, C.E., Bock, J.H., Kennedy, L. and Jones, Z.F., 2007. Spread of non-native grasses into grazed versus ungrazed desert grasslands. Journal of Arid Environments, 71, 229-235.

Borer, E.T., Seabloom, E.W., Gruner, D.S., et al., 2014. Herbivores and nutrients control grassland plant diversity via light limitation. Nature, 508,517-520.

Borer, E.T. and Stevens, C.J., 2022. Nitrogen deposition and climate: an integrated synthesis. Trends in Ecology & Evolution 37: 541-552.

Bradford, J.B., Schlaepfer, D.R., Lauenroth, W.K. and Palmquist, K.A., 2020. Robust ecological drought projections for drylands in the 21st century. Global change biology, 26, 3906-3919.

Bret-Harte, M.S., García, E.A., Sacré, V.M., Whorley, J.R., Wagner, J.L., Lippert, S.C. and Chapin, F.S., 2004. Plant and soil responses to neighbour removal and fertilization in Alaskan tussock tundra. Journal of Ecology, 92, 635-647.

Brookes, P.C., Landman, A., Pruden, G. and Jenkinson, D.S., 1985. Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil biology and biochemistry, 17, 837-842.

Brooks, M.L., 2003. Effects of increased soil nitrogen on the dominance of alien annual plants in the Mojave Desert. Journal of Applied Ecology, 40, 344-353.

Brooks, M.L., D'antonio, C.M., Richardson, D.M., Grace, J.B., Keeley, J.E., DiTomaso, J.M., Hobbs, R.J., Pellant, M. and Pyke, D., 2004. Effects of invasive alien plants on fire regimes. BioScience, 54, 677-688.

Brooks, M.E., Kristensen, K., Van Benthem, K.J., Magnusson, A., Berg, C.W., Nielsen, A., Skaug, H.J., Machler, M. and Bolker, B.M., 2017. glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. The R journal, 9, 378-400.

Brooks, M., Bolker, B., Kristensen, K., Maechler, M., Magnusson, A. and McGillycuddy, M., 2023. Package 'glmmtmb'. R Packag Vers, 1.

Brown, R.F., Sala, O.E., Sinsabaugh, R.L. and Collins, S.L., 2022. Temporal effects of monsoon rainfall pulses on plant available nitrogen in a Chihuahuan Desert grassland. Journal of Geophysical Research: Biogeosciences, 127, e2022JG006938

Buerdsell, S.L. and Lehnhoff, E.A., 2023. Lehmann lovegrass (Eragrostis lehmanniana) removal and black grama (Bouteloua eriopoda) restoration in the Chihuahuan desert. Journal of Arid Environments, 213, p.104976.

Burruss, N.D., Peters, D.P., Huang, H. and Yao, J., 2022. Simulated distribution of Eragrostis lehmanniana (Lehmann lovegrass): Soil–climate interactions complicate predictions. Ecosphere, 13, p.e3974.

Bukovsky, M.S., Carrillo, C.M., Gochis, D.J., Hammerling, D.M., McCrary, R.R. and Mearns, L.O., 2015. Toward assessing NARCCAP regional climate model credibility for the North American monsoon: future climate simulations. Journal of Climate, 28, 6707-6728.

Byrne, K.M., Lauenroth, W.K. and Adler, P.B., 2013. Contrasting effects of precipitation manipulations on production in two sites within the central grassland region, USA. Ecosystems, 16,1039-1051.

Carey, C.S., Jones, J.W., Butler, R.S. and Hallerman, E.M., 2015. Restoring the endangered oyster mussel (Epioblasma capsaeformis) to the upper Clinch River, Virginia: an evaluation of population restoration techniques. Restoration Ecology, 23, 447-454.

Carpenter, A.T., Moore, J.C., Redente, E.F. and Stark, J.C., 1990. Plant community dynamics in a semi-arid ecosystem in relation to nutrient addition following a major disturbance. Plant and Soil, 126,91-99.

Chapin, F. S., Matson, P. A., and Mooney, H.A., 2002. Principals of terrestrial ecosystem ecology. Springer-Verlag, New York.

Chaudhary, V.B., Bowker, M.A., O'Dell, T.E., Grace, J.B., Redman, A.E., Rillig, M.C. and Johnson, N.C., 2009. Untangling the biological contributions to soil stability in semiarid shrublands. Ecological Applications, 19, 110-122.

Chen, D., Lan, Z., Hu, S. and Bai, Y., 2015. Effects of nitrogen enrichment on belowground communities in grassland: Relative role of soil nitrogen availability vs. soil acidification. Soil Biology and Biochemistry, 89, 99-108.

Chen, D., Pan, Q., Bai, Y., Hu, S., Huang, J., Wang, Q., et al., 2016. Effects of plant functional group loss on soil biota and net ecosystem exchange: A plant removal experiment in the Mongolian grassland. Journal of Ecology, 104, 734–743

Chen, H., Li, D., Zhao, J., Xiao, K. and Wang, K., 2018. Effects of nitrogen addition on activities of soil nitrogen acquisition enzymes: A meta-analysis. Agriculture, Ecosystems & Environment, 252, 126-131.

Choi, R.T., Reed, S.C. and Tucker, C.L., 2022. Multiple resource limitation of dryland soil microbial carbon cycling on the Colorado Plateau. Ecology, 103, e3671.

Clark, C.M., Simkin, S.M., Allen, E.B., et al., 2019. Potential vulnerability of 348 herbaceous species to atmospheric deposition of nitrogen and sulfur in the United States. Nature Plants 5: 697-705.

Cleland, E.E., Lind, E.M., DeCrappeo, N.M., DeLorenze, et al., 2019. Belowground biomass response to nutrient enrichment depends on light limitation across globally distributed grasslands. Ecosystems, 22, 1466-1477.

Cleveland, C.C. and Liptzin, D., 2007. C: N: P stoichiometry in soil: is there a “Redfield ratio” for the microbial biomass?. Biogeochemistry, 85, 235-252.

Collins SL, Sinsabaugh RL, Crenshaw C, Green L, Porras-Alfaro A, Stursova M, Zeglin LH. 2008. Pulse dynamics and microbial processes in aridland ecosystems. Journal of Ecology, 96, 413-420.

Collins, S.L., Belnap, J., Grimm, N.B., Rudgers, J.A., Dahm, C.N., D'odorico, P., Litvak, M., Natvig, D.O., Peters, D.C., Pockman, W.T. and Sinsabaugh, R.L., 2014. A multiscale, hierarchical model of pulse dynamics in arid-land ecosystems. Annual Review of Ecology, Evolution, and Systematics 45, 397-419.

Cook, B.I., Ault, T.R. and Smerdon, J.E., 2015. Unprecedented 21st century drought risk in the American Southwest and Central Plains. Science Advances, 1, e1400082.

Cook, E.M., Sponseller, R., Grimm, N.B. and Hall, S.J., 2018. Mixed method approach to assess atmospheric nitrogen deposition in arid and semi-arid ecosystems. Environmental Pollution, 239, 617-630.

Cox, J.R., Morton, H.L., Johnsen, T.N., Jordan, G.L., Martin, S.C. and Fierro, L.C., 1984. Vegetation restoration in the Chihuahuan and Sonoran deserts of North America. Rangelands Archives, 6, 112-115.

Cox, J.R., Ruyle, G.B. and Roundy, B.A., 1990. Lehmann lovegrass in southeastern Arizona: biomass production and disappearance. Rangeland Ecology & Management/Journal of Range Management Archives, 43, 367-372.

Crain, G.M., McLaren, J.R., Brunner, B. and Darrouzet-Nardi, A., 2018. Biologically available phosphorus in biocrust-dominated soils of the Chihuahuan Desert. Soil Systems, 2, 56.

Craine, J.M., Morrow, C. and Fierer, N., 2007. Microbial nitrogen limitation increases decomposition. Ecology, 88, 2105-2113.

Crimmins, T.M. and McPherson, G.R., 2008. Vegetation and seedbank response to Eragrostis lehmanniana removal in semi-desert communities. Weed research, 48, 542-551.

Cui, Y., Fang, L., Guo, X., Wang, X., Wang, Y., Li, P., Zhang, Y. and Zhang, X., 2018. Responses of soil microbial communities to nutrient limitation in the desert-grassland ecological transition zone. Science of the Total Environment, 642, 45-55.

Cui, H., Sun, W., Delgado-Baquerizo, M., Song, W., Ma, J.Y., Wang, K. and Ling, X., 2021. Cascading effects of N fertilization activate biologically driven mechanisms promoting P availability in a semi-arid grassland ecosystem. Functional Ecology, 35(4), pp.1001-1011.

Darrouzet-Nardi, A., Reed, S.C., Grote, E.E. and Belnap, J., 2015. Observations of net soil exchange of CO_2 in a dryland show experimental warming increases carbon losses in biocrust soils. Biogeochemistry, 126, 363-378.

D'Angelo, E., Crutchfield, J. and Vandiviere, M., 2001. Rapid, sensitive, microscale determination of phosphate in water and soil. Journal of environmental quality, 30, 2206-2209.

de-Bashan, L.E., Magallon-Servin, P., Lopez, B.R. and Nannipieri, P., 2022. Biological activities affect the dynamic of P in dryland soils. Biology and Fertility of Soils, 58, 105-119

Delgado-Baquerizo, M., Maestre, F., Gallardo, A. et al., 2013. Decoupling of soil nutrient cycles as a function of aridity in global drylands. Nature 502, 672–676.

Dix, B., de Bruin, J., Roosenbrand, E., Vlemmix, T., Francoeur, C., Gorchov-Negron, A., McDonald, B., Zhizhin, M., Elvidge, C., Veefkind, P. and Levelt, P., 2020. Nitrogen oxide emissions from US oil and gas production: Recent trends and source attribution. Geophysical Research Letters, 47, e2019GL085866.

Doane, T.A. and Horwáth, W.R., 2003. Spectrophotometric determination of nitrate with a single reagent. Analytical letters, 36, 2713-2722.

Dong, C., Wang, W., Liu, H., Xu, X. and Zeng, H., 2019. Temperate grassland shifted from nitrogen to phosphorus limitation induced by degradation and nitrogen deposition: Evidence from soil extracellular enzyme stoichiometry. Ecological Indicators, 101, 453-464.

Duniway, M.C., Benson, C., Nauman, T.W., Knight, A., Bradford, J.B., Munson, S.M., Witwicki, D., Livensperger, C., Van Scoyoc, M., Fisk, T.T. and Thoma, D., 2022. Geologic, geomorphic, and edaphic underpinnings of dryland ecosystems: Colorado Plateau landscapes in a changing world. Ecosphere, 13, e4273.

Eberwein, J.R., Homyak, P.M., Carey, C.J., Aronson, E.L. and Jenerette, G.D., 2020. Large nitrogen oxide emission pulses from desert soils and associated microbiomes. Biogeochemistry, 149, 239-250.

Elser, J.J., Bracken, M.E., Cleland, E.E., Gruner, D.S., Harpole, W.S., Hillebrand, H., Ngai, J.T., Seabloom, E.W., Shurin, J.B. and Smith, J.E., 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. Ecology letters, 10, 1135-1142.

Elsheikh, E.A., El-Keblawy, A., Mosa, K.A., Okoh, A.I. and Saadoun, I., 2021. Role of endophytes and rhizosphere microbes in promoting the invasion of exotic plants in arid and semi-arid areas: A review. Sustainability, 13, 13081.

Ettershank, G., Ettershank, J., Bryant, M. and Whitford, W.G., 1978. Effects of nitrogen fertilization on primary production in a Chihuahuan Desert ecosystem. Journal of Arid Environments, 1,135-139.

Fang, C., Li, F., Pei, J., Ren, J., Gong, Y., Yuan, Z., Ke, W., Zheng, Y., Bai, X. and Ye, J.S., 2018. Impacts of warming and nitrogen addition on soil autotrophic and heterotrophic respiration in a semi-arid environment. Agricultural and Forest Meteorology, 248, 449-457.

Fay, P.A., Prober, S.M., Harpole, W.S., Knops, J.M., Bakker, J.D., Borer, E.T., Lind, E.M., MacDougall, A.S., Seabloom, E.W., Wragg, P.D. and Adler, P.B., 2015. Grassland productivity limited by multiple nutrients. Nature plants, 1, 1-5.

Fenn, M.E., Baron, J.S., Allen, E.B., Rueth, H.M., Nydick, K.R., Geiser, L., Bowman, W.D., Sickman, J.O., Meixner, T., Johnson, D.W. and Neitlich, P., 2003. Ecological effects of nitrogen deposition in the western United States. BioScience, 53, 404-420.

Fenn, M.E., Allen, E.B., Weiss, S.B., Jovan, S., Geiser, L.H., Tonnesen, G.S., Johnson, R.F., Rao, L.E., Gimeno, B.S., Yuan, F. and Meixner, T., 2010. Nitrogen critical loads and management alternatives for N-impacted ecosystems in California. Journal of Environmental Management, 91, 2404-2423.

Feng, H., Guo, J., Peng, C., Kneeshaw, D., Roberge, G., Pan, C., Ma, X., Zhou, D. and Wang, W., 2023. Nitrogen addition promotes terrestrial plants to allocate more biomass to aboveground organs: A global meta-analysis. Global Change Biology, 29, 3970-3989.

Fernandez-Giménez, M.E. and Smith, S.E., 2004. Research observation: Nitrogen effects on Arizona cottontop and Lehmann lovegrass seedlings. Journal of Range Management, 57, 76-81.

Ferrenberg, S., Tucker, C.L. and Reed, S.C., 2017. Biological soil crusts: diminutive communities of potential global importance. Frontiers in Ecology and the Environment, 15, 160-167.

Finzi, A.C., Moore, D.J., DeLucia, E.H., Lichter, J., Hofmockel, K.S., Jackson, R.B., Kim, H.S., Matamala, R., McCarthy, H.R., Oren, R. and Pippen, J.S., 2006. Progressive nitrogen limitation of ecosystem processes under elevated CO2 in a warm-temperate forest. Ecology, 87, 15-25.

Fisher, F. M., J. C. Zak, G. L. Cunningham, and W. G. Whitford . 1988. Water and nitrogen effects on growth and allocation patterns of creosotebush in the northern Chihuahuan Desert. Journal of Range Management 41, 387- 391.

Frasier, G.W. and Cox, J.R., 1994. Water balance in pure stand of Lehmann lovegrass. Rangeland Ecology & Management/Journal of Range Management Archives, 47, 373-378.

Gallardo, A. and Schlesinger, W.H., 1995. Factors determining soil microbial biomass and nutrient immobilization in desert soils. Biogeochemistry, 28, 55-68.

Galloway, J.N., Aber, J.D., Erisman, J.W., et al., 2003. The nitrogen cascade. BioScience, 53, 341-356.

Galloway, J.N., Townsend, A.R., Erisman, J.W., et al., 2008. Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. Science, 320, 889-892.

Geiger, E.L., 2006. The role of fire and a nonnative grass as disturbances in semi-desert grasslands of southeastern Arizona (Doctoral dissertation, The University of Arizona).

Geisseler, D., Horwath, W.R., Joergensen, R.G. and Ludwig, B., 2010. Pathways of nitrogen utilization by soil microorganisms–a review. Soil Biology and Biochemistry, 42, 2058-2067.

Gherardi, L.A. and Sala, O.E., 2015. Enhanced precipitation variability decreases grass-and increases shrub-productivity. Proceedings of the National Academy of Sciences, 112, 12735-12740.

Gleeson, S.K. and Tilman, D., 1992. Plant allocation and the multiple limitation hypothesis. The American Naturalist, 139, 1322-1343.

Gori, D.F. and Enquist, C.A.F., 2003. An assessment of the spatial extent and condition of grasslands in central and southern Arizona, southwestern New Mexico and northern Mexico. Tucson, Arizona, USA: Nature Conservancy.

Gornish, E.S., Franklin, K., Rowe, J. and Barberán, A., 2020. Buffelgrass invasion and glyphosate effects on desert soil microbiome communities. Biological Invasions, 22, 2587-2597.

Guo X., Hu Y., Ma J.Y., Wang H., Wang K.L., et al., 2023. Nitrogen Deposition Effects on Invasive and Native Plant Competition: Implications for Future Invasions. Ecotoxicology and Environmental Safety, 259, 115029.

Griffin-Nolan, R.J., Blumenthal, D.M., Collins, S.L., Farkas, T.E., Hoffman, A.M., Mueller, K.E., Ocheltree, T.W., Smith, M.D., Whitney, K.D. and Knapp, A.K., 2019. Shifts in plant functional composition following long-term drought in grasslands. Journal of Ecology, 107, 2133-2148.

Grime, J.P., 1973. Competitive exclusion in herbaceous vegetation. Nature, 242(5396), pp.344-347.

Grime, J.P., 1998. Benefits of plant diversity to ecosystems: immediate, filter and founder effects. Journal of Ecology, 86(6), pp.902-910.

Hall, S.J., Sponseller, R.A., Grimm, N.B., Huber, D., Kaye, J.P., Clark, C. and Collins, S.L. 2011. Ecosystem response to nutrient enrichment across an urban airshed in the Sonoran Desert. Ecological Applications, 21, 640-660.

Hall, S.J., Reyes, L., Huang, W. and Homyak, P.M., 2018. Wet spots as hotspots: Moisture responses of nitric and nitrous oxide emissions from poorly drained agricultural soils. Journal of Geophysical Research: Biogeosciences, 123, 3589-3602.

Hanan, N.P., Milne, E., Aynekulu, E., Yu, Q. and Anchang, J., 2021. A role for drylands in a carbon neutral world?. Frontiers in Environmental Science, 9, 786087.

Hartley, A.E. and Schlesinger, W.H., 2000. Environmental controls on nitric oxide emission from northern Chihuahuan desert soils. Biogeochemistry, 50, 279-300.

Hartig, F. and Hartig, M.F., 2021. Package 'DHARMa'. R package.

Harpole, W.S., Sullivan, L.L., Lind, E.M., Firn, J., Adler, P.B., Borer, E.T., Chase, J., Fay, P.A., Hautier, Y., Hillebrand, H. and MacDougall, A.S., 2016. Addition of multiple limiting resources reduces grassland diversity. Nature, 537, 93-96.

Harris, D., Horwáth, W.R. and Van Kessel, C., 2001. Acid fumigation of soils to remove carbonates prior to total organic carbon or carbon-13 isotopic analysis. Soil Science Society of America Journal, 65,1853-1856.

Hooper, D. U. and Johnson, L., 1999. Nitrogen limitation in dryland ecosystems: Response to geographical and temporal variation in precipitation. Biogeochemistry, 46, 247-293.

Holguin, J., Collins, S.L. and McLaren, J.R., 2022. Belowground responses to altered precipitation regimes in two semi-arid grasslands. Soil Biology and Biochemistry, 171, 108725.

Hoover, D.L., Bestelmeyer, B., Grimm, N.B., Huxman, T.E., Reed, S.C., Sala, O., Seastedt, T.R., Wilmer, H. and Ferrenberg, S., 2020. Traversing the wasteland: a framework for assessing ecological threats to drylands. BioScience, 70, 35-47.

Homyak, P.M., Kamiyama, M., Sickman, J.O. and Schimel, J.P., 2017. Acidity and organic matter promote abiotic nitric oxide production in drying soils. Global Change Biology, 23, 1735-1747.

Horswill, P., O'Sullivan, O., Phoenix, G.K., Lee, J.A. and Leake, J.R., 2008. Base cation depletion, eutrophication and acidification of species-rich grasslands in response to long-term simulated nitrogen deposition. Environmental Pollution, 155, 336-349.

Huang, J., Yu, H., Guan, X., Wang, G. and Guo, R., 2016. Accelerated dryland expansion under climate change. Nature climate change, 6, 166-171.

Huang, J., Yu, H., Dai, A., Wei, Y. and Kang, L., 2017. Drylands face potential threat under 2° C global warming target. Nature Climate Change, 7, 417-422.

Inderjit S, van der Putten WH (2010) Impacts of soil microbial communities on exotic plant invasions. Trends in Ecology & Evolution, 25, 512- 519

James, J.J., Tiller, R.L. and Richards, J.H., 2005. Multiple resources limit plant growth and function in a saline-alkaline desert community. Journal of Ecology, 93, 113-126.

Jia, J., Dai, Z., Li, F. and Liu, Y., 2016. How will global environmental changes affect the growth of alien plants?. Frontiers in plant science, 7, 1623.

Jia, X., Zhong, Y., Liu, J., Zhu, G., Shangguan, Z. and Yan, W., 2020. Effects of nitrogen enrichment on soil microbial characteristics: From biomass to enzyme activities. Geoderma, 366, 114256.

Jian, S., Li, J., Chen, J.I., Wang, G., Mayes, M.A., Dzantor, K.E., Hui, D. and Luo, Y., 2016. Soil extracellular enzyme activities, soil carbon and nitrogen storage under nitrogen fertilization: A meta-analysis. Soil Biology and Biochemistry, 101, 32-43.

Johnson, P.C., 2014. Extension of Nakagawa & Schielzeth's R2 GLMM to random slopes models. Methods in ecology and evolution, 5, 944-946.

Kozak, M. and Piepho, H.P., 2018. What's normal anyway? Residual plots are more telling than significance tests when checking ANOVA assumptions. Journal of agronomy and crop science, 204, 86-98.

Krichels, A.H., Homyak, P.M., Aronson, E.L., Sickman, J.O., Botthoff, J., Shulman, H., Piper, S., Andrews, H.M. and Jenerette, G.D., 2022. Rapid nitrate reduction produces pulsed NO and N_2O emissions following wetting of dryland soils. Biogeochemistry, 158, 233-250.

Kuske, C.R., Ticknor, L.O., Miller, M.E., Dunbar, J.M., Davis, J.A., Barns, S.M. and Belnap, J., 2002. Comparison of soil bacterial communities in rhizospheres of three plant species and the interspaces in an arid grassland. Applied and Environmental Microbiology, 68, 1854-1863.

Ladwig, L.M., Collins, S.L., Swann, A.L., Xia, Y., Allen, M.F. and Allen, E.B., 2012. Above-and belowground responses to nitrogen addition in a Chihuahuan Desert grassland. Oecologia, 169, 177-185.

Lajtha, K. and W. G. Whitford . 1989. The effect of water and nitrogen amendments on photosynthesis, leaf demography, and resource-use efficiency in Larrea tridentata, a desert evergreen shrub. Oecologia, 80, 341– 348.

LeBauer, D. S. and Treseder, K.K., 2008. Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. Ecology, 89, 371-379.

Lenth, R. (2020). emmeans: Estimated Marginal Means, aka Least-Squares Means (R package version 1.5.1).

Li, L.J., Zeng, D.H., Yu, Z.Y., Fan, Z.P. and Mao, R., 2010. Soil microbial properties under N and P additions in a semi-arid, sandy grassland. Biology and Fertility of Soils, 46, 653-658.

Lightfoot, D. C., and W. G. Whitford. 1987. Variation in insect densities on desert creosotebush: is nitrogen a factor? Ecology 68:547–557.

Liu, L. and Greaver, T.L., 2010. A global perspective on belowground carbon dynamics under nitrogen enrichment. Ecology letters, 13, 819-828.

Litchman, E., Edwards, K.F. and Klausmeier, C.A., 2015. Microbial resource utilization traits and trade-offs: implications for community structure, functioning, and biogeochemical impacts at present and in the future. Frontiers in microbiology, 6, 254.

Ludwig, J.A., Whitford, W.G. and Cornelius, J.M., 1989. Effects of water, nitrogen and sulfur amendments on cover, density and size of Chihuahuan Desert ephemerals. Journal of arid environments, 16, 35-42.

Luo, W., Li, M.H., Sardans, J., Lü, X.T., Wang, C., Peñuelas, J., Wang, Z., Han, X.G. and Jiang, Y., 2017. Carbon and nitrogen allocation shifts in plants and soils along aridity and fertility gradients in grasslands of China. Ecology and Evolution, 7, 6927-6934.

Lyons, K.G., Maldonado-Leal, B.G. and Owen, G., 2013. Community and ecosystem effects of buffelgrass (Pennisetum ciliare) and nitrogen deposition in the Sonoran Desert. Invasive Plant Science and Management, 6(1), pp.65-78.

Ma, B., Zhou, Z.Y., Zhang, C.P., Zhang, G. and Hu, Y.J., 2009. Inorganic phosphorus fractions in the rhizosphere of xerophytic shrubs in the Alxa Desert. Journal of Arid Environments, 73, 55-61.

Ma, Q., Liu, X., Li, Y., Li, L., Yu, H., Qi, M., Zhou, G. and Xu, Z., 2020. Nitrogen deposition magnifies the sensitivity of desert steppe plant communities to large changes in precipitation. Journal of Ecology, 108, 598-610.

Manzoni, S., Taylor, P., Richter, A., Porporato, A. and Ågren, G.I., 2012. Environmental and stoichiometric controls on microbial carbon-use efficiency in soils. New Phytologist, 196, 79-91.

Marklein AR, Houlton BZ. 2012. Nitrogen inputs accelerate phosphorus cycling rates across a wide variety of terrestrial ecosystems. New Phytologist, 193, 696-704.

Marshall, V.M., Lewis, M.M. and Ostendorf, B., 2012. Buffel grass (Cenchrus ciliaris) as an invader and threat to biodiversity in arid environments: a review. Journal of Arid Environments, 78, 1-12.

Mau-Crimmins, T., Hubbard, A., McPherson, G.R. and Network, S.D., 2004. Non-native Plant Mapping at Montezuma Castle and Tuzigoot National Monuments. Sonoran Desert Network, Inventory and Monitoring, National Park Service and School of Natural Resources, The University of Arizona.

McGlone, C.M. and Huenneke, L.F., 2004. The impact of a prescribed burn on introduced Lehmann lovegrass versus native vegetation in the northern Chihuahuan Desert. Journal of Arid Environments, 57, 297-310.

McHugh, T.A., Morrissey, E.M., Mueller, R.C., Gallegos-Graves, L.V., Kuske, C.R. and Reed, S.C., 2017. Bacterial, fungal, and plant communities exhibit no biomass or compositional response to two years of simulated nitrogen deposition in a semiarid grassland. Environmental Microbiology, 19, 1600-1611.

McLaren, J.R. and Turkington, R., 2010. Ecosystem properties determined by plant functional group identity. Journal of Ecology, 98, 459-469.

McLaren, J.R., Buckeridge, K.M., van de Weg, M.J., Shaver, G.R., Schimel, J.P. and Gough, L., 2017. Shrub encroachment in Arctic tundra: Betula nana effects on above-and belowground litter decomposition. Ecology, 98, 1361-1376.

Melle, C., Wallenstein, M., Darrouzet-Nardi, A. and Weintraub, M.N., 2015. Microbial activity is not always limited by nitrogen in Arctic tundra soils. Soil Biology and Biochemistry, 90, 52-61.

Melendez Gonzalez, M., Crofts, A.L. and McLaren, J.R., 2019. Plant biomass, rather than species composition, determines ecosystem properties: Results from a long-term graminoid removal experiment in a northern Canadian grassland. Journal of Ecology, 107, 2211-2225.

Meunier, C.L., Gundale, M.J., Sánchez, I.S. and Liess, A., 2016. Impact of nitrogen deposition on forest and lake food webs in nitrogen-limited environments. Global Change Biology 22: 164-179.

Midolo, G., Alkemade, R., Schipper, A.M., Benítez-López, A., Perring, M.P. and De Vries, W., 2019. Impacts of nitrogen addition on plant species richness and abundance: A global meta-analysis. Global Ecology and Biogeography, 28, 398-413.

Millennium Ecosystem Assessment, 2005. Ecosystems and Human Well-Being: Synthesis. Island Press, Washington.

Mori, T., Lu, X., Aoyagi, R. and Mo, J., 2018. Reconsidering the phosphorus limitation of soil microbial activity in tropical forests. Functional Ecology, 32, 1145-1154.

Moyes, A.B., Gaines, S.J., Siegwolf, R.T. and Bowling, D.R., 2010. Diffusive fractionation complicates isotopic partitioning of autotrophic and heterotrophic sources of soil respiration. Plant, Cell & Environment, 33,1804-1819.

Mueller, R.C., Belnap, J. and Kuske, C.R., 2015. Soil bacterial and fungal community responses to nitrogen addition across soil depth and microhabitat in an arid shrubland. Frontiers in microbiology, 6, 891.

Mun, H.T. and Whitford, W.G., 1989. Effects of nitrogen amendment on annual plants in the Chihuahuan Desert. Plant and Soil, 120, pp.225-231.

Naimie, L.E., Sullivan, A.P., Benedict, K.B., Prenni, A.J., Sive, B.C., Schichtel, B.A., Fischer, E.V., Pollack, I. and Collett, J., 2022. PM2. 5 in Carlsbad Caverns National Park: Composition, sources, and visibility impacts. Journal of the Air & Waste Management Association, 72, 1201-1218.

Nakagawa, S. and Schielzeth, H., 2013. A general and simple method for obtaining R2 from generalized linear mixed-effects models. Methods in ecology and evolution, 4, 133-142.

Nielsen, U.N. and Ball, B.A., 2015. Impacts of altered precipitation regimes on soil communities and biogeochemistry in arid and semi-arid ecosystems. Global change biology, 21, 1407-1421.

Noy-Meir, I., 1973. Desert ecosystems: environment and producers. Annual review of ecology and systematics, 4, 25-51.

Niu, D., Yuan, X., Cease, A.J., Wen, H., Zhang, C., Fu, H. and Elser, J.J., 2018. The impact of nitrogen enrichment on grassland ecosystem stability depends on nitrogen addition level. Science of the Total Environment, 618, 1529-1538.

Ochoa-Hueso, R., Allen, E.B., Branquinho, C., Cruz, C., Dias, T., Fenn, M.E., Manrique, E., Pérez-Corona, M.E., Sheppard, L.J. and Stock, W.D., 2011. Nitrogen deposition effects on Mediterranean-type ecosystems: an ecological assessment. Environmental Pollution, 159, 2265-2279.

Ochoa-Hueso, R., Maestre, F.T., de Los Ríos, A., Valea, S., Theobald, M.R., Vivanco, M.G., Manrique, E. and Bowker, M.A., 2013. Nitrogen deposition alters nitrogen cycling and reduces soil carbon content in low-productivity semiarid Mediterranean ecosystems. Environmental Pollution, 179, 185-193.

Ochoa-Hueso R., Manrique E., 2013. Effects of nitrogen deposition on growth and physiology of Pleurochaete squarrosa (Brid) Lindb., a terricolous moss from Mediterranean ecosystems. Water Air Soil Pollution, 224, 1492.

Ochoa-Hueso, R., Bell, M.D. and Manrique, E., 2014. Impacts of increased nitrogen deposition and altered precipitation regimes on soil fertility and functioning in semiarid Mediterranean shrublands. Journal of Arid Environments, 104, 106-115.

Ochoa-Hueso, R., Delgado-Baquerizo, M., Gallardo, A., Bowker, M.A. and Maestre, F.T., 2016. Climatic conditions, soil fertility and atmospheric nitrogen deposition largely determine the structure and functioning of microbial communities in biocrust-dominated Mediterranean drylands. Plant and Soil, 399, 271-282

Ochoa-Hueso, R., Borer, E.T., Seabloom, E.W., Hobbie, S.E., Risch, A.C., Collins, S.L., Alberti, J., Bahamonde, H.A., Brown, C.S., Caldeira, M.C. and Daleo, P., 2020. Microbial processing of plant remains is co-limited by multiple nutrients in global grasslands. Global change biology, 26, 4572-4582.

Oduor, A.M., 2013. Evolutionary responses of native plant species to invasive plants: a review. New Phytologist, 200, 986-992.

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P. and Stevens, M.H.H., 2022. vegan: Community Ecology Package. R package version 2.5-7. 2020.

Osborne, B.B., Roybal, C.M., Reibold, R., Collier, C.D., Geiger, E., Phillips, M.L., Weintraub, M.N. and Reed, S.C., 2022a. Biogeochemical and ecosystem properties in three adjacent semi-arid grasslands are resistant to nitrogen deposition but sensitive to edaphic variability. Journal of Ecology, 110, 1615-1631.

Osborne, B.B., Bestelmeyer, B.T., Currier, C.M., Homyak, P.M., Throop, H.L., Young, K. and Reed, S.C., 2022b. The consequences of climate change for dryland biogeochemistry. New Phytologist, 236, 15-20.

Pardo, L. H., M. Fenn, C. L. Goodale, L. H. et al., 2011. Effects of nitrogen deposition and empirical nitrogen critical loads for ecoregions of the United States. Ecological Applications, 21, 3049-3082.

Pascale, S., Boos, W.R., Bordoni, S., Delworth, T.L., Kapnick, S.B., Murakami, H., Vecchi, G.A. and Zhang, W., 2017. Weakening of the North American monsoon with global warming. Nature Climate Change, 7, 806-812.

Payne, R.J., Dise, N.B., Field, C.D., Dore, A.J., Caporn, S.J. and Stevens, C.J., 2017. Nitrogen deposition and plant biodiversity: past, present, and future. Frontiers in Ecology and the Environment, 15, 431-436.

Peterjohn, W.T. and Schlesinger, W.H., 1990. Nitrogen loss from deserts in the southwestern United States. Biogeochemistry, 10, 67-79.

Phoenix, G.K., Hicks, W.K., Cinderby, S., et al., 2006. Atmospheric nitrogen deposition in world biodiversity hotspots: the need for a greater global perspective in assessing N deposition impacts. Global Change Biology 12:470-476.

Phillips, M.L., Winkler, D.E., Reibold, R.H., Osborne, B.B. and Reed, S.C., 2021. Muted responses to chronic experimental nitrogen deposition on the Colorado Plateau. Oecologia, 195, 513-524.

Pinheiro, J.C. and Bates, D.M., 2000. Linear Mixed-Effects Models: Basic Concepts and Examples. In: Mixed-Effects Models in S and S-PLUS. Statistics and Computing. Springer, New York, NY.

Plaza, C., Zaccone, C., Sawicka, K., Méndez, A.M., Tarquis, A., Gascó, G., Heuvelink, G., Schuur, E.A. and Maestre, F.T., 2018. Soil resources and element stocks in drylands to face global issues. Scientific Reports, 8, 13788.

Porras-Alfaro, A., Herrera, J., Natvig, D.O., Lipinski, K. and Sinsabaugh, R.L., 2011. Diversity and distribution of soil fungal communities in a semiarid grassland. Mycologia, 103, 10-21.

Poulter, B., Frank, D., Ciais, P., Myneni, R.B., Andela, N., Bi, J., Broquet, G., Canadell, J.G., Chevallier, F., Liu, Y.Y. and Running, S.W., 2014. Contribution of semi-arid ecosystems to interannual variability of the global carbon cycle. Nature, 509, 600-603.

Potthoff, M., Steenwerth, K.L., Jackson, L.E., Drenovsky, R.E., Scow, K.M. and Joergensen, R.G., 2006. Soil microbial community composition as affected by restoration practices in California grassland. Soil Biology and Biochemistry, 38,1851-1860.

Prăvălie, R., Bandoc, G., Patriche, C. and Sternberg, T., 2019. Recent changes in global drylands: Evidences from two major aridity databases. Catena, 178, 209-231.

Püspök, J.F., Zhao, S., Calma, A.D., Vourlitis, G.L., Allison, S.D., Aronson, E.L., Schimel, J.P., Hanan, E.J. and Homyak, P.M., 2023. Effects of experimental nitrogen deposition on soil organic carbon storage in Southern California drylands. Global Change Biology, 29, 1660-1679.

R Core Development Team., 2021. A language and environment for statistical computing. http://www. R-project. org.

R Studio Team 2021. RStudio: Integrated Development for R. RStudio, PBC, Boston, MA URL http://www.rstudio.com/.

Ramirez, K.S., Lauber, C.L., Knight, R., Bradford, M.A. and Fierer, N., 2010. Consistent effects of nitrogen fertilization on soil bacterial communities in contrasting systems. Ecology, 91, 3463-3470.

Ramirez, K.S., Craine, J.M. and Fierer, N., 2012. Consistent effects of nitrogen amendments on soil microbial communities and processes across biomes. Global Change Biology, 18, 1918-1927.

Rao, L.E. and Allen, E.B., 2010. Combined effects of precipitation and nitrogen deposition on native and invasive winter annual production in California deserts. Oecologia, 162,1035-1046.

Reed, S.C., Vitousek, P.M. and Cleveland, C.C., 2011. Are patterns in nutrient limitation belowground consistent with those aboveground: results from a 4 million year chronosequence. Biogeochemistry, 106, 323-336.

Reay, D.S., Dentener, F., Smith, P., Grace, J. and Feely, R.A., 2008. Global nitrogen deposition and carbon sinks. Nature geoscience, 1, 430-437.

Reed, S.C., Yang, X. and Thornton, P.E., 2015. Incorporating phosphorus cycling into global modeling efforts: a worthwhile, tractable endeavor. New Phytologist, 208, 324-329.

Reynolds, J.F., Kemp, P.R., Ogle, K. and Fernández, R.J., 2004. Modifying the 'pulse–reserve' paradigm for deserts of North America: precipitation pulses, soil water, and plant responses. Oecologia, 141, 194-210.

Reichmann, L.G., Sala, O.E. and Peters, D.P., 2013. Water controls on nitrogen transformations and stocks in an arid ecosystem. Ecosphere, 4(1), pp.1-17.

Rong, X., Zhou, X., Li, X., Yao, M., Lu, Y., Xu, P., Yin, B., Li, Y., Aanderud, Z.T. and Zhang, Y., 2022. Biocrust diazotrophs and bacteria rather than fungi are sensitive to chronic low N deposition. Environmental Microbiology, 24, 450-5466.

Romney, E., A. Wallace, and R. Hunger . 1978. Plant response to nitrogen fertilization in the northern Mohave Desert and its relationship to water manipulation. Pages 232– 243. in N. West and J. Skujins . editors. Nitrogen in desert ecosystems. Dowden, Hutchinson, and Ross. Stroudsburg, Pennsylvania, USA.

Saiya-Cork, K.R., Sinsabaugh, R.L. and Zak, D.R., 2002. The effects of long term nitrogen deposition on extracellular enzyme activity in an Acer saccharum forest soil. Soil Biology and Biochemistry, 34,1309-1315.

Sharifi, M. R., F. C. Meinzer, E. T. Nilsen, P. W. Rundel, R. A. Virginia, W. M. Jarrell, D. J. Herman, and P. C. Clark . 1988. Effect of manipulation of water and nitrogen supplies on the quantitative phenology of Larrea tridentata (Creosote Bush) in the Sonoran Desert of California. American Journal of Botany, 75, 1163-1174.

Schaeffer, S.M., Billings, S.A. and Evans, R.D., 2003. Responses of soil nitrogen dynamics in a Mojave Desert ecosystem to manipulations in soil carbon and nitrogen availability. Oecologia, 134, 547-553.

Schaeffer, S.M. and Evans, R.D., 2005. Pulse additions of soil carbon and nitrogen affect soil nitrogen dynamics in an arid Colorado Plateau shrubland. Oecologia, 145, 425-433.

Schenk, H.J. and Jackson, R.B., 2002. Rooting depths, lateral root spreads and below-ground/above-ground allometries of plants in water-limited ecosystems. Journal of Ecology, 90, 480-494.

Schimel, J.P. and Schaeffer, S.M., 2012. Microbial control over carbon cycling in soil. Frontiers in microbiology, 3, 348.

Schussman, H., Geiger, E., Mau-Crimmins, T. and Ward, J., 2006. Spread and current potential distribution of an alien grass, Eragrostis lehmanniana Nees, in the southwestern USA: comparing historical data and ecological niche models. Diversity and Distributions, 12, 582-592.

Schwinning, S., Starr, B.I., Wojcik, N.J., Miller, M.E., Ehleringer, J.E. and Sanford Jr, R.L., 2005. Effects of nitrogen deposition on an arid grassland in the Colorado plateau cold desert. Rangeland Ecology & Management, 58, 565-574.

Seager, R., Ting, M., Held, I., Kushnir, Y., Lu, J., Vecchi, G., Huang, H.P., Harnik, N., Leetmaa, A., Lau, N.C. and Li, C., 2007. Model projections of an imminent transition to a more arid climate in southwestern North America. Science, 316, 1181-1184.

Sellers, W.D., 1964. Potential evapotranspiration in arid regions. Journal of Applied Meteorology and Climatology, 3, 98-104.

She, W., Chen, N., Zhang, Y., Qin, S., Bai, Y., Feng, W., Lai, Z., Qiao, Y., Liu, L., Zhang, W. and Miao, C., 2022. Precipitation and nitrogen deposition alter biocrust–vascular plant coexistence in a desert ecosystem: Threshold and mechanisms. Journal of Ecology, 110, 772-783.

Steers, R.J., Funk, J.L. and Allen, E.B., 2011. Can resource-use traits predict native vs. exotic plant success in carbon amended soils?. Ecological Applications, 21,1211-1224.

Simonin, K. A. 2001. Bromus rubens, Bromus madritensis. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Fire Sciences Laboratory.

Sinsabaugh, R. L., Belnap, J., Rudgers, J., Kuske, C. R., Martinez, N., and Sandquist, D. 2015. Soil microbial responses to nitrogen addition in arid ecosystems. Frontiers in microbiology, 6, 819.

Simkin, S.M., Allen, E.B., Bowman, W.D., et al., 2016. Conditional vulnerability of plant diversity to atmospheric nitrogen deposition across the United States. Proceedings of the National Academy of Sciences 113: 4086-4091.

Smith, P., Ashmore, M.R., Black, H.I., Burgess, P.J., Evans, C.D., Quine, T.A., Thomson, A.M., Hicks, K. and Orr, H.G., 2013. The role of ecosystems and their management in regulating climate, and soil, water and air quality. Journal of Applied Ecology, 50, 812-829.

Song, J., Wan, S., Piao, S., Knapp, A.K., Classen, A.T., Vicca, S., Ciais, P., Hovenden, M.J., Leuzinger, S., Beier, C. and Kardol, P., 2019. A meta-analysis of 1,119 manipulative experiments on terrestrial carbon-cycling responses to global change. Nature ecology & evolution, 3, 1309-1320.

Sterner, R. W., and Elser, J.J., 2003.Ecological Stoichiometry: The Biology of Elements from Molecules to the Biosphere, Princeton: Princeton University Press

Steven B. 2017. An introduction to arid soils and their biology. In: Blaire S, ed. The biology of arid soils. New Haven, CT, USA: Walter de Gruyter, 1–14.

Stevens, C.J., David, T.I. and Storkey, J., 2018. Atmospheric nitrogen deposition in terrestrial ecosystems: Its impact on plant communities and consequences across trophic levels. Functional Ecology 32: 1757-1769.

Stursova M, Crenshaw CL and Sinsabaugh RL. 2006. Microbial responses to long-term N deposition in a semiarid grassland. Microbial Ecology 51: 90–98.

Su, Y.G., Huang, G., Lin, Y.J. and Zhang, Y.M., 2016. No synergistic effects of water and nitrogen addition on soil microbial communities and soil respiration in a temperate desert. Catena, 142, 126-133.

Sullivan, T.J., McDonnell, T.C., Moore, D.L., and Barna. M.G., 2016. Mapping of nitrogen critical loads for selected national parks in the western United States. Natural Resource Report NPS/NRSS/ARD/NRR—2016/1286. National Park Service, Fort Collins, Colorado.

Sun, Y., Yang, Y., Zhao, X., Tang, Z., Wang, S. and Fang, J., 2021. Global patterns and climatic drivers of above-and belowground net primary productivity in grasslands. Science China Life Sciences, 64, 739-751.

Tian, D. and Niu, S., 2015. A global analysis of soil acidification caused by nitrogen addition. Environmental Research Letters, 10, 024019.

Tilman, D., 1987. On the meaning of competition and the mechanisms of competitive superiority. Functional Ecology, 1, 304-315.

Torres, N., Herrera, I., Fajardo, L. and Bustamante, R.O., 2021. Meta-analysis of the impact of plant invasions on soil microbial communities. BMC Ecology and Evolution, 21, 1-8.

Treseder, K.K., 2008. Nitrogen additions and microbial biomass: A meta-analysis of ecosystem studies. Ecology letters, 11, 1111-1120.

Throop, H. L. and Lerdau, M. T., 2004. Effects of nitrogen deposition on insect herbivory: Implications for community and ecosystem processes. Ecosystems, 7,109-133.

Vila M, Espinar JL, Hejda M, Hulme PE, Jarosık V, Maron JL, Pergl J, Schaffner U, Sun Y, Pysek P. 2011. Ecological impacts of invasive alien plants: a meta-analysis of their effects on species, communities and ecosystems. Ecology Letters, 14, 702-708.

Vourlitis, G.L., Pasquini, S.C. and Mustard, R., 2009. Effects of dry-season N input on the productivity and N storage of Mediterranean-type shrublands. Ecosystems, 12, 473-488.

Vourlitis, G.L., 2017. Chronic N enrichment and drought alter plant cover and community composition in a Mediterranean-type semi-arid shrubland. Oecologia, 184, 267-277.

Vourlitis, G.L., Jaureguy, J., Marin, L. and Rodriguez, C., 2021a. Shoot and root biomass production in semi-arid shrublands exposed to long-term experimental N input. Science of the Total Environment, 754, p.142204.

Vourlitis, G.L., Kirby, K., Vallejo, I., Asaeli, J. and Holloway, J.M., 2021b. Potential soil extracellular enzyme activity is altered by long-term experimental nitrogen deposition in semiarid shrublands. Applied Soil Ecology, 158, p.103779.

Vitousek, P.M., Aber, J.D., Howarth, R.W., et al., 1997. Human alteration of the global nitrogen cycle: sources and consequences. Ecological applications 7: 737-750.

Vitousek, P.M., Porder, S., Houlton, B.Z. and Chadwick, O.A., 2010. Terrestrial phosphorus limitation: mechanisms, implications, and nitrogen–phosphorus interactions. Ecological applications, 20, 5-15.

Waldrop, M.P., Zak, D.R. and Sinsabaugh, R.L., 2004. Microbial community response to nitrogen deposition in northern forest ecosystems. Soil Biology and Biochemistry, 36, 1443-1451.

Wang, R., Filley, T.R., Xu, Z., Wang, X., Li, M.H., Zhang, Y., Luo, W. and Jiang, Y., 2014. Coupled response of soil carbon and nitrogen pools and enzyme activities to nitrogen and water addition in a semi-arid grassland of Inner Mongolia. Plant and soil, 381, 323-336.

Wang, J., Bao, J., Su, J., Li, X., Chen, G. & Ma, X., 2015. Impact of inorganic nitrogen additions on microbes in biological soil crusts. Soil Biology and Biochemistry, 88, 303–313.

Wang, Z., Yang, S., Wang, R., Xu, Z., Feng, K., Feng, X., Li, T., Liu, H., Ma, R., Li, H. and Jiang, Y., 2020. Compositional and functional responses of soil microbial communities to long-term nitrogen and phosphorus addition in a calcareous grassland. Pedobiologia, 78, p.150612.

Wang, R., Bicharanloo, B., Hou, E., Jiang, Y. and Dijkstra, F.A., 2022. Phosphorus Supply Increases Nitrogen Transformation Rates and Retention in Soil: A Global Meta-Analysis. Earth's Future, 10, e2021EF002479.

Wang, X., Feng, J., Ao, G., Qin, W., Han, M., Shen, Y., Liu, M., Chen, Y. and Zhu, B., 2023. Globally nitrogen addition alters soil microbial community structure, but has minor effects on soil microbial diversity and richness. Soil Biology and Biochemistry, 179, p.108982.

Wardle, D.A. and Zackrisson, O., 2005. Effects of species and functional group loss on island ecosystem properties. Nature, 435, 806-810.

Weber, B., Belnap, J., Büdel, B., Antoninka, A.J., Barger, N.N., Chaudhary, V.B., Darrouzet-Nardi, A., Eldridge, D.J., Faist, A.M., Ferrenberg, S. and Havrilla, C.A., 2022. What is a biocrust? A refined, contemporary definition for a broadening research community. Biological Reviews, 97, 1768-1785.

Wei, C., Yu, Q., Bai, E., Lü, X., Li, Q.I., Xia, J., Kardol, P., Liang, W., Wang, Z. and Han, X., 2013. Nitrogen deposition weakens plant–microbe interactions in grassland ecosystems. Global change biology, 19, 3688-3697.

Wheeler, M.M., Collins, S.L., Grimm, N.B., Cook, E.M., Clark, C., Sponseller, R.A. and Hall, S.J., 2021. Water and nitrogen shape winter annual plant diversity and community composition in near-urban Sonoran Desert preserves. Ecological monographs, 91, e014

Whitford, W.G. and Steinberger, Y., 2011. Effects of simulated storm sizes and nitrogen on three Chihuahuan Desert perennial herbs and a grass. Journal of arid environments, 75, 861-864.

Wickham, H. and Wickham, H., 2016. Data analysis. ggplot2: elegant graphics for data analysis, 189-201.

Williams, R.B. and Bell, K.L., 1981. Nitrogen allocation in Mojave Desert winter annuals. Oecologia, 48, 145-150.

Williams, D.G. and Baruch, Z., 2000. African grass invasion in the Americas: ecosystem consequences and the role of ecophysiology. Biological invasions, 2, 123-140.

Williams, J.P., Gornish, E.S. and Barberán, A., 2022. Effects of buffelgrass removal and nitrogen addition on soil microbial communities during an extreme drought in the Sonoran Desert. Restoration Ecology, 30, e13570.

Wolfe B.E., Klironomos J.N., 2005. Breaking new ground: soil communities and exotic plant invasion. Bioscience, 55, 477– 487

Xiao, W., Chen, X., Jing, X. and Zhu, B., 2018. A meta-analysis of soil extracellular enzyme activities in response to global change. Soil Biology and Biochemistry, 123, 21-32.

Xu, C., Xu, X., Ju, C., Chen, H. Y. H., Wilsey, B. J., Luo, Y., and Fan, W., 2021. Long-term, amplified responses of soil organic carbon to nitrogen addition worldwide. Global Change Biology, 27, 1170–1180.

Xu, H., Liu, Q., Wang, S., Yang, G. and Xue, S., 2022. A global meta-analysis of the impacts of exotic plant species invasion on plant diversity and soil properties. Science of the Total Environment, 810, 152286.

Yang, S., Xu, Z., Wang, R., Zhang, Y., Yao, F., Zhang, Y., Turco, R.F., Jiang, Y., Zou, H. and Li, H., 2017. Variations in soil microbial community composition and enzymatic activities in response to increased N deposition and precipitation in Inner Mongolian grassland. Applied Soil Ecology, 119, 275-285.

Yang, X., Liu, Y., Tian, H. and Shen, Y., 2021. Short-term nitrogen and phosphorus additions rather than mycorrhizal suppression determine plant community composition and productivity in desert steppe. Applied Soil Ecology, 168,104144.

Yahdjian, L., Gherardi, L. and Sala, O.E., 2011. Nitrogen limitation in arid-subhumid ecosystems: a meta-analysis of fertilization studies. Journal of Arid Environments, 75, 675-680.

Yahdjian, L., Gherardi, L. and Sala, O.E., 2014. Grasses have larger response than shrubs to increased nitrogen availability: a fertilization experiment in the Patagonian steppe. Journal of Arid Environments, 102, 17-20.

Young, K.E., Ferrenberg, S., Reibold, R., Reed, S.C., Swenson, T., Northen, T. and Darrouzet-Nardi, A., 2022. Vertical movement of soluble carbon and nutrients from biocrusts to subsurface mineral soils. Geoderma, 405, 115495.

Yuan, Z.Y. and Chen, H.Y., 2015. Decoupling of nitrogen and phosphorus in terrestrial plants associated with global changes. Nature Climate Change, 5, 465-469.

Yue, K., Peng, Y., Peng, C., Yang, W., Peng, X. and Wu, F., 2016. Stimulation of terrestrial ecosystem carbon storage by nitrogen addition: a meta-analysis. Scientific reports, 6, 1-10.

Yue, P., Cui, X., Gong, Y., Li, K., Goulding, K. and Liu, X., 2018. Impact of elevated precipitation, nitrogen deposition and warming on soil respiration in a temperate desert. Biogeosciences, 15, 2007-2019.

Zeglin, Lydia H., Martina Stursova, Robert L. Sinsabaugh, and Scott L. Collins., 2007. "Microbial responses to nitrogen addition in three contrasting grassland ecosystems." Oecologia 154, 349-359.

Zhang, N., Xu, W., Yu, X., Lin, D., Wan, S. and Ma, K., 2013. Impact of topography, annual burning, and nitrogen addition on soil microbial communities in a semiarid grassland. Soil Science Society of America Journal, 77, 1214-1224.

Zhang, C., Niu, D., Hall, S.J., Wen, H., Li, X., Fu, H., Wan, C. and Elser, J.J., 2014. Effects of simulated nitrogen deposition on soil respiration components and their temperature sensitivities in a semiarid grassland. Soil Biology and Biochemistry, 75, 113-123.

Zhang, T.A., Chen, H.Y. and Ruan, H., 2018. Global negative effects of nitrogen deposition on soil microbes. The ISME journal, 12, 1817-1825.

Zhang, Y., Tariq, A., Hughes, A.C., Hong, D., Wei, F., Sun, H., Sardans, J., Peñuelas, J., Perry, G., Qiao, J. and Kurban, A., 2023a. Challenges and solutions to biodiversity conservation in arid lands. Science of the Total Environment, 857, 159695.

Zhang, J., Zuo, X. and Lv, P., 2023b. Effects of Grazing, Extreme Drought, Extreme Rainfall and Nitrogen Addition on Vegetation Characteristics and Productivity of Semiarid Grassland. International Journal of Environmental Research and Public Health, 20(2), p.960.

Zeng, D. H., Li, L. J., Fahey, T. J., Yu, Z. Y., Fan, Z. P., & Chen, F. S. 2010. Effects of nitrogen addition on vegetation and ecosystem carbon in a semi-arid grassland. Biogeochemistry, 98, 185-193.

Zhou, X.B., Zhang, Y.M. and Yin, B.F., 2016. Divergence in physiological responses between cyanobacterial and lichen crusts to a gradient of simulated nitrogen deposition. Plant and Soil, 399, pp.121-134.

Zhou, Z., Wang, C., Zheng, M., Jiang, L. and Luo, Y., 2017. Patterns and mechanisms of responses by soil microbial communities to nitrogen addition. Soil Biology and Biochemistry, 115, 433-441.

Zhu, C., Ma, Y., Wu, H., Sun, T., La Pierre, K.J., Sun, Z. and Yu, Q., 2016. Divergent effects of nitrogen addition on soil respiration in a semiarid grassland. Scientific reports, 6, 33541.

Zuur, A.F., Ieno, E.N., Walker, N.J., Saveliev, A.A. and Smith, G.M., 2009. Mixed effects models and extensions in ecology with R (Vol. 574, p. 574). New York: springer.

Zuur, A.F., Ieno, E.N. and Elphick, C.S., 2010. A protocol for data exploration to avoid common statistical problems. Methods in ecology and evolution, 1, 3-14.

SUPPLEMENTARY TABLES

Table S1.1. Mean annual precipitation (MAP), annual summer (June- September) precipitation, mean maximum and mean minimum annual temperature for Carlsbad Caverns National Park. Data were obtained from mesowest.utah.edu BATN5 station.

Year	MAP (mm)	Annual summer precipitation June- Sept. (mm)	Mean Annual Max Temp. (°C)	Mean Annual Min. Temp. (°C)
2018	297	125	24	12
2019	201	115	24	13
2020	180	72	25	14
2021	409	316	25	13

Table S4.1. Soil texture and average soil pH for all sites (measured in 2018 before the start of the experiment).

Characteristic	Invasive Lovegrass	Native Muhly	Native Grama
Texture Class	Sandy-loam	Sandy-loam	Sandy-loam
Sand (%)	71	49	46
Silt (%)	25	45	49
Clay (%)	4	7	5
pH	7.8	7.8	7.6

Table S4.2. Experimental type and sampling scheme for measured variables. *Variables measured three times per year were only measured during the monsoon season of 2018.

Experimental Type	Variables	Sampling period (s)	Sampling frequency
Field	% Cover	Peak biomass (October)	1x/ annually
	Foliar C and N	Monsoon 2021	1x (4th year only)
	Soil moisture	Pre-monsoon, monsoon, and winter	3x/ year* (during soil collection)
	Soil pH	Monsoon	1x/ annually
	Available nutrients	Pre-monsoon, monsoon, and winter	3x/ annually*
	EOC	Pre-monsoon, monsoon, and winter	3x/ annually*
	ETN	Pre-monsoon, monsoon, and winter	3x/ annually*
	Soil %OC	Monsoon	1x/ annually
	Soil %N	Monsoon	1x/ annually
	MBC	Pre-monsoon, monsoon, and winter	3x/ annually*
	MBN	Pre-monsoon, monsoon, and winter	3x/ annually*
	MBP	Pre-monsoon, monsoon, and winter	3x/ annually*
	Extracellular enzymes	Monsoon	1x/ annually
Laboratory incubation	N-mineralization, nitrification, and ammonification	Monsoon 2019	30-day incubation
	NOx (NO and NO_2)	October 2021	1 and 24 hr. incubation

Table S4.3. Chi-square estimates and p-values from type 3 Wald chi-squared tests for relative plant cover models derived from mixed effects models (LMM) or generalized linear mixed-effects models (GLMM) for non-Gaussian distributions. Backward step-wise model selection was used to select the best fitting, simplest model based on a 5 AICc improvement (the maximal model is listed above relevant response variables). Statistical analyses did not include 2020 due to a severe drought that resulted in zero live plant cover values in most plots. *E. lehmanniana* cover was only analyzed in the Invasive Lovegrass site, as it was not present in the Native Muhly or Native Grama sites. Annual species plant cover was only analyzed in 2021, using a 2-way ANOVA (interaction was eliminated from the model due to insignificance). Bold values indicate significance at p<0.05. Significance codes: *** < 0.001, ** p<0.01, * p< 0.05, . p<0.1.

Maximal model: ~ Treatment * Site * Year

Total vegetation cover: Final model [(LMM, family= gaussian (link="ldentity")]: ~ Treatment +Site + Year + Treatment: Year + Site :Year

	χ2	**Df**	**Pr(>Chisq)**	
Treatment	10.56	3	**0.014**	*
Site	19.25	2	**<0.001**	***
Year	11.45	2	**0.003**	**
Treatment: Year	21.15	6	**0.002**	**
Site: Year	67.80	4	**<0.001**	***

C4 perennial grass cover: Final model [(LMM, family= gaussian (link="ldentity")]: ~ Site + Year + Site :Year

	χ2	**Df**	**Pr(>Chisq)**	
Site	35.87	2	**<0.001**	***
Year	274.33	2	**<0.001**	***
Site: Year	156.21	4	**<0.001**	***

Perennial forb cover: Final model [(GLMM, family= beta (link="logit")]: ~ Treatment +Site + Year + Treatment: Year + Site :Year

	χ2	**Df**	**Pr(>Chisq)**	
Treatment	6.86	3	0.076	.
Site	12.58	2	**0.002**	**
Year	0.69	2	0.706	
Treatment: Year	15.43	6	**0.017**	*
Site: Year	46.92	4	**<0.001**	***

***Eragrostis lehmanniana* cover:** Final model [(LMM, family= gaussian (link="ldentity")]: ~ Year

	χ2	**Df**	**Pr(>Chisq)**	
Year	178.36	2	**<0.001**	***

Maximal model: ~ Treatment * Site

Annual cover: Final model [(2-way ANOVA]: ~ Treatment + Site

	Df	**Sum Sq**	**Mean Sq**	**F value**	**P(>F)**		**Residuals**
Treatment	3	0.10	0.03	0.93	0.435		54
Site	2	1.58	0.79	20.94	**<0.001**	***	

Table S4.4. Chi-square estimates and p-values from type 3 Wald chi-squared tests for Shannon's diversity (H), species richness (SR), Foliar %C, and Foliar %N derived from mixed effects models (LMM) or generalized linear mixed-effects models (GLMM) for non-Gaussian distributions. Backward step-wise model selection was used to select the best fitting, simplest model based on a 5 AICc improvement (the maximal model is listed above relevant response variables). Statistical analyses did not include 2020 due to a severe drought that resulted in zero live plant cover values in most plots. Annual species H was only analyzed in 2021, using a 2-way ANOVA (interaction was eliminated from the model due to insignificance). Bold values indicate significance at $p<0.05$. Significance codes: *** < 0.001, ** $p<0.01$, * $p< 0.05$, . $p<0.1$.

Maximal model: ~ Treatment *Site * Year

H: Final model [(LMM, family= gaussian (link="identity")]: ~ Site + Year + Site :Year

	χ2	Df	Pr(>Chisq)	
Site	38.17	2	**<0.001**	***
Year	39.02	2	**<0.001**	***
Site: Year	55.67	4	**<0.001**	***

H C4 Perennial grass: Final model [(LMM, family= gaussian (link="identity")]: ~ Treatment+ Site + Year + Site :Year

	χ2	Df	Pr(>Chisq)	
Treatment	4.16	3	0.244	
Site	14.88	2	**0.001**	***
Year	1.11	2	0.575	
Site: Year	11.56	4	**0.021**	*

H Perennial forbs: Final model [(LMM, family= gaussian (link="identity")]: ~ Treatment+ Site + Year + Site :Year

	χ2	Df	Pr(>Chisq)	
Treatment	3.47	3	0.324	
Site	4.49	2	0.106	
Year	0.57	2	0.751	
Site: Year	32.28	4	**<0.001**	***

SR: Final model [(GLMM, family= poisson (link="log")]: ~ Site + Year + Site :Year

	χ2	Df	Pr(>Chisq)	
Site	16.46	2	**<0.001**	***
Year	23.65	2	**<0.001**	***
Site: Year	39.56	4	**<0.001**	***

Maximal model: ~ Treatment * Site

H Annuals: Final model [(2-way ANOVA]: ~ Treatment + Site

	Df	Sum Sq	Mean Sq	F value	P(>F)		Residuals
Teatment	3	0.14	0.05	0.38	0.767		54
Site	2	7.79	3.90	30.95	**<0.001**	***	

Maximal model: ~ Treatment *Site * Functional Group

Foliar %C: Final model [(LMM, family= gaussian (link="identity")]: ~ Treatment+ Site + Functional Group + Site :Functional Group

	χ2	Df	Pr(>Chisq)	
Treatment	3.44	3	0.329	
Site	14.11	2	**<0.001**	***
Functional Group	10.83	1	**<0.001**	***
Site: Functional Group	192.09	2	**<0.001**	***

Foliar %N: Final model [(LMM, family= gaussian (link="identity")]: ~ Treatment: Site:Functional Group

	χ2	Df	Pr(>Chisq)	
Treatment	0.98	3	0.806	
Site	42.12	2	**<0.001**	***
Functional Group	6.85	1	**0.009**	**
Treatment: Site	8.43	6	0.209	
Treatment: Fucntional Group	3.95	3	0.266	
Site: Functional Group	11.76	2	**0.003**	**
Treatment: Site: Functional Group	16.38	6	**0.012**	*

Table S4.5. Chi-square estimates and p-values from type 3 Wald chi-squared tests for field soil ecosystem properties soil pH, available soil nitrate (NO_3^-), ammonium (NH_4^+), phosphate (PO_4^{3-}), extractable organic carbon (EOC), extractable total nitrogen

(ETN), soil %OC, and soil %N, derived from mixed effects models (LMM) or generalized linear mixed-effects models (GLMM) for non-Gaussian distributions. Backward step-wise model selection was used to select the best fitting, simplest model based on a 5 AICc improvement (maximal model is listed in bold above relevant response variables). Bold values indicate significance at p<0.05. Significance codes: *** < 0.001, ** p<0.01, * p< 0.05, . p<0.1.

Maximal model: ~Treatment * Site * Year				
pH: Final model [(LMM, family= gaussian (link="Identity")]: ~ Site + Year + Site : Year				
	χ2	**Df**	**Pr(>Chisq)**	
Site	94.99	2	**<0.001**	***
Year	136.85	3	**<0.001**	***
Site: Year	138.89	6	**<0.001**	***
Maximal model: ~ Treatment * Site * Year+ Treatment * Site* Season				
NO_3^- Final model [(GLMM, family= gamma (link="log")]: ~Treatment + Site + Year+ Season+ Treatment: Year + Site:Season+ Site: Year				
	χ2	**Df**	**Pr(>Chisq)**	
Treatment	4.39	3	0.222	
Site	9.54	2	**0.008**	**
Year	8.48	3	**0.037**	*
Season	83.73	2	**<0.001**	***
Treatment: Year	17.50	9	**0.041**	*
Site: Season	7.04	4	0.134	
Site: Year	28.66	6	**<0.001**	***
NH_4^+ Final model [(GLMM, family= gamma (link="log")]: ~Treatment + Site + Year+ Season+ Site: Season + Site: Year				
	χ2	**Df**	**Pr(>Chisq)**	
Treatment	5.84	3	0.120	
Site	27.83	2	**<0.001**	***
Year	48.97	3	**<0.001**	***
Season	42.42	2	**<0.001**	***
Site: Season	17.08	4	**0.002**	**
Site: Year	116.22	6	**<0.001**	***
PO_4^{3-} Final model [(GLMM, family= gamma (link="log")]: ~Treatment + Site + Year+ Season+ Site: Season +Site: Year				
	χ2	**Df**	**Pr(>Chisq)**	
Treatment	4.3	3	0.235	
Site	185.92	2	**<0.001**	***
Year	10.31	3	**0.016**	*
Season	18.47	2	**<0.001**	***
Site: Season	18.97	4	**0.001**	***
Site: Year	35.09	6	**<0.001**	***
EOC: Final model [(GLMM, family= gamma (link="log")]: Treatment + Site + Year+ Season+ Treatment: Season+ Site: Season + Site: Year				
	χ2	**Df**	**Pr(>Chisq)**	
Treatment	1.54	3	0.672	
Site	49.45	2	**<0.001**	***
Year	6.20	3	0.102	
Season	31.88	2	**<0.001**	***
Treatment: Season	3.33	6	0.767	
Site: Season	33.38	4	**<0.001**	***
Site: Year	76.67	6	**<0.001**	***
ETN: Final model [(GLMM, family= gamma (link="log")]: Treatment + Site + Year+ Season+ Site: Season + Site: Year				
	χ2	**Df**	**Pr(>Chisq)**	
Treatment	4.11	3	0.250	
Site	35.09	2	**<0.001**	***
Year	18.34	3	**<0.001**	***
Season	46.88	2	**<0.001**	***
Site: Season	17.01	4	**0.002**	**
Site: Year	105.75	6	**<0.001**	***
Soil %OC: Final model [(LMM, family= gaussian (link="Identity")]: ~ Site + Year + Site : Year				
	χ2	**Df**	**Pr(>Chisq)**	
Site	1090.04	2	**<0.001**	***
Year	112.04	3	**<0.001**	***
Site: Year	141.28	6	**<0.001**	***
Soil %N: Final model [(LMM, family= gaussian (link="Identity")]: ~ Site + Year + Site : Year				
	χ2	**Df**	**Pr(>Chisq)**	
Site	91.88	2	**<0.001**	***
Year	40.58	3	**<0.001**	***
Site: Year	79.33	6	**<0.001**	***

Table S4.6. Chi-square estimates and p-values from type 3 Wald chi-squared tests for field microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), and microbial biomass phosphorus (MBP), derived from mixed effects models (LMM) or generalized linear mixed-effects models (GLMM) for non-Gaussian distributions. Backward step-wise model selection was used to select the best fitting, simplest model based on a 5 AICc improvement (maximal model is listed in bold above relevant response variables). Bold values indicate significance at $p<0.05$. Significance codes: *** < 0.001, ** $p<0.01$, * $p< 0.05$, . $p<0.1$.

MBC: Final model [(GLMM, family= gamma (link="log")]: ~ Treatment+ Site + Year+ Season+ Site: Season + Site: Year

	χ^2	**Df**	**Pr(>Chisq)**	
Treatment	2.50	3	0.476	
Site	35.57	2	**<0.001**	***
Year	2.68	3	0.443	
Season	13.83	2	**0.001**	***
Site: Season	16.14	4	**0.003**	**
Site: Year	91.06	6	**<0.001**	***

MBN: Final model [(GLMM, family= gamma (link="log")]: ~ Treatment+ Site + Year+ Season+ Site: Season + Site: Year

	χ^2	**Df**	**Pr(>Chisq)**	
Treatment	1.31	3	0.727	
Site	24.42	2	**<0.001**	***
Year	35.19	3	**<0.001**	***
Season	61.95	2	**<0.001**	***
Site: Season	8.24	4	0.083	.
Site: Year	105.31	6	**<0.001**	

MBP: Final model [(GLMM, family= gamma (link="log")]: ~ Treatment+ Site + Year+ Season+ Site: Season + Site: Year

	χ^2	**Df**	**Pr(>Chisq)**	
Treatment	1.98	3	0.577	
Site	2.08	2	0.353	
Year	14.22	3	**0.003**	**
Season	12.65	2	**0.002**	**
Site: Season	11.09	4	**0.026**	*
Site: Year	10.30	6	0.113	

Table S4.7. Chi-square estimates and p-values from type 3 Wald chi-squared tests for field hydrolytic extracellular enzyme potentials β -D-cellobioside (β -cello), β-glucosidase (β-gluc), α-Glucosidase (α-gluc), β-xylosidase (β -xylo), N-acetylglucosaminidase (NAG), leucyl aminopeptidase (LAP), acid phosphatase (Phos) and phosphodiesterase (Phos-D), and oxidative enzymes peroxidase (Perox), and phenol oxidase (Phenol), derived from mixed effects models (LMM) or generalized linear mixed-effects models (GLMM) for non-Gaussian distributions. Backward step-wise model selection was used to select the best fitting, simplest model based on a 5 AICc improvement (maximal model is listed in bold above relevant response variables). Bold values indicate significance at p<0.05. Significance codes: *** < 0.001, ** p<0.01, * p< 0.05, . p<0.1.

Maximal model: ~ Treatment * Site * Year				
β-cello: Final model[(GLMM, family= gamma (link="log")]: ~ Site + Year+ Site: Year				
	χ2	**Df**	**Pr(>Chisq)**	
Site	26.62	2	**<0.001**	***
Year	23.69	3	**<0.001**	***
Site: Year	53.25	6	**<0.001**	***
β-gluc: Final model [(GLMM, family= gamma (link="log")]: ~ Site + Year + Site: Year				
	χ2	**Df**	**Pr(>Chisq)**	
Site	28.86	2	**<0.001**	***
Year	65.63	3	**<0.001**	***
Site: Year	58.71	6	**<0.001**	***
α-gluc: Final model [(GLMM, family= gamma (link="log")]: ~ Treatment + Site + Year+ Site: Year				
	χ2	**Df**	**Pr(>Chisq)**	
Treatment	2.46	3	0.482	
Site	22.70	2	**<0.001**	***
Year	41.84	3	**<0.001**	***
Site: Year	58.57	6	**<0.001**	***
β-xylo: Final model [(GLMM, family= gamma (link="log")]: ~ Site + Year+ Site: Year				
	χ2	**Df**	**Pr(>Chisq)**	
Site	50.06	2	**<0.001**	***
Year	52.30	3	**<0.001**	***
Site: Year	72.87	6	**<0.001**	***
NAG: Final model [(GLMM, family= gamma (link="log")]: ~ Treatment + Site + Year+ Site: Year				
	χ2	**Df**	**Pr(>Chisq)**	
Treatment	2.42	3	0.490	
Site	5.68	2	0.058	.
Year	99.31	3	**<0.001**	***
Site: Year	33.14	6	**<0.001**	***
LAP: Final model [(GLMM, family= gamma (link=""log"")]: ~ Treatment + Site + Year+ Site: Year				
	χ2	**Df**	**Pr(>Chisq)**	
Treatment	2.71	3	0.438	
Site	7.63	2	**0.022**	*
Year	1.37	3	0.713	
Site: Year	83.04	6	**<0.001**	***
Phos: Final model [(GLMM, family= gamma (link=""log"")]: ~ Treatment + Site + Year+ Site: Year				
	χ2	**Df**	**Pr(>Chisq)**	
Treatment	3.49	3	0.321	
Site	14.67	2	**0.001**	***
Year	110.45	3	**<0.001**	***
Site: Year	73.34	6	**<0.001**	***
Phos-D: Final model [(GLMM, family= gamma (link=""log"")]: ~ Treatment + Site + Year+ Site: Year				
	χ2	**Df**	**Pr(>Chisq)**	
Treatment	7.48	3	0.058	.
Site	19.32	2	**<0.001**	***
Year	32.57	3	**<0.001**	***
Site: Year	65.30	6	**<0.001**	***
Phenol: Final model [(LMM, family= gaussian (link="ldentity")]: ~ Treatment + Site + Year + Site : Year				
	χ2	**Df**	**Pr(>Chisq)**	
Treatment	3.60	3	0.308	
Site	2.66	2	0.264	
Year	20.68	3	**<0.001**	***
Site: Year	28.65	6	**<0.001**	***
Perox: Final model [(GLMM, family= gamma (link="log")]: ~ Treatment + Site + Year + Site : Year				
	χ2	**Df**	**Pr(>Chisq)**	
Treatment	0.67	3	0.881	
Site	5.81	2	0.055	.
Year	38.08	3	**<0.001**	***
Site: Year	27.78	6	**<0.001**	***

Table S4.8. Summary of 2-way ANOVA analyses for net N-mineralization, nitrification, and ammonification in a 30-day laboratory incubation experiment. Initial nitrate (NO_3^-) and ammonium (NH_4^+) were assessed before the start of the incubation. Interaction terms were eliminated from the model if they were not significant. Bold values indicate significance at $p<0.05$. Significance codes: *** < 0.001, ** p<0.01, * p< 0.05, . p<0.1.

Maximal model: ~ Treatment * Site							
N-mineralization: Final model [(2-way ANOVA]: ~ Treatment + Site							
	Df	**Sum Sq**	**Mean Sq**	**F value**	**P(>F)**		**Residuals**
Treatment	3	4.76	1.59	3.70	**0.017**	*	54
Site	2	132.59	66.30	154.42	**<0.001**	***	
Net nitrification: Final model [(2-way ANOVA]: ~ Treatment + Site							
	Df	**Sum Sq**	**Mean Sq**	**F value**	**P(>F)**		**Residuals**
Treatment	3	3.04	1.01	3.76	**0.016**	*	54
Site	2	50.55	25.28	93.87	**<0.001**	***	
Net ammonification : Final model [(2-way ANOVA]: ~ Treatment : Site							
	Df	**Sum Sq**	**Mean Sq**	**F value**	**P(>F)**		**Residuals**
Treatment	3	0.202	0.0672	0.631	0.599		48
Site	2	0.657	0.3284	3.082	0.055	.	
Treatment : Site	6	1.576	0.2627	2.466	**0.037**	*	
Initial NO_3- : Final model [(2-way ANOVA]: ~ Treatment + Site							
	Df	**Sum Sq**	**Mean Sq**	**F value**	**P(>F)**		**Residuals**
Treatment	3	2.41	0.80	1.28	0.290		54
Site	2	1.93	0.96	1.54	0.224		
Initial NH_4+ : Final model [(2-way ANOVA]: ~ Treatment + Site							
	Df	**Sum Sq**	**Mean Sq**	**F value**	**P(>F)**		**Residuals**
Treatment	3	0.06	0.02	0.18	0.911		54
Site	2	10.18	5.09	49.66	**<0.001**	***	

Table S4.9. Summary statistics nitric oxide (NO) and initial nitrate (NO_3^-), ammonium (NH_4^+), and phosphate (PO_4^{3+}) concentrations in a 1 and 24-hour laboratory incubation experiment using soils gathered from a 4-year field N addition treatment (Ambient, +N low, and +N high treatment plots). Soils were treated with water (DI) or N (+N high) in the laboratory. NO efflux was measured at 1 and 24 hours. For NO efflux, chi-square estimates and p-values from type 3 Wald chi-squared tests were derived from a generalized linear mixed-effects model (GLMM). Backward step-wise model selection was used to select the best fitting, simplest model based on a 5 AICc improvement (maximal model is listed in bold above relevant response variables). Initial NO_3^-,

NH_4^+, and PO_4^{3+} were assessed before the start of the incubation and were analyzed using 2-way ANOVA. For 2-way ANOVA analyses, interaction terms were eliminated from models if they were not significant. Bold values indicate significance at p<0.05. Significance codes: *** < 0.001, ** p<0.01, * p< 0.05, . p<0.1.

Maximal model: ~ Lab N treatment * Field N treatment* Site * Incubation period

NO flux: Final model [(GLMM, family= gamma (link=""log"")]: ~ Lab N treatment+ Field N treatment + Site +Incubation period + Lab N treatment: Field N treatment+ Field N treatment: Site+ Lab N treatment: Incubation period+ Field N treatment : Incubation period+ Site: Incubation period+ Lab N treatment: Field N treatment: Incubaiotn period

	χ2	**Df**	**Pr(>Chisq)**	
Lab N treatment	0.40	1	0.525	
Field N treatment	0.03	2	0.985	
Site	0.37	2	0.830	
Incubation Period	23.02	1	**<0.001**	***
Lab N treatment: Field N treatment	0.01	2	0.994	
Lab N treatment: Site	0.01	2	0.996	
Lab N treatment: Incubation period	22.23	1	**<0.001**	***
Field N treatment: Incubation period	8.19	2	**0.017**	*
Site: Incubation period	11.45	2	**0.003**	**
Lab N treatment: Field N treatment: Incubation period	6.33	2	**0.042**	*
Lab N treatment: Site: Incubation period	2.74	2	0.254	

Maximal model: ~ Field N treatment * Site

Initial NO_3- : Final model [(2-way ANOVA]: ~ Field N treatment + Site

	Df	**Sum Sq**	**Mean Sq**	**F value**	**P(>F)**		**Residuals**
Field N treatment	2	1.35	0.68	3.38	**0.044**	*	40
Site	2	20.20	10.10	50.55	**<0.001**	***	

Initial NH_4+ : Final model [(2-way ANOVA]: ~ Field N treatment + Site

	Df	**Sum Sq**	**Mean Sq**	**F value**	**P(>F)**		**Residuals**
Field N treatment	2	0.86	0.43	1.98	0.151		40
Site	2	4.88	2.44	11.30	**<0.001**	***	

Initial PO_4^{3-} : Final model [(2-way ANOVA]: ~ Field N treatment + Site

	Df	**Sum Sq**	**Mean Sq**	**F value**	**P(>F)**		**Residuals**
Field N treatment	2	1.61	0.81	1.63	0.208		40
Site	2	75.18	37.59	75.99	**<0.001**	***	

Table S5.1. Sampling scheme for measured variables. * Variables measured three times per year were only measured during the monsoon season of 2018.

Variables	Sampling period	Sampling frequency
% Cover	Peak biomass (October)	1x/ annually
Foliar %C and %N	Monsoon 2021	1x (4th year only)
Soil moisture	Pre-monsoon, monsoon, and winter	3x/ annually*
Soil pH	Monsoon	1x/ annually
Available nutrients	Pre-monsoon, monsoon, and winter	3x/ annually*
EOC & ETN	Pre-monsoon, monsoon, and winter	3x/ annually*
Soil %OC & %N	Monsoon	1x/ annually
MBC, MBN, & MBP	Pre-monsoon, monsoon, and winter	3x/ annually*
Extracellular enzyme potentials	Monsoon	1x/ annually

Table S5.2. Best predictor variables for relative cover and foliar %C and %N from multiple mixed effects models including N addition treatments (Ambient N, +C, +N low, and +N high), *Eragrostis lehmanniana* removal treatments (G= non-removal and GR= removal treatment), and year (2018, 2019, and 2021). All estimates shown are relative proportions. In 2020, most cover values were zero due to extreme drought; thus, 2020 data was omitted from analyses. For total relative % cover, the Ambient N and G treatments in 2018 were set as the reference and are represented by the intercept (highlighted in grey). For all other % cover, the reference level was set as the G treatments in 2018 and is represented by the intercept. Bold values indicate significant effects (95% confidence intervals that do not encompass zero). P(>|z|) significance codes: *** < 0.001, ** p<0.01, * p< 0.05, **.** p<0.1. All treatment effects are relative to the intercept, i.e., Ambient N, G plots in 2018. A significant treatment: year interaction indicates that the treatment effect was significantly different from the treatment effect in 2018; a non-significant treatment: year interaction means the effect in that year was the same as in 2018.

Maximal model: ~ N * Removal * Year + (1 | plot.id)

Total vegetation: *Final model [(LMM, family= gaussian (link="Identity")]: ~ N + Removal + Year + Removal :Year + (1 | plot.id)*

	Estimate	Std. Error	z value	Pr(>|z|)		Min CI	Max CI	R^2 Marginal	R^2 Conditional	AICc
(Intercept)	0.32	0.03	11.69	< 0.001	***	0.26	0.37	0.68	0.68	-147.65
+C	0.02	0.03	0.68	0.498		-0.03	0.07			
+N Low	-0.01	0.03	-0.22	0.822		-0.06	0.04			
+N High	0.03	0.03	1.17	0.244		-0.02	0.08			
GR	-0.21	0.03	-6.82	**< 0.001**	***	**-0.28**	**-0.16**			
Year 2019	-0.18	0.03	-5.77	**< 0.001**	***	**-0.24**	**-0.12**			
Year 2021	0.10	0.03	3.34	**0.001**	***	**0.04**	**0.16**			
GR: Year 2019	0.13	0.04	2.97	**0.003**	**	**0.05**	**0.22**			
GR: Year 2021	0.18	0.04	3.99	**< 0.001**	***	**0.09**	**0.27**			

Eragrostis lehmanniana: *Final model [(GLMM, family= beta (link="logit")]: ~ Removal + Year + Removal :Year + (1 | plot.id)*

	Estimate	Std. Error	z value	Pr(>|z|)		Min CI	Max CI	R^2 Marginal	R^2 Conditional	AICc
(Intercept)	0.84	0.24	3.53	< 0.001	***	0.37	1.33	0.81	0.94	-128.96
GR	-1.99	0.34	-5.90	**< 0.001**	***	**-2.77**	**-1.30**			
Year 2019	0.56	0.30	1.84	0.065	.	0.005	1.19			
Year 2021	-2.47	0.36	-6.89	**< 0.001**	***	**-3.15**	**-1.87**			
GR: Year 2019	-0.70	0.43	-1.62	0.105		-1.64	0.15			
GR: Year 2021	1.86	0.44	4.22	**< 0.001**	***	**1.04**	**2.81**			

Native perennial C4 grasses: *Final model [(GLMM, family= beta (link="logit")]: ~ Removal + Year + Removal :Year + (1 | plot.id)*

	Estimate	Std. Error	z value	Pr(>|z|)		Min CI	Max CI	R^2 Marginal	R^2 Conditional	AICc
(Intercept)	-1.28	0.26	-4.93	< 0.001	***	-1.85	-0.74	0.22	0.55	-108.00
GR	0.84	0.35	2.38	**0.017**	*	**0.11**	**1.57**			
Year 2019	-0.38	0.32	-1.17	0.240		-1.02	0.30			
Year 2021	0.01	0.33	0.04	0.966		-0.62	0.68			
GR: Year 2019	0.11	0.44	0.25	0.802		-0.75	1.05			
GR: Year 2021	-1.03	0.46	-2.23	**0.026**	*	**-1.94**	**-0.11**			

Perennial forbs: *Final model [(GLMM, family= beta (link="logit")]: ~ Removal + Year + Removal :Year + (1 | plot.id)*

	Estimate	Std. Error	z value	Pr(>|z|)		Min CI	Max CI	R^2 Marginal	R^2 Conditional	AICc
(Intercept)	-2.00	0.27	-7.30	< 0.001	***	-2.59	-1.55	0.32	0.56	-161.51
GR	0.91	0.35	2.63	**0.009**	**	**0.27**	**1.67**			
Year 2019	-0.31	0.32	-0.97	0.334		-0.93	0.32			
Year 2021	0.54	0.31	1.714	0.086	.	-0.10	1.17			
GR: Year 2019	0.60	0.43	1.39	0.165		-0.30	1.48			
GR: Year 2021	-0.30	0.43	-0.71	0.476		-1.21	0.53			

Annuals: *Final model [(GLMM, family= beta (link="logit")]: ~ Removal + Year + Removal :Year + (1 | plot.id)*

	Estimate	Std. Error	z value	Pr(>|z|)		Min CI	Max CI	R^2 Marginal	R^2 Conditional	AICc
(Intercept)	-4.12	0.31	-13.23	< 0.001	***	-4.68	-3.59	0.55	0.67	-467.99
GR	0.05	0.37	0.14	0.891		-0.67	0.81			
Year 2019	-0.35	0.28	-1.26	0.209		-0.93	0.21			
Year 2021	4.27	0.27	15.70	**< 0.001**	***	**3.81**	**4.79**			
GR: Year 2019	0.46	0.38	1.21	0.226		-0.32	1.29			
GR: Year 2021	-0.24	0.29	-0.81	0.420		-0.86	0.37			

Maximal model: ~ N * Removal * Species +(1 | plot.id)

Foliar %C: *Final model [(LMM, family= gaussian (link="Identity")]: ~ N + Removal + Species + (1 | plot.id)*

	Estimate	Std. Error	z value	Pr(>|z|)		Min CI	Max CI	R^2 Marginal	R^2 Conditional	AICc
(Intercept)	43.34	0.33	131.20	<0.001	***	42.72	43.98	0.66	0.68	343.38
+C	0.31	0.36	0.85	0.394		-0.39	1.05			
+N Low	0.67	0.35	1.91	0.056	.	-0.06	1.35			
+N High	0.48	0.36	1.33	0.182		-0.24	1.14			
GR	0.22	0.26	0.86	0.388		-0.27	0.71			
Species ENDE	1.05	0.28	3.70	**<0.001**	***	**0.49**	**1.59**			
Species SIAB	-2.88	0.29	-9.86	**<0.001**	***	**-3.46**	**-2.35**			

Foliar %N: *Final model [(LMM, family= gaussian (link="Identity")]: ~ N + Removal + Species +Removal: Species + (1 | plot.id)*

	Estimate	Std. Error	z value	Pr(>|z|)		Min CI	Max CI	R^2 Marginal	R^2 Conditional	AICc
(Intercept)	2.18	0.07	30.58	<0.001	***	2.05	2.32	0.83	0.89	16.46
+C	-0.14	0.08	-1.86	0.063	.	-0.29	0.02			
+N Low	-0.03	0.08	-0.40	0.686		-0.17	0.11			
+N High	-0.12	0.08	-1.55	0.120		-0.28	0.03			
GR	0.00	0.07	0.05	0.958		-0.14	0.15			
Species ENDE	-0.09	0.06	-1.45	0.148		-0.21	0.02			
Species SIAB	0.91	0.06	14.84	**<0.001**	***	**0.78**	**1.03**			
GR: Species ENDE	0.00	0.08	0.01	0.992		-0.15	0.16			
GR: Species SIAB	0.11	0.09	1.22	0.223		-0.07	0.28			

Table S5.3. Best predictor variables for species richness (SR), Shannon's diversity (H), and H for functional groups from multiple mixed effects models and 2-way ANOVA (H Annuals only), which assessed *Eragrostis lehmanniana* removal treatments (G= *Eragrostis lehmanniana* present and GR= *Eragrostis lehmanniana* removed), N addition treatments: Ambient N (background N deposition, ~4kg N ha^{-1} yr^{-1}), C addition treatment (+C, 6g of sucrose), +N low treatment (+2 kg N ha^{-1} yr^{-1}), and +N high treatment (+4 kg N ha^{-1} yr^{-1}), and year (2018, 2019, and 2021). In 2020, most values were zero due to extreme drought. Thus 2020 data was omitted from analyses. Additionally, annual species H was only analyzed in 2021 due to the low number of annual species from 2018-2020. The reference level for mixed effects models was set as the G, Ambient N treatments in 2018 and is represented by the intercept (highlighted in grey). Bold values indicate significant effects (95% confidence intervals that do not encompass zero). P(>|z|) significance codes: *** < 0.001, ** p<0.01, * p< 0.05, **.** p<0.1. A significant treatment: year interaction indicates that the treatment effect was significantly different from 2018; a non-significant treatment: year interaction means the effect in that year was the same as in 2018.

Full model: ~ N *Removal * Year +(1 | plot.id)

H: Final model [(LMM, family= gaussian (link="identity")]: ~ N + Removal + Year + Removal :Year + (1 | plot.id)

	Estimate	Std. Error	z value	Pr(>|z|)		Min CI	Max CI	R^2 Marginal	R^2 Conditional	AICc
(Intercept)	0.79	0.10	7.59	< 0.001	***	0.58	0.99	0.55	0.68	122.72
+C	-0.13	0.11	-1.15	0.248		-0.35	0.09			
+N Low	-0.04	0.11	-0.38	0.706		-0.26	0.18			
+N High	-0.09	0.11	-0.80	0.424		-0.31	0.13			
GR	0.38	0.11	3.42	**0.001**	***	**0.16**	**0.59**			
Year 2019	-0.22	0.09	-2.37	**0.018**	*	**-0.41**	**-0.04**			
Year 2021	0.86	0.09	9.18	**< 0.001**	***	**0.68**	**1.04**			
GR: Year 2019	0.09	0.13	0.70	0.487		-0.17	0.35			
GR: Year 2021	-0.50	0.13	-3.76	**< 0.001**	***	**-0.76**	**-0.24**			

H C$_4$ grasses [(LMM, family= gaussian (link="identity")]: ~ Removal + Year (1 | plot.id)

	Estimate	Std. Error	z value	Pr(>|z|)		Min CI	Max CI	R^2 Marginal	R^2 Conditional	AICc
(Intercept)	0.39	0.06	6.88	< 0.001	***	0.29	0.50	0.15	0.34	59.11
GR	0.09	0.06	1.45	0.147		-0.03	0.22			
Year 2019	-0.07	0.06	-1.18	**0.240**		**-0.18**	**0.04**			
Year 2021	0.19	0.06	3.37	**0.001**	***	**0.07**	**0.30**			

H Perennial forbs: Final model [(LMM, family= gaussian (link="identity")]: ~ N + Removal + Year + Removal :Year + (1 | plot.id)

	Estimate	Std. Error	z value	Pr(>|z|)		Min CI	Max CI	R^2 Marginal	R^2 Conditional	AICc
(Intercept)	0.88	0.15	5.87	< 0.001	***	0.58	1.18	0.27	0.41	199.27
+C	-0.02	0.15	-0.16	0.873		-0.36	0.29			
+N Low	-0.18	0.15	-1.16	0.246		-0.49	0.11			
+N High	0.05	0.15	0.30	0.766		-0.28	0.34			
GR	-0.26	0.16	-1.65	0.098	.	-0.56	0.06			
Year 2019	-0.64	0.14	-4.49	**< 0.001**	***	**-0.90**	**-0.36**			
Year 2021	0.20	0.14	1.41	0.158		-0.05	0.48			
GR: Year 2019	0.39	0.20	1.93	0.053	.	-0.01	0.75			
GR: Year 2021	-0.09	0.20	-0.43	0.668		-0.51	0.27			

H Annuals (2021 only): Final model ANOVA: ~ N+ Removal

	df	Sum Sq	Mean Sq	F value	P(>F)
N	3	0.249	0.08286	0.542	0.657
Removal	1	0.095	0.09484	0.621	0.436
Residuals	34				

SR Final model [(GLMM, family= poisson (link="log")]: ~ N + Removal + Year + Removal :Year + (1 | plot.id)

	Estimate	Std. Error	z value	Pr(>|z|)		Min CI	Max CI	R^2 Marginal	R^2 Conditional	AICc
(Intercept)	1.79	0.11	16.12	< 0.001	***	1.58	2.01	0.51	0.56	520.67
+C	-0.02	0.10	-0.15	0.878		-0.22	0.19			
+N Low	-0.05	0.10	-0.52	0.604		-0.26	0.15			
+N High	-0.01	0.10	-0.05	0.959		-0.21	0.20			
GR	-0.16	0.14	-1.15	0.251		-0.42	0.11			
Year 2019	-0.64	0.16	-4.10	**< 0.001**	***	**-0.95**	**-0.34**			
Year 2021	0.62	0.11	5.42	**< 0.001**	***	**0.39**	**0.84**			
GR: Year 2019	0.40	0.22	1.83	0.067	.	-0.03	0.82			
GR: Year 2021	-0.09	0.17	-0.54	0.588		-0.42	0.24			

Table S5.4: Best predictor variables for soil ecosystem properties from multiple mixed effects models which assessed *Eragrostis lehmanniana* removal treatments (G= *Eragrostis lehmanniana* present and GR= *Eragrostis lehmanniana* removed), N addition treatments: Ambient N (background N deposition, ~4kg N ha^{-1} yr^{-1}), C addition treatment (+C, 6g of sucrose), +N low treatment (+2 kg N ha^{-1} yr^{-1}), and +N high treatment (+4 kg N ha^{-1} yr^{-1}), season (pre-monsoon, monsoon, and winter), and year (2018 - 2021). Soil % organic C (%OC) and total N (%TN) were only measured annually during the monsoon season. For all variables measured each year and season, the reference level was set as the G, Ambient N treatments during the pre-monsoon season in 2018 and is represented by the intercept (highlighted in grey). The reference level for soil %OC and %TN was set as the G, Ambient N treatments in 2018 and is represented by the intercept (highlighted in grey). Bold values indicate significant effects (95% confidence intervals that do not encompass zero). P(>|z|) significance codes: *** < 0.001, ** p<0.01, * p< 0.05, **.** p<0.1. A significant treatment: year interaction indicates that the treatment effect was significantly different from 2018; a non-significant treatment: year interaction means the effect in that year was the same as in 2018.

Maximal model: ~ N * Removal * Year +(1 | plot.id)

pH: *Final model [(LMM, family= gaussian (link="Identity")]: ~ N + Removal + Year + N : Removal + (1 | plot.id)*

	Estimate	Std. Error	z value	Pr(>|z|)		Min CI	Max CI	R^2 Marginal	R^2 Conditional	AICc
(Intercept)	7.65	0.04	213.87	< 0.001	***	7.59	7.72	0.60	0.60	-108.38
+C	0.02	0.04	0.35	0.729		-0.07	0.11			
+N Low	0.03	0.04	0.77	0.442		-0.06	0.12			
+N High	0.05	0.04	1.04	0.298		-0.04	0.13			
GR	0.05	0.04	1.04	0.298		-0.04	0.13			
Year 2019	0.29	0.03	9.38	**< 0.001**	***	**0.23**	**0.35**			
Year 2020	0.45	0.03	14.89	**< 0.001**	***	**0.39**	**0.51**			
Year 2021	0.23	0.03	7.57	**< 0.001**	***	**0.17**	**0.28**			
+C: GR	-0.01	0.06	-0.08	0.935		-0.13	0.12			
+N Low: GR	0.01	0.06	0.18	0.854		-0.10	0.13			
+N High: GR	-0.12	0.06	-1.88	0.060	.	-0.23	0.00			

Full model: ~ N treatment * Removal * Year+ N treatment * Removal* Season+ (1 | plot.id)

NO_3^- Final model [(GLMM, family= gamma (link="log")]: ~ N treatment + Removal + Year+ Season+ N treatment: Removal + Removal: Season+ Removal: Year+ (1 | plot.id)

	Estimate	Std. Error	z value	Pr(>|z|)		Min CI	Max CI	R^2 Marginal	R^2 Conditional	AICc
(Intercept)	1.35	0.31	4.44	<0.001	***	0.77	1.94	0.22	0.53	1802.23
+C	-0.34	0.34	-0.99	0.322		-1.08	0.27			
+N Low	-0.45	0.34	-1.30	0.194		-1.12	0.18			
+N High	-0.26	0.34	-0.75	0.455		-0.94	0.41			
GR	0.57	0.43	1.33	0.184		-0.29	1.46			
Year 2019	-0.08	0.17	-0.46	0.645		-0.43	0.28			
Year 2020	0.54	0.18	3.00	**0.003**	**	**0.20**	**0.89**			
Year 2021	0.58	0.18	3.18	**0.001**	**	**0.23**	**0.94**			
Season Winter	-0.72	0.12	-6.12	**< 0.001**	***	**-0.95**	**-0.50**			
Season Monsoon	-0.04	0.12	-0.33	0.740		-0.27	0.19			
+C: GR	0.21	0.49	0.42	0.672		-0.70	1.23			
+N Low: GR	0.81	0.49	1.66	0.097	.	-0.14	1.77			
+N High: GR	0.71	0.49	1.47	0.143		-0.28	1.67			
GR: Season Winter	0.25	0.16	1.51	0.132		-0.09	0.56			
GR: Season Monsoon	-0.30	0.16	-1.83	0.068	.	-0.63	0.02			
GR: Year 2019	-0.44	0.25	-1.77	0.076	.	-0.92	0.09			
GR: Year 2020	-0.89	0.25	-3.52	**< 0.001**	***	**-1.40**	**-0.42**			
GR: Year 2021	-1.01	0.25	-3.98	**< 0.001**	***	**-1.52**	**-0.53**			

NH_4^+ Final model [(GLMM, family= gamma (link="log")]: ~ N treatment + Removal + Year+ Season+ N treatment: Removal + Removal: Season+ Removal: Year + (1 | plot.id)

	Estimate	Std. Error	z value	Pr(>|z|)		Min CI	Max CI	R^2 Marginal	R^2 Conditional	AICc
(Intercept)	0.86	0.25	3.47	0.001	***	0.38	1.35	0.39	0.43	1656.78
+C	-0.11	0.20	-0.57	0.570		-0.50	0.27			
+N Low	-0.18	0.20	-0.91	0.361		-0.57	0.21			
+N High	-0.34	0.20	-1.73	0.084	.	-0.72	0.05			
GR	-0.48	0.36	-1.35	0.177		-1.17	0.22			
Year 2019	-0.12	0.21	-0.56	0.572		-0.53	0.29			
Year 2020	1.13	0.20	5.72	**< 0.001**	***	**0.74**	**1.52**			
Year 2021	0.61	0.22	2.74	**0.006**	**	**0.17**	**1.04**			
Season Winter	0.16	0.15	1.07	0.283		-0.13	0.45			
Season Monsoon	-0.26	0.14	-1.94	0.052	.	-0.53	0.00			
+C: GR	0.23	0.28	0.83	0.407		-0.31	0.78			
+N Low: GR	0.35	0.28	1.24	0.214		-0.20	0.89			
+N High: GR	0.40	0.28	1.43	0.152		-0.15	0.94			
GR: Year 2019	0.28	0.30	0.94	0.346		-0.31	0.87			
GR: Year 2020	0.35	0.28	1.22	0.221		-0.21	0.90			
GR: Year 2021	0.16	0.32	0.50	0.619		-0.47	0.78			
GR: Season Winter	-0.15	0.21	-0.70	0.485		-0.56	0.27			
GR: Season Monsoon	-0.07	0.19	-0.35	0.726		-0.45	0.31			

PO_4^{3-} Final model [(GLMM, family= gamma (link="log")]: ~ N treatment + Removal + Year+ Season+ N treatment: Removal + Removal: Season+ Removal: Year + (1 | plot.id)

	Estimate	Std. Error	z value	Pr(>|z|)		Min CI	Max CI	R^2 Marginal	R^2 Conditional	AICc
(Intercept)	1.61	0.15	10.90	<0.001	***	1.32	1.89	0.34	0.72	1260.36
+C	-0.13	0.18	-0.69	0.489		-0.49	0.23			
+N Low	-0.27	0.18	-1.48	0.140		-0.63	0.09			
+N High	-0.31	0.18	-1.70	0.089	.	-0.68	0.05			
GR	-0.20	0.21	-0.96	0.335		-0.61	0.21			
Year 2019	-0.23	0.07	-3.32	**0.001**	***	**-0.36**	**-0.09**			
Year 2020	0.29	0.07	4.26	**< 0.001**	***	**0.15**	**0.42**			
Year 2021	0.26	0.07	3.87	**< 0.001**	***	**0.13**	**0.40**			
Season Winter	-0.27	0.04	-6.17	**< 0.001**	***	**-0.36**	**-0.19**			
Season Monsoon	-0.11	0.04	-2.59	**0.010**	**	**-0.20**	**-0.03**			
+C: GR	-0.13	0.26	-0.50	0.615		-0.64	0.38			
+N Low: GR	0.34	0.26	1.30	0.194		-0.17	0.85			
+N High: GR	0.21	0.26	0.81	0.416		-0.30	0.72			
GR: Season Winter	-0.01	0.06	-0.15	0.878		-0.13	0.11			
GR: Season Monsoon	0.08	0.06	1.24	0.216		-0.05	0.20			
GR: Year 2019	0.17	0.10	1.76	0.079	.	-0.02	0.36			
GR: Year 2020	0.08	0.10	0.82	0.410		-0.11	0.27			
GR: Year 2021	0.07	0.10	0.73	0.463		-0.12	0.26			

EOC: Final model [(GLMM, family= gamma (link="log")]: ~ N treatment + Removal + Year+ Season+ N treatment: Removal + Removal: Season+ (1 | plot.id)

	Estimate	Std. Error	z value	Pr(>|z|)		Min CI	Max CI	R^2 Marginal	R^2 Conditional	AICc
(Intercept)	5.34	0.16	32.77	< 0.001	***	5.02	5.66	0.32	0.65	4705.76
+C	-0.13	0.21	-0.60	0.546		-0.53	0.28			
+N Low	-0.35	0.21	-1.67	0.095	.	-0.76	0.06			
+N High	-0.32	0.21	-1.55	0.121		-0.73	0.09			
GR	-0.25	0.21	-1.19	0.235		-0.67	0.17			
Year 2019	0.33	0.06	5.16	**< 0.001**	***	**0.21**	**0.46**			
Year 2020	0.86	0.06	13.54	**< 0.001**	***	**0.74**	**0.99**			
Year 2021	0.52	0.07	7.88	**< 0.001**	***	**0.39**	**0.65**			
Season Winter	-0.22	0.06	-3.79	**< 0.001**	***	**-0.34**	**-0.11**			
Season Monsoon	-0.16	0.06	-2.82	**0.005**	**	**-0.27**	**-0.05**			
+C: GR	-0.03	0.29	-0.09	0.926		-0.60	0.55			
+N Low: GR	0.41	0.29	1.40	0.160		-0.16	0.99			
+N High: GR	0.27	0.29	0.90	0.367		-0.31	0.84			
GR: Season Winter	0.01	0.08	0.10	0.923		-0.16	0.17			
GR: Season Monsoon	0.06	0.08	0.83	0.407		-0.09	0.22			

ETN: Final model [(GLMM, family= gamma (link="log")]: ~ N treatment + Removal + Year+ Season+ N treatment: Removal + Removal: Season+ Removal: Year + (1 | plot.id)

	Estimate	Std. Error	z value	Pr(>|z|)		Min CI	Max CI	R^2 Marginal	R^2 Conditional	AICc
(Intercept)	2.98	0.18	16.77	0.000	***	2.64	3.33	0.48	0.74	3129.37
+C	-0.16	0.21	-0.73	0.463		-0.58	0.26			
+N Low	-0.34	0.21	-1.58	0.114		-0.76	0.08			
+N High	-0.32	0.21	-1.47	0.141		-0.74	0.10			
GR	0.00	0.25	0.01	0.996		-0.49	0.50			
Year 2019	0.45	0.09	4.88	**< 0.001**	***	**0.27**	**0.62**			
Year 2020	1.23	0.09	13.66	**< 0.001**	***	**1.06**	**1.41**			
Year 2021	1.23	0.09	13.07	**< 0.001**	***	**1.05**	**1.42**			
Season Winter	-0.45	0.06	-7.46	**< 0.001**	***	**-0.57**	**-0.33**			
Season Monsoon	-0.07	0.06	-1.17	0.241		-0.19	0.05			
+C: GR	0.09	0.30	0.30	0.761		-0.50	0.69			
+N Low: GR	0.53	0.30	1.74	0.082	.	-0.07	1.12			
+N High: GR	0.43	0.30	1.43	0.153		-0.16	1.03			
GR: Season Winter	0.05	0.09	0.57	0.571		-0.12	0.21			
GR: Season Monsoon	-0.04	0.09	-0.49	0.621		-0.21	0.12			
GR: Year 2019	-0.18	0.13	-1.36	0.174		-0.44	0.08			
GR: Year 2020	-0.35	0.13	-2.69	**0.007**	**	**-0.60**	**-0.10**			
GR: Year 2021	-0.34	0.14	-2.53	**0.011**	*	**-0.61**	**-0.08**			

Maximal model: ~ N * Removal * Year +(1 | plot.id)

Soil %OC: *Final model [(LMM, family= gaussian (link="Identity")]: ~ N + Removal + Year + N : Removal + (1 | plot.id)*

	Estimate	Std. Error	z value	Pr(>|z|)		Min CI	Max CI	R^2 Marginal	R^2 Conditional	AICc
(Intercept)	7.30	0.45	16.22	<0.001	***	6.50	8.21	0.19	0.63	477.75
+C	-0.38	0.62	-0.61	0.543		-1.57	0.85			
+N Low	0.19	0.62	0.31	0.758		-1.04	1.42			
+N High	-0.04	0.62	-0.06	0.952		-1.29	1.09			
GR	0.32	0.62	0.52	0.604		-0.90	1.54			
Year 2019	-0.97	0.18	-5.36	**< 0.001**	***	**-1.30**	**-0.64**			
Year 2020	-0.53	0.18	-2.94	**< 0.001**	**	**-0.85**	**-0.20**			
Year 2021	-0.95	0.18	-5.25	**< 0.001**	***	**-1.26**	**-0.59**			
+C: GR	0.26	0.87	0.30	0.762		-1.47	2.01			
+N Low: GR	-0.14	0.87	-0.16	0.876		-1.82	1.56			
+N High: GR	-1.23	0.87	-1.41	0.157		-3.02	0.58			

Soil %TN: *Final model [(LMM, family= gaussian (link="Identity")]: ~ Removal + Year + Remova: Yearl + (1 | plot.id)*

	Estimate	Std. Error	z value	Pr(>|z|)		Min CI	Max CI	R^2 Marginal	R^2 Conditional	AICc
(Intercept)	0.13	0.01	10.39	<0.001	***	0.11	0.16	0.09	0.73	-492.26
GR	0.00	0.02	0.17	0.867		-0.03	0.04			
Year 2019	0.02	0.01	1.60	0.111		0.00	0.03			
Year 2020	0.05	0.01	5.56	**< 0.001**	***	**0.03**	**0.07**			
Year 2021	0.04	0.01	4.48	**< 0.001**	***	**0.02**	**0.06**			
GR: Year 2019	-0.01	0.01	-0.62	0.536		-0.03	0.02			
GR: Year 2020	-0.03	0.01	-1.96	**0.050**	*	-0.05	0.001			
GR: Year 2021	-0.02	0.01	-1.60	0.110		-0.05	0.01			

Table S5.5. Best predictor variables for microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), and microbial biomass phosphorous (MBP) from multiple mixed effects models which assessed *Eragrostis lehmanniana* removal treatments (G= *Eragrostis lehmanniana* present and GR= *Eragrostis lehmanniana* removed), N addition treatments: Ambient N (background N deposition, ~4kg N ha^{-1} yr^{-1}), C addition treatment (+C, 6g of sucrose), +N low treatment (+2 kg N ha^{-1} yr^{-1}), and +N high treatment (+4 kg N ha^{-1} yr^{-1}), season (pre-monsoon, monsoon, and winter), and year (2018 - 2021). The reference level was set as the G, Ambient N treatments during the pre-monsoon season in 2018 and is represented by the intercept (highlighted in grey). Bold values indicate significant effects (95% confidence intervals that do not encompass zero). P(>|z|) significance codes: *** < 0.001, ** p<0.01, * p< 0.05, **.** p<0.1. A significant treatment: year interaction indicates that the treatment effect was significantly different from 2018; a non-significant treatment: year interaction means the effect in that year was the same as in 2018.

Full model: ~ N * Removal * Year+ N treatment * Removal* Seasonf+ (1 | plot.id)

MBC: Final model [(GLMM, family= gamma (link="log")]: ~ N + Removal + Year+ Season+ N: Removal + Removal: Season+ Removal: Year + (1 | plot.id)

	Estimate	Std. Error	z value	Pr(>|z|)		Min CI	Max CI	R^2 Marginal	R^2 Conditional	AICc
(Intercept)	5.54	0.22	25.40	< 0.001	***	5.09	5.96	0.35	0.40	4799.62
+C	-0.08	0.17	-0.44	0.661		-0.40	0.27			
+N Low	-0.29	0.17	-1.67	0.096	.	-0.63	0.05			
+N High	-0.23	0.17	-1.33	0.184		-0.57	0.10			
GR	-0.10	0.31	-0.32	0.750		-0.71	0.50			
Year 2019	-1.07	0.17	-6.14	**< 0.001**	***	**-1.42**	**-0.72**			
Year 2020	-0.59	0.18	-3.21	**0.001**	**	**-0.95**	**-0.24**			
Year 2021	-0.04	0.18	-0.24	0.813		-0.39	0.30			
Season Winter	0.56	0.12	4.51	**< 0.001**	***	**0.34**	**0.79**			
Season Monsoon	0.26	0.12	2.23	**0.026**	*	**0.01**	**0.48**			
+C: GR	-0.13	0.24	-0.53	0.597		-0.61	0.35			
+N Low: GR	0.19	0.24	0.77	0.444		-0.30	0.69			
+N High: GR	0.33	0.24	1.34	0.179		-0.15	0.82			
GR: Season Winter	0.03	0.18	0.20	0.846		-0.28	0.31			
GR: Season Monsoon	0.02	0.16	0.14	0.886		-0.32	0.34			
GR: Year 2019	-0.01	0.25	-0.05	0.963		-0.52	0.50			
GR: Year 2020	-0.15	0.26	-0.56	0.574		-0.64	0.36			
GR: Year 2021	-0.23	0.25	-0.95	0.341		-0.72	0.29			

MBN: Final model [(GLMM, family= gamma (link="log")]: ~ N + Removal + Year+ Season+ N: Removal + Removal: Season+ Removal: Year + (1 | plot.id)

	Estimate	Std. Error	z value	Pr(>|z|)		Min CI	Max CI	R^2 Marginal	R^2 Conditional	AICc
(Intercept)	3.48	0.24	14.46	< 0.001	***	2.96	3.92	0.48	0.52	3161.88
+C	-0.06	0.19	-0.31	0.760		-0.42	0.33			
+N Low	-0.22	0.19	-1.14	0.255		-0.57	0.16			
+N High	-0.19	0.19	-1.02	0.306		-0.58	0.19			
GR	-0.19	0.34	-0.57	0.570		-0.84	0.54			
Year 2019	-1.40	0.20	-6.94	**< 0.001**	***	**-1.76**	**-1.01**			
Year 2020	-0.21	0.19	-1.07	0.285		-0.58	0.20			
Year 2021	0.13	0.19	0.68	0.496		-0.24	0.56			
Season Winter	-0.22	0.13	-1.70	0.090	.	-0.48	0.04			
Season Monsoon	0.49	0.13	3.92	**< 0.001**	***	**0.24**	**0.75**			
+C: GR	-0.14	0.27	-0.53	0.594		-0.71	0.38			
+N Low: GR	0.13	0.27	0.47	0.638		-0.49	0.63			
+N High: GR	0.30	0.27	1.10	0.271		-0.24	0.83			
GR: Season Winter	0.11	0.18	0.61	0.543		-0.26	0.47			
GR: Season Monsoon	0.12	0.18	0.65	0.515		-0.24	0.48			
GR: Year 2019	0.18	0.29	0.62	0.535		-0.37	0.69			
GR: Year 2020	-0.07	0.28	-0.26	0.792		-0.65	0.42			
GR: Year 2021	-0.28	0.27	-1.06	0.291		-0.80	0.24			

MBP: Final model [(GLMM, family= tweedie (link="log")]: ~ N + Removal + Year+ Season+ N: Removal + Removal: Season+ (1 | plot.id)

	Estimate	Std. Error	z value	Pr(>|z|)		Min CI	Max CI	R^2 Marginal	R^2 Conditional	AICc
(Intercept)	0.46	0.22	2.08	0.037	*	-0.01	0.87	0.44	0.42	1337.56
+C	0.21	0.17	1.24	0.215		-0.13	0.56			
+N Low	0.00	0.18	-0.02	0.984		-0.35	0.33			
+N High	-0.04	0.18	-0.20	0.844		-0.39	0.32			
GR	-0.25	0.23	-1.09	0.276		-0.76	0.20			
Year 2019	-0.77	0.18	-4.19	0.000	***	**-1.13**	**-0.41**			
Year 2020	-0.31	0.18	-1.76	0.078	.	-0.63	0.02			
Year 2021	0.62	0.16	3.81	0.000	***	**0.33**	**0.94**			
Season Winter	-0.03	0.16	-0.21	0.835		-0.35	0.30			
Season Monsoon	0.10	0.15	0.68	0.494		-0.21	0.43			
+C: GR	-0.27	0.26	-1.07	0.285		-0.78	0.26			
+N Low: GR	0.13	0.26	0.50	0.617		-0.37	0.64			
+N High: GR	0.32	0.25	1.28	0.201		-0.17	0.82			
GR: Season Winter	0.02	0.24	0.07	0.943		-0.44	0.49			
GR: Season Monsoon	0.04	0.22	0.18	0.857		-0.41	0.46			

Table S5.6: Best predictor variables for soil extracellular enzyme potentials which assessed *Eragrostis lehmanniana* removal treatments (G= *Eragrostis lehmanniana* present and GR= *Eragrostis lehmanniana* removed), N addition treatments: Ambient N (background N deposition, ~4kg N ha^{-1} yr^{-1}), C addition treatment (+C, 6g of sucrose), +N low treatment (+2 kg N ha^{-1} yr^{-1}), and +N high treatment (+4 kg N ha^{-1} yr^{-1}), and year (2018 - 2021). The reference level was set as the G, Ambient N treatments in 2018 and is represented by the intercept (highlighted in grey). Bold values indicate significant effects (95% confidence intervals that do not encompass zero). P(>|z|) significance codes: *** < 0.001, ** p<0.01, * p< 0.05, **.** p<0.1. A significant treatment: year interaction indicates that the treatment effect was significantly different from 2018; a non-significant treatment: year interaction means the effect in that year was the same as in 2018.

Maximal model: ~ N * Removal * Year +(1 | plot.id)

β-cello: Final model [(LMM, family= gaussian (link="Identity")]: ~ Removal + Year+ Removal; Year + (1 | plot.id)

	Estimate	Std. Error	z value	Pr(>|z|)		Min CI	Max CI	R^2 Marginal	R^2 Conditional	AICc
(Intercept)	0.014	0.002	6.96	< 0.001	***	0.0101	0.0180	0.13	0.41	-945.99
GR	-0.002	0.003	-0.58	0.560		-0.0073	0.0040			
Year 2019	0.008	0.002	3.39	**0.001**	***	**0.0034**	**0.0129**			
Year 2020	-0.001	0.002	-0.62	0.532		-0.0060	0.0031			
Year 2021	0.003	0.002	1.42	0.157		-0.0013	0.0079			
GR: Year 2019	-0.007	0.003	-2.00	**0.045**	*	**-0.0134**	**-0.0002**			
GR: Year 2020	-0.001	0.003	-0.22	0.828		-0.0072	0.0058			
GR: Year 2021	-0.002	0.003	-0.48	0.630		-0.0081	0.0049			

β-gluc: Final model [(LMM, family= gaussian (link="Identity")]: ~ N + Removal + Year + Removal: Year + (1 | plot.id)

	Estimate	Std. Error	z value	Pr(>|z|)		Min CI	Max CI	R^2 Marginal	R^2 Conditional	AICc
(Intercept)	0.23	0.03	7.90	< 0.001	***	0.16	0.28	0.19	0.52	-248.94
+C	0.02	0.03	0.53	0.598		-0.04	0.08			
+N Low	-0.02	0.03	-0.60	0.546		-0.08	0.04			
+N High	-0.03	0.03	-0.91	0.363		-0.09	0.03			
GR	-0.01	0.03	-0.26	0.797		-0.06	0.05			
Year 2019	0.10	0.02	4.27	**< 0.001**	***	**0.05**	**0.15**			
Year 2020	-0.03	0.02	-1.40	0.162		-0.07	0.01			
Year 2021	0.03	0.02	1.33	0.185		-0.01	0.08			
GR: Year 2019	-0.07	0.03	-2.18	**0.029**	*	**-0.14**	**-0.01**			
GR: Year 2020	-0.01	0.03	-0.20	0.840		-0.07	0.05			
GR: Year 2021	-0.03	0.03	-0.88	0.382		-0.09	0.03			

α-gluc: Final model [(GLMM, family= gamma (link="log")]: ~ N + Removal + Year+ N: Removal + Removal: Year (1 | plot.id)

	Estimate	Std. Error	z value	Pr(>|z|)		Min CI	Max CI	R^2 Marginal	R^2 Conditional	AICc
(Intercept)	-4.90	0.19	-25.73	< 0.001	***	-5.28	-4.56	0.24	0.39	-1226.38
+C	0.13	0.23	0.58	0.564		-0.31	0.58			
+N Low	-0.27	0.23	-1.14	0.253		-0.69	0.20			
+N High	-0.29	0.23	-1.26	0.208		-0.75	0.17			
GR	-0.28	0.27	-1.04	0.299		-0.82	0.25			
Year 2019	0.85	0.17	5.01	**< 0.001**	***	**0.53**	**1.18**			
Year 2020	0.16	0.17	0.97	0.334		-0.15	0.49			
Year 2021	0.50	0.16	3.05	**0.002**	**	**0.16**	**0.81**			
+C: GR	-0.13	0.32	-0.40	0.688		-0.71	0.51			
+N Low: GR	0.31	0.33	0.94	0.348		-0.34	0.96			
+N High: GR	0.44	0.32	1.36	0.173		-0.20	1.05			
GR: Year 2019	-0.25	0.24	-1.03	0.301		-0.72	0.18			
GR: Year 2020	0.14	0.23	0.58	0.560		-0.36	0.58			
GR: Year 2021	-0.01	0.23	-0.05	0.957		-0.50	0.46			

β-xylo: Final model [(GLMM, family= gamma (link="log")]: ~ N + Removal + Year+ (1 | plot.id)

	Estimate	Std. Error	z value	Pr(>|z|)		Min CI	Max CI	R^2 Marginal	R^2 Conditional	AICc
(Intercept)	-3.03	0.13	-23.43	< 0.001	***	-3.291	-2.771	0.23	0.42	-752.08
+C	0.02	0.15	0.13	0.896		-0.271	0.306			
+N Low	-0.14	0.15	-0.93	0.352		-0.446	0.144			
+N High	-0.09	0.15	-0.63	0.527		-0.394	0.188			
GR	-0.15	0.10	-1.48	0.139		-0.370	0.041			
Year 2019	0.48	0.10	4.91	**< 0.001**	***	**0.280**	**0.673**			
Year 2020	-0.19	0.10	-2.00	0.046	*	**-0.384**	**0.005**			
Year 2021	0.17	0.10	1.76	0.079	.	-0.012	0.365			

NAG: Final model [(GLMM, family= gamma (link="log")]: ~ N + Removal + Year+ N: Year + (1 | plot.id)

	Estimate	Std. Error	z value	Pr(>|z|)		Min CI	Max CI	R^2 Marginal	R^2 Conditional	AICc
(Intercept)	-4.10	0.17	-24.73	< 0.001	***	-4.43	-3.80	0.32	0.43	-1076.59
+C	0.18	0.22	0.80	0.423		-0.25	0.60			
+N Low	0.75	0.23	3.34	**0.001**	***	**0.35**	**1.17**			
+N High	-0.42	0.22	-1.90	0.058	.	-0.85	0.00			
GR	-0.20	0.10	-2.05	**0.040**	*	**-0.38**	**-0.02**			
Year 2019	0.14	0.20	0.70	0.484		-0.27	0.57			
Year 2020	-0.20	0.20	-0.96	0.335		-0.59	0.19			
Year 2021	0.02	0.20	0.09	0.930		-0.38	0.44			
+C Year 2019	0.11	0.29	0.38	0.708		-0.48	0.69			
+N low: Year 2019	-0.60	0.30	-2.02	**0.044**	*	**-1.18**	**-0.08**			
+N high: Year 2019	0.77	0.29	2.68	**0.007**	**	**0.23**	**1.31**			
+C Year 2020	-0.40	0.29	-1.39	0.164		-0.99	0.15			
+N low: Year 2020	-0.83	0.29	-2.86	**0.004**	**	**-1.39**	**-0.27**			
+N high: Year 2020	0.37	0.29	1.31	0.191		-0.21	0.91			
+C :Year 2021	0.01	0.29	0.03	0.978		-0.59	0.57			
+N low: Year 2021	-0.83	0.29	-2.84	**0.005**	**	**-1.42**	**-0.25**			
+N high: Year 2021	0.47	0.29	1.65	0.099	.	-0.13	1.03			

LAP: Final model [(GLMM, family= gamma (link=""log"")]: ~ N + Removal + Year+ N: Removal + Removal: Year (1 | plot.id)

	Estimate	Std. Error	z value	Pr(>|z|)		Min CI	Max CI	R^2 Marginal	R^2 Conditional	AICc
(Intercept)	-0.80	0.10	-8.04	< 0.001	***	-1.00	-0.61	0.50	0.63	-152.01
+C	0.07	0.11	0.60	0.547		-0.15	0.29			
+N Low	-0.03	0.11	-0.28	0.782		-0.25	0.20			
+N High	0.04	0.11	0.33	0.743		-0.18	0.27			
GR	-0.06	0.08	-0.72	0.471		-0.21	0.10			
Year 2019	0.46	0.07	6.22	**< 0.001**	***	**0.31**	**0.60**			
Year 2020	-0.50	0.07	-6.80	**< 0.001**	***	**-0.64**	**-0.36**			
Year 2021	-0.37	0.07	-5.08	**< 0.001**	***	**-0.51**	**-0.24**			

Phos: Final model [(LMM, family= gaussian (link="Identity")]: ~ N + Removal + Year + Removal: Year + (1 | plot.id)

	Estimate	Std. Error	z value	Pr(>|z|)		Min CI	Max CI	R^2 Marginal	R^2 Conditional	AICc
(Intercept)	0.06	0.01	5.96	< 0.001	***	0.040	0.083	0.45	0.62	-535.57
+C	0.01	0.01	0.76	0.448		-0.012	0.029			
+N Low	-0.01	0.01	-0.55	0.581		-0.027	0.015			
+N High	0.00	0.01	0.05	0.962		-0.020	0.021			
GR	-0.01	0.01	-0.91	0.363		-0.033	0.011			
Year 2019	0.09	0.01	9.54	**< 0.001**	***	**0.070**	**0.104**			
Year 2020	0.01	0.01	1.40	0.160		-0.005	0.032			
Year 2021	0.01	0.01	1.12	0.262		-0.008	0.028			
GR: Year 2019	-0.03	0.01	-2.22	**0.027**	*	**-0.052**	**-0.002**			
GR: Year 2020	-0.01	0.01	-0.60	0.550		-0.032	0.018			
GR: Year 2021	0.00	0.01	0.05	0.961		-0.025	0.025			

Phos-D: Final model [(GLMM, family= gamma (link="log")]: ~ N + Removal + Year+ N: Removal + (1 | plot.id)

	Estimate	Std. Error	z value	Pr(>|z|)		Min CI	Max CI	R^2 Marginal	R^2 Conditional	AICc
(Intercept)	-4.48	0.14	-31.11	< 0.001	***	-4.81	-4.19	0.35	0.47	-1225.67
+C	0.16	0.19	0.88	0.380		-0.21	0.56			
+N Low	-0.20	0.19	-1.05	0.292		-0.55	0.19			
+N High	-0.19	0.19	-1.04	0.301		-0.56	0.19			
GR	-0.30	0.19	-1.58	0.113		-0.66	0.08			
Year 2019	0.34	0.10	3.50	**< 0.001**	***	**0.14**	**0.52**			
Year 2020	-0.38	0.10	-3.96	**< 0.001**	***	**-0.56**	**-0.19**			
Year 2021	0.41	0.10	4.25	**< 0.001**	***	**0.22**	**0.61**			
+C: GR	-0.09	0.26	-0.33	0.739		-0.62	0.43			
+N low: GR	0.36	0.27	1.36	0.174		-0.17	0.88			
+N High: GR	0.28	0.26	1.08	0.282		-0.25	0.82			

Phenol: Final model [(GLMM, family= gamma (link="log")]: ~ N + Removal + Year+ N: Removal + (1 | plot.id)

	Estimate	Std. Error	z value	Pr(>|z|)		Min CI	Max CI	R^2 Marginal	R^2 Conditional	AICc
(Intercept)	2.72	0.16	16.83	< 0.001	***	2.35	3.03	0.36	0.40	1282.11
+C	0.24	0.20	1.21	0.226		-0.18	0.69			
+N Low	-0.19	0.20	-0.93	0.355		-0.55	0.23			
+N High	0.04	0.20	0.20	0.842		-0.36	0.42			
GR	-0.03	0.20	-0.14	0.889		-0.41	0.40			
Year 2019	1.08	0.13	8.54	**< 0.001**	***	**0.84**	**1.33**			
Year 2020	0.69	0.13	5.40	**< 0.001**	***	**0.46**	**0.93**			
Year 2021	0.24	0.13	1.89	0.059	.	-0.01	0.50			
+C: GR	-0.39	0.28	-1.38	0.167		-1.02	0.19			
+N low: GR	0.10	0.29	0.36	0.720		-0.50	0.62			
+N High: GR	-0.05	0.28	-0.17	0.865		-0.63	0.47			

Perox: Final model [(GLMM, family= gamma (link="log")]: ~ N + Removal + Year+ (1 | plot.id)

	Estimate	Std. Error	z value	Pr(>|z|)		Min CI	Max CI	R^2 Marginal	R^2 Conditional	AICc
(Intercept)	3.22	0.07	43.58	< 0.001	***	3.08	3.36	0.55	0.65	1355.70
GR	-0.04	0.08	-0.44	0.662		-0.20	0.13			
Year 2019	1.18	0.08	15.21	**< 0.001**	***	**1.03**	**1.34**			
Year 2020	0.76	0.08	9.80	**< 0.001**	***	**0.61**	**0.92**			
Year 2021	0.49	0.08	6.34	**< 0.001**	***	**0.34**	**0.64**			

SUPPLEMENTARY FIGURES

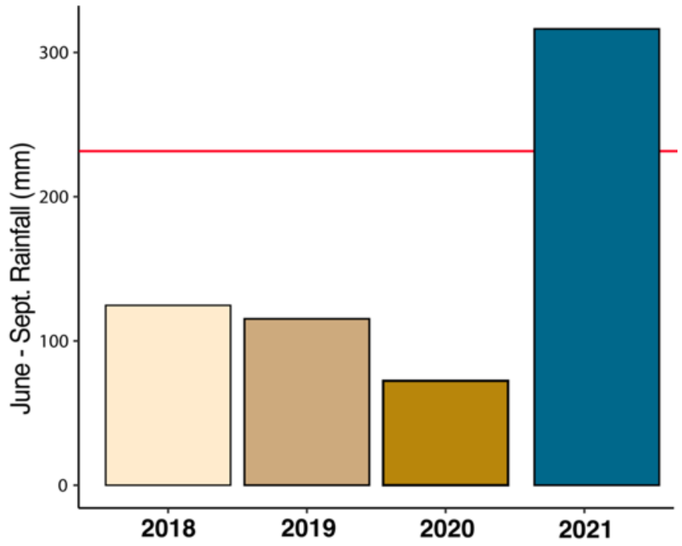

Fig. S1.1. Average summer rainfall from June to September. The red line represents the 30-year average rainfall (227 mm) from June to September. Annual 2018- 2021 rainfall data was acquired from BATN5 station http://mesowest.utah.edu. The 30-yr normal rainfall data (252 mm June- July 1981-2010) was acquired from the National Weather Service Cooperative Network (Station ID #: 291480-7; wrcc.dri.edu).

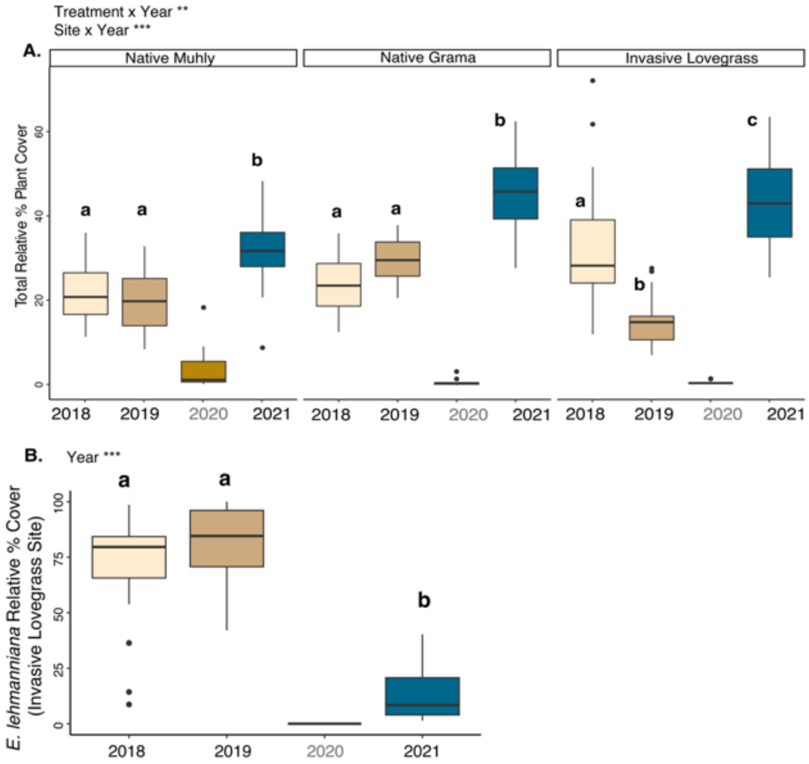

Fig. S4.1. Boxplot of total relative % plant cover (A) and *Eragrostis lehmanniana* relative % cover (B; Invasive Lovegrass site only) in a 4-year N addition manipulation experiment in the Chihuahuan Desert. Statistical analyses did not include 2020. Bold lowercase letters indicate significant differences between years (estimated marginal means, $p<0.05$). See the top left for significant main and interaction effects (significance codes: *** < 0.001, ** $p<0.01$, * $p< 0.05$, . $p<0.1$).

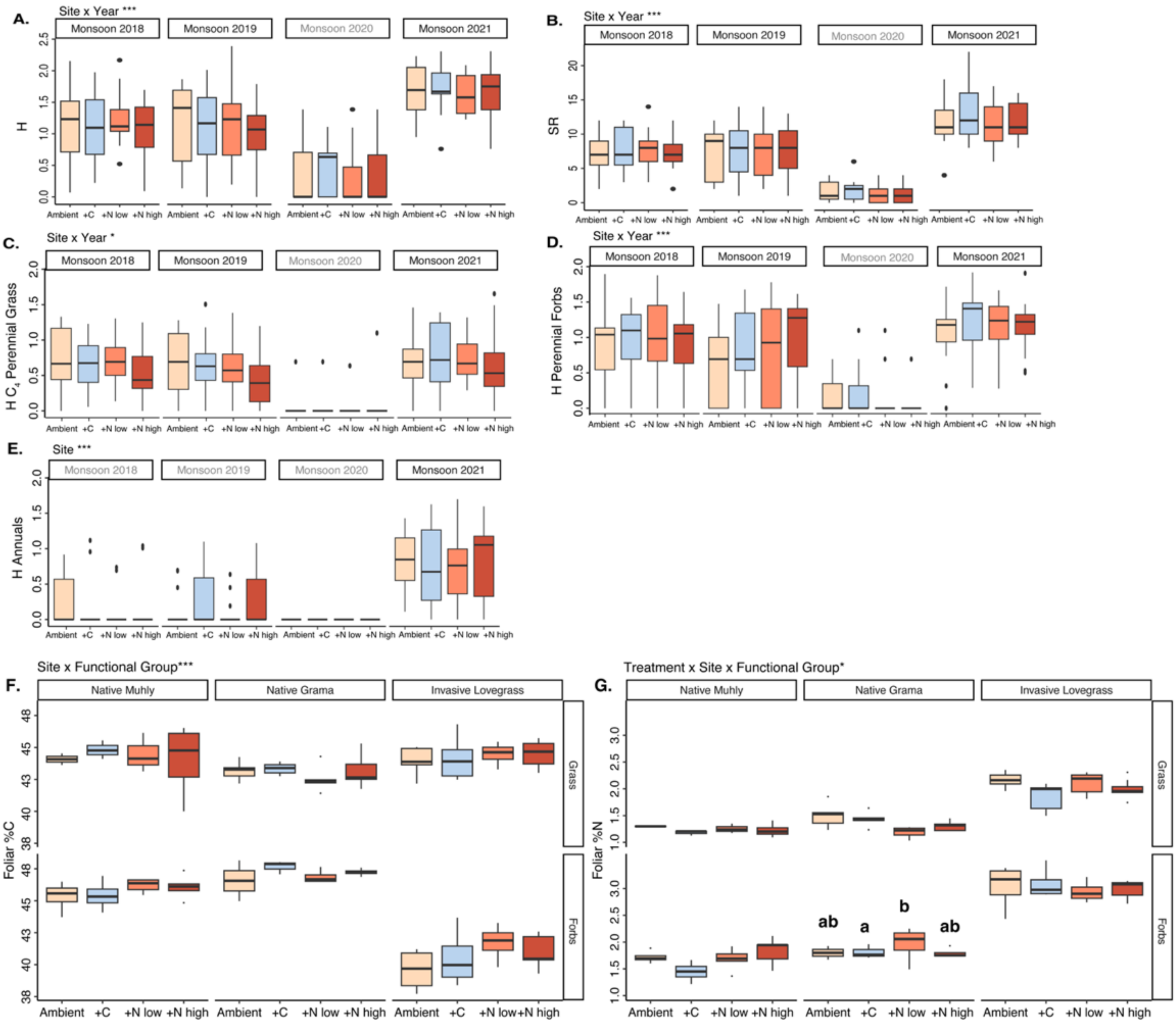

Fig. S4.2 Boxplots of community Shannon's diversity (H, A), species richness (SR, B), C4 perennial grass H (C), perennial forb H (D), annual species H (E), foliar %C (F), and foliar %N (G) in a 4-year field N addition experiment conducted in the Chihuahuan Desert N.M. USA. H and SR analyses (A- D) did not include 2020. Annual species H (E) was only analyzed in 2021. Foliar %C and %N concentrations were only measured in 2021. Bold lowercase letters indicate significant treatment effects (estimated marginal means, $p<0.05$). See the top left for significant main and interaction effects (significance codes: *** < 0.001, ** p<0.01, * p< 0.05, . p<0.1).

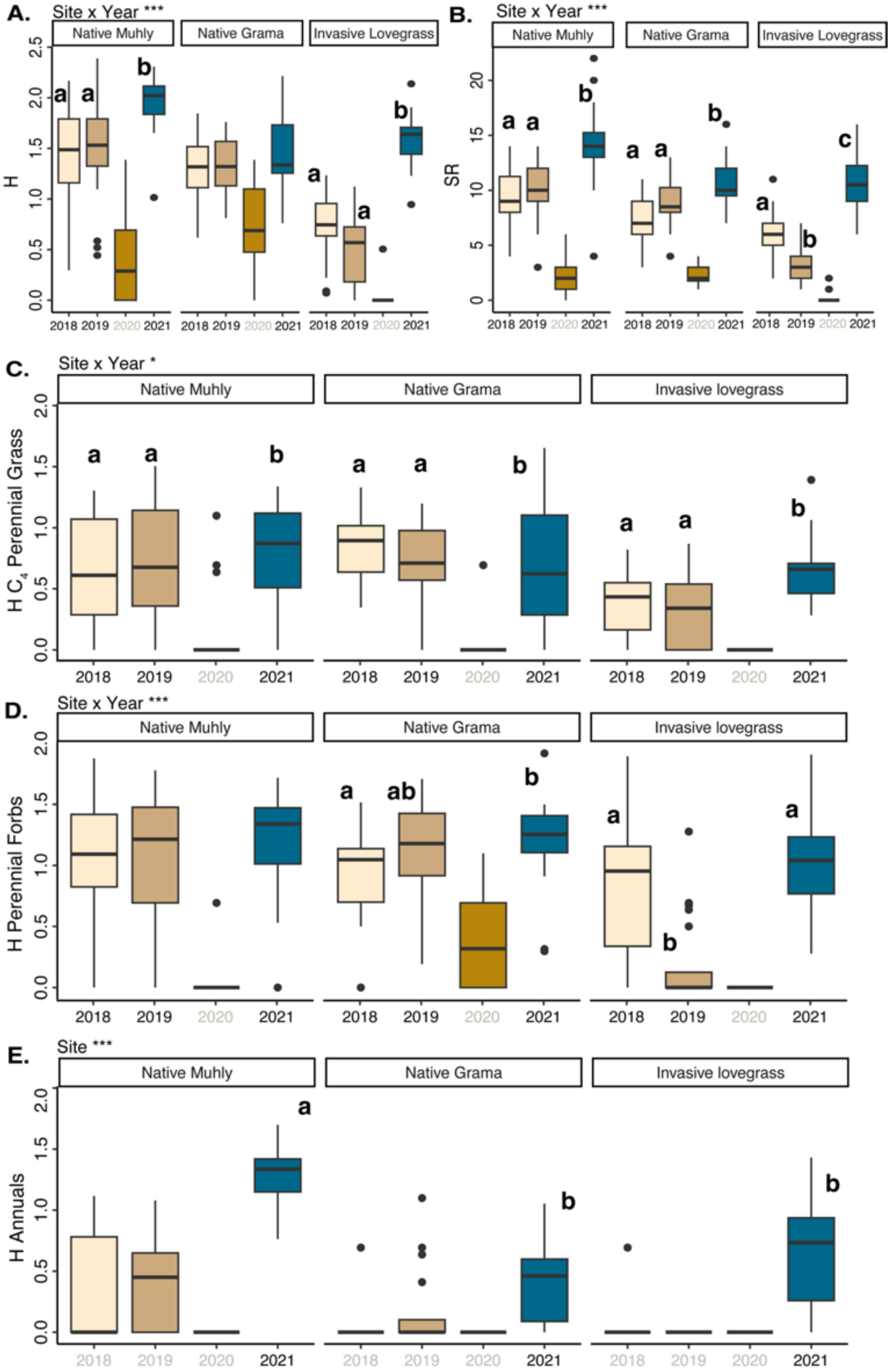

Fig. S4.3. Boxplots of community Shannon's diversity (H, A), species richness (SR, B), C4 perennial grass H (C), perennial forb H (D) and annual plant H (E), in a 4-year field N addition experiment conducted in three adjacent Chihuahuan Desert grasslands. H and SR analyses (A- D) did not include 2020. Annual species H (E) was only analyzed in 2021. Bold lowercase letters indicate significant treatment effects (estimated marginal means, p<0.05). See the top left for significant main and interaction effects (significance codes: *** < 0.001, ** p<0.01, * p< 0.05, . p<0.1).

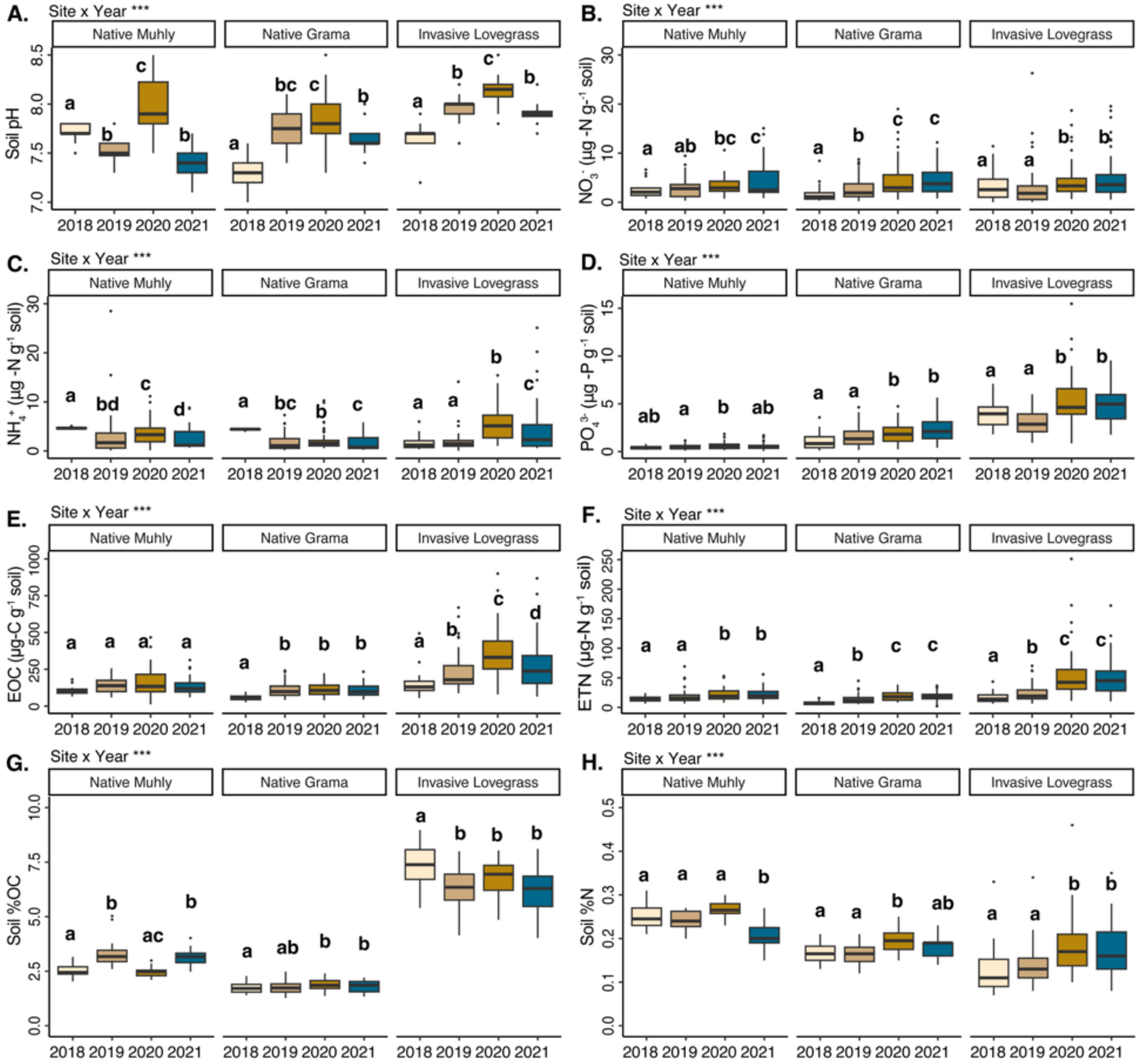

Fig. S4.4. Boxplots of soil ecosystem properties: soil pH (A) available soil nitrate (NO_3^-, B), ammonium (NH_4^+, C), phosphate (PO_4^{3-}, D), extractable organic carbon (EOC, E), extractable total nitrogen (ETN, F), soil %OC (G), and soil %N (H) in a 4-year N addition experiment conducted three adjacent Chihuahuan Desert grasslands. Bold lowercase letters indicate significant treatment effects (estimated marginal means, p<0.05). See the top left for significant main and interaction effects (significance codes: *** < 0.001, ** p<0.01, * p< 0.05, . p<0.1).

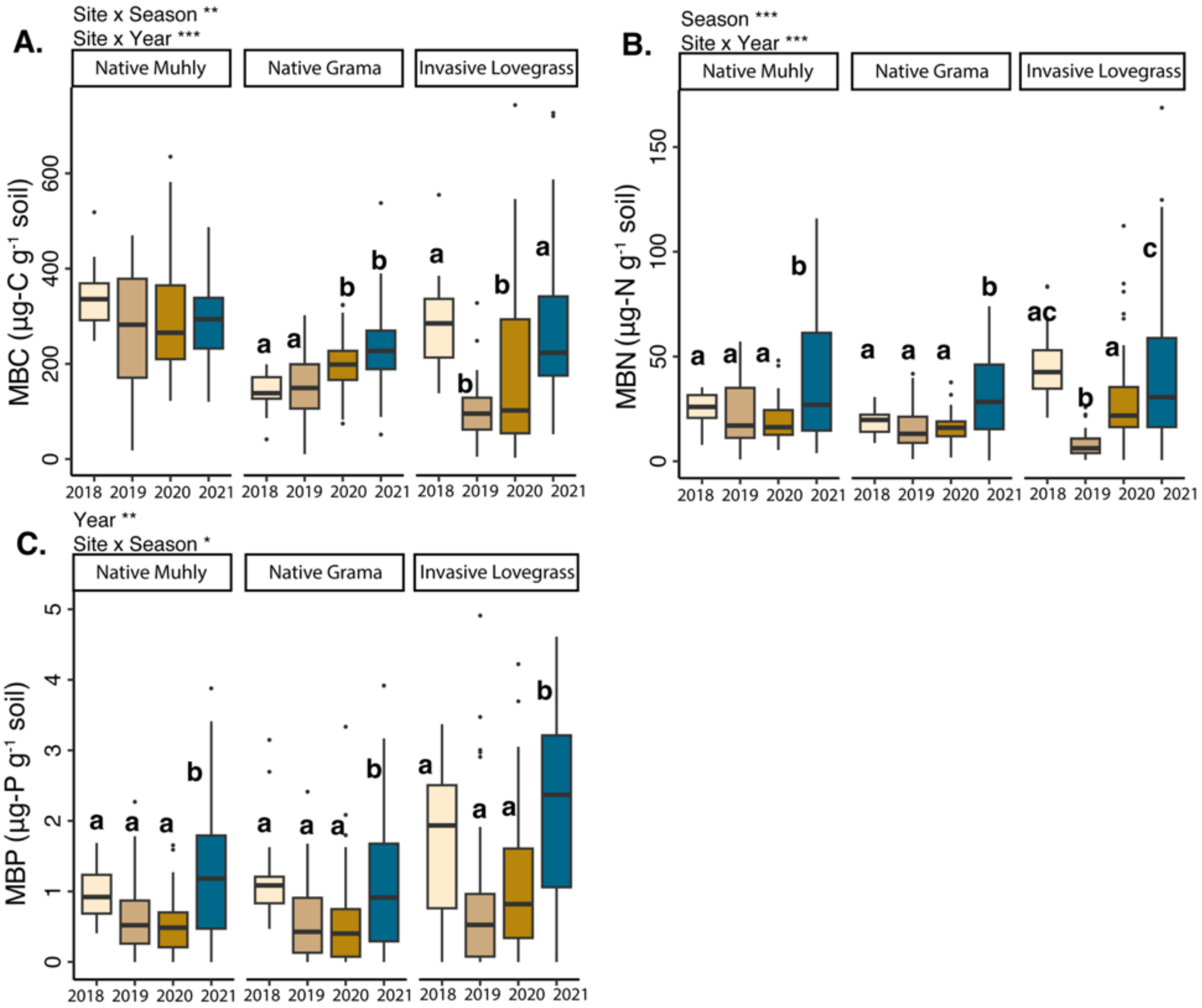

Fig. S4.5.Boxplots of soil microbial biomass carbon (MBC, A), soil microbial biomass nitrogen (MBN, B), microbial biomass phosphorus (MBP, C), in a 4-year N addition experiment conducted three adjacent Chihuahuan Desert grasslands. Bold lowercase letters indicate significant treatment effects (estimated marginal means, $p<0.05$). See the top left for significant main and interaction effects (Significance codes: *** < 0.001, ** $p<0.01$, * $p< 0.05$, . $p<0.1$).

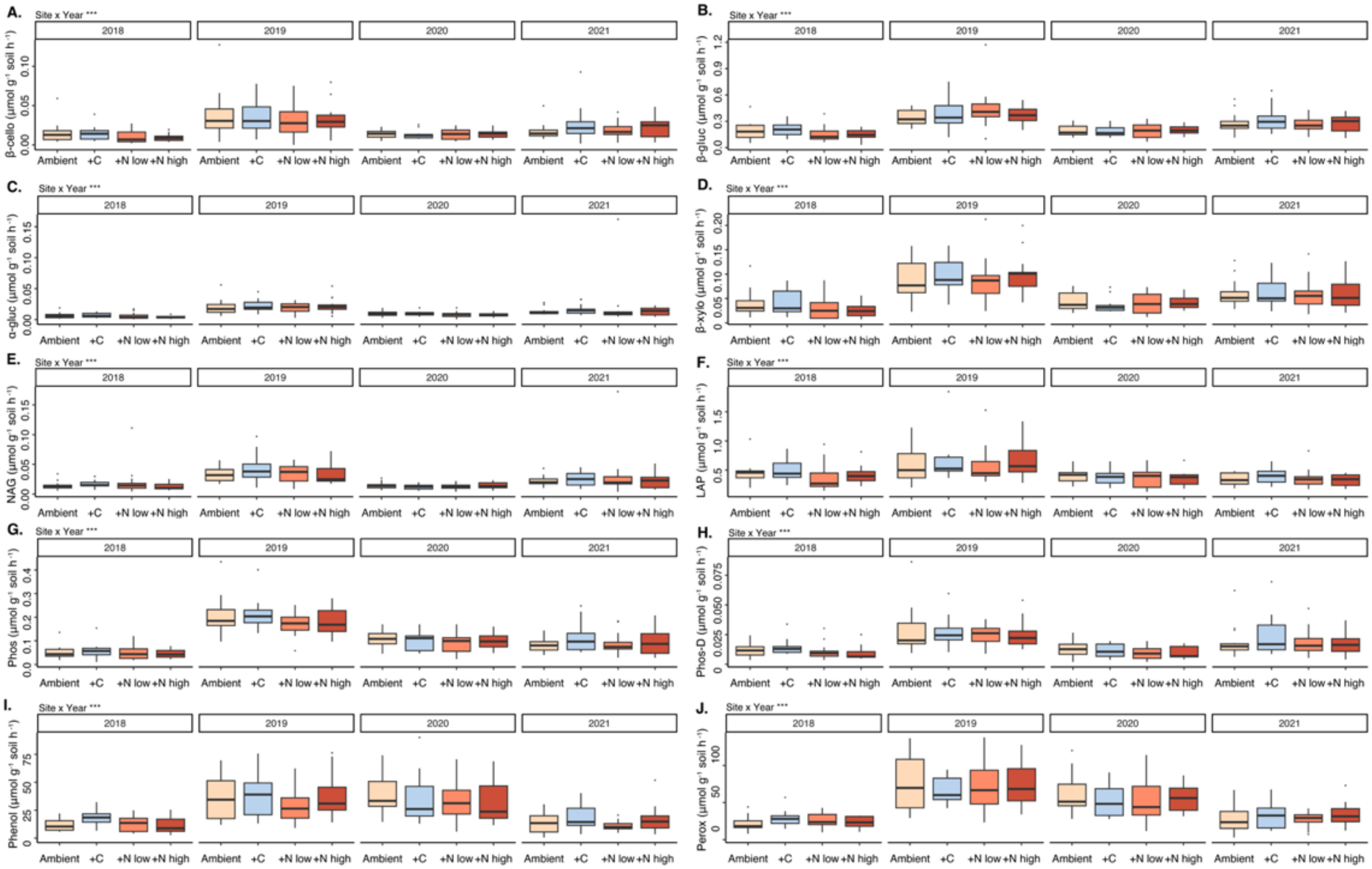

Fig. S4.6. Boxplots of hydrolytic extracellular enzyme potentials β -D-cellobioside (β -cello, A), β-glucosidase (β-gluc, B), α-Glucosidase (α-gluc, C), β-xylosidase (β -xylo, D), N-acetylglucosaminidase (NAG, E), leucyl aminopeptidase (LAP, F), acid phosphatase (Phos, G) and phosphodiesterase (Phos-D, H), and oxidative enzymes peroxidase (Perox, I), and phenol oxidase (Phenol, J) in a 4-year N addition experiment conducted three adjacent Chihuahuan Desert grasslands. See the top left for significant main and interaction effects (significance codes: *** < 0.001, ** p<0.01, * p< 0.05, . p<0.1).

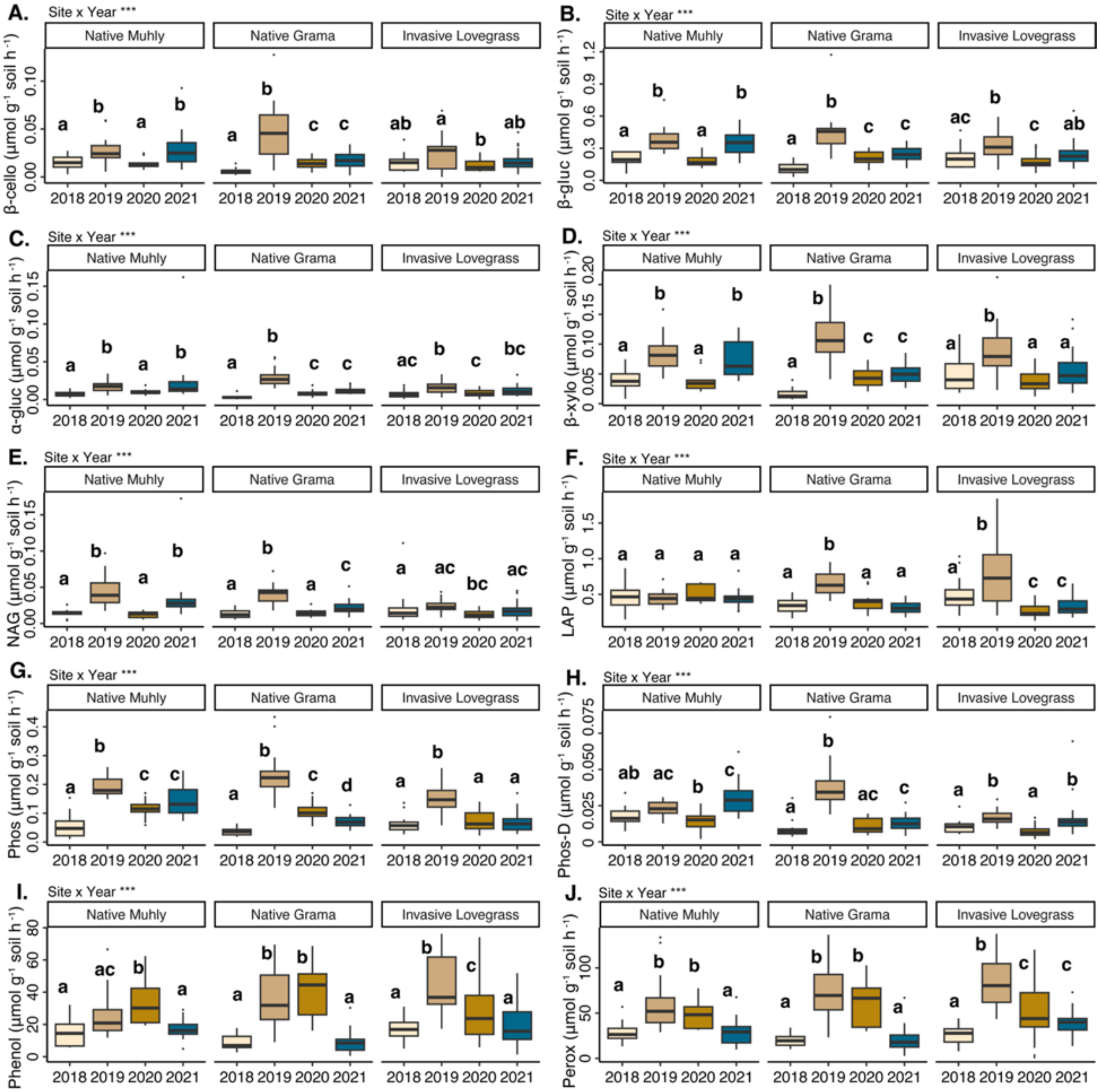

Fig. S4.7.Boxplots of hydrolytic extracellular enzyme potentials β -D-cellobioside (β -cello, A), β-glucosidase (β-gluc, B), α-Glucosidase (α-gluc, C), β-xylosidase (β -xylo, D), N-acetylglucosaminidase (NAG, E), leucyl aminopeptidase (LAP, F), acid phosphatase (Phos, G) and phosphodiesterase (Phos-D, H), and oxidative enzymes peroxidase (Perox, I), and phenol oxidase (Phenol, J) in a 4-year N addition experiment conducted three adjacent Chihuahuan Desert grasslands. Bold lowercase letters indicate significant treatment effects (estimated marginal means, $p<0.05$). See the top left for significant main and interaction effects (significance codes: *** < 0.001, ** $p<0.01$, * $p< 0.05$, . $p<0.1$).

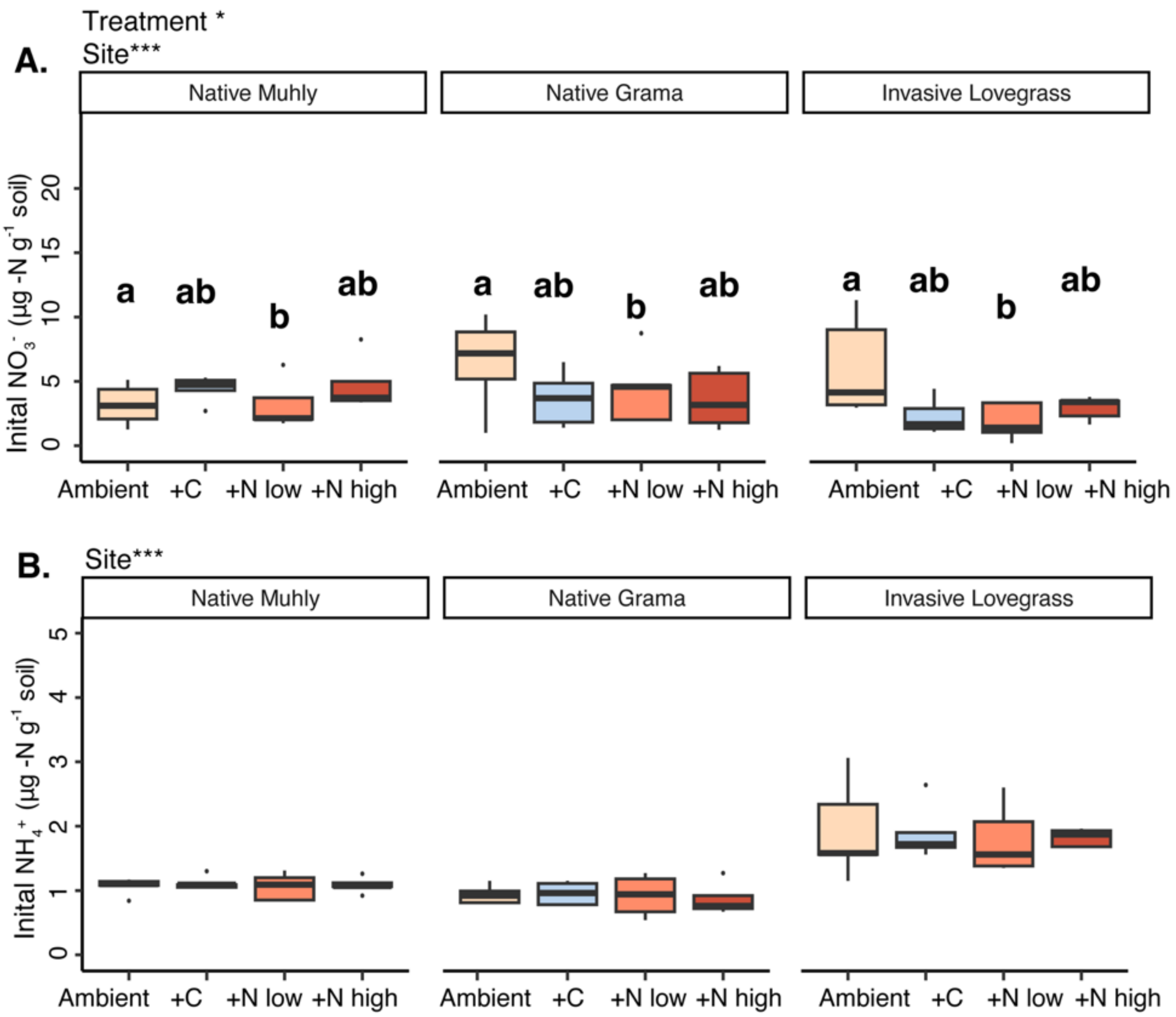

Fig. S4.8. Boxplots of initial nitrate (A; NO_3^-) and ammonium (B; NH_4^+) concentrations before the 30-day net N-mineralization, nitrification, and ammonification laboratory incubation experiment incubation. Interaction terms were eliminated from the model if they were not significant. Bold values indicate significance at $p<0.05$. See the top left for significant main and interaction effects (significance codes: *** < 0.001, ** $p<0.01$, * $p<0.05$, . $p<0.1$).

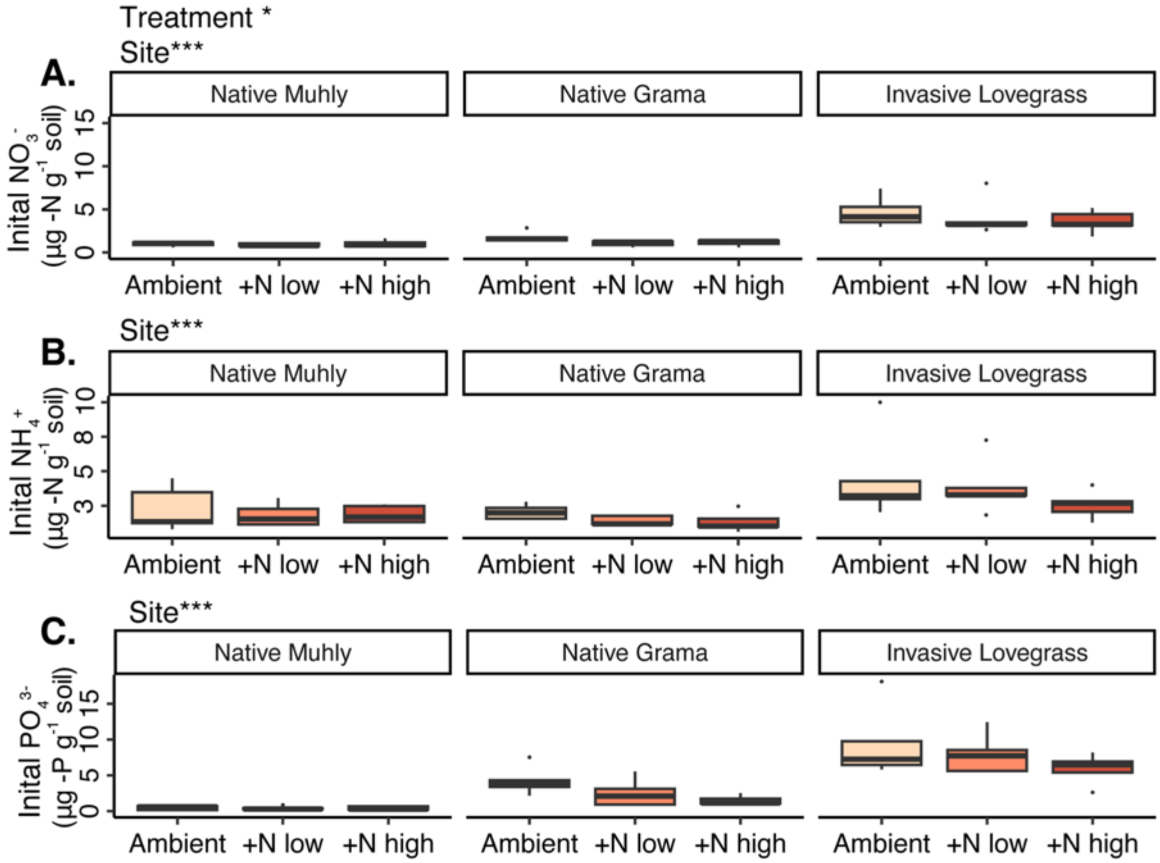

Fig. S4.9. Boxplots of initial nitrate (A; NO_3^-), ammonium (B; NH_4^+), and phosphate (C; PO_4^{3-}) concentrations for the 1and 24-hour laboratory incubation experiment assessing nitric oxide efflux in soils gathered from a 4-year field N addition treatment (Ambient, +N low, and +N high treatment plots). Initial NO_3^-, NH_4^+, and PO_4^{3-} were assessed before the start of the incubation. See the top left for significant main and interaction effects (significance codes: *** < 0.001, ** p<0.01, * p< 0.05, . p<0.1).

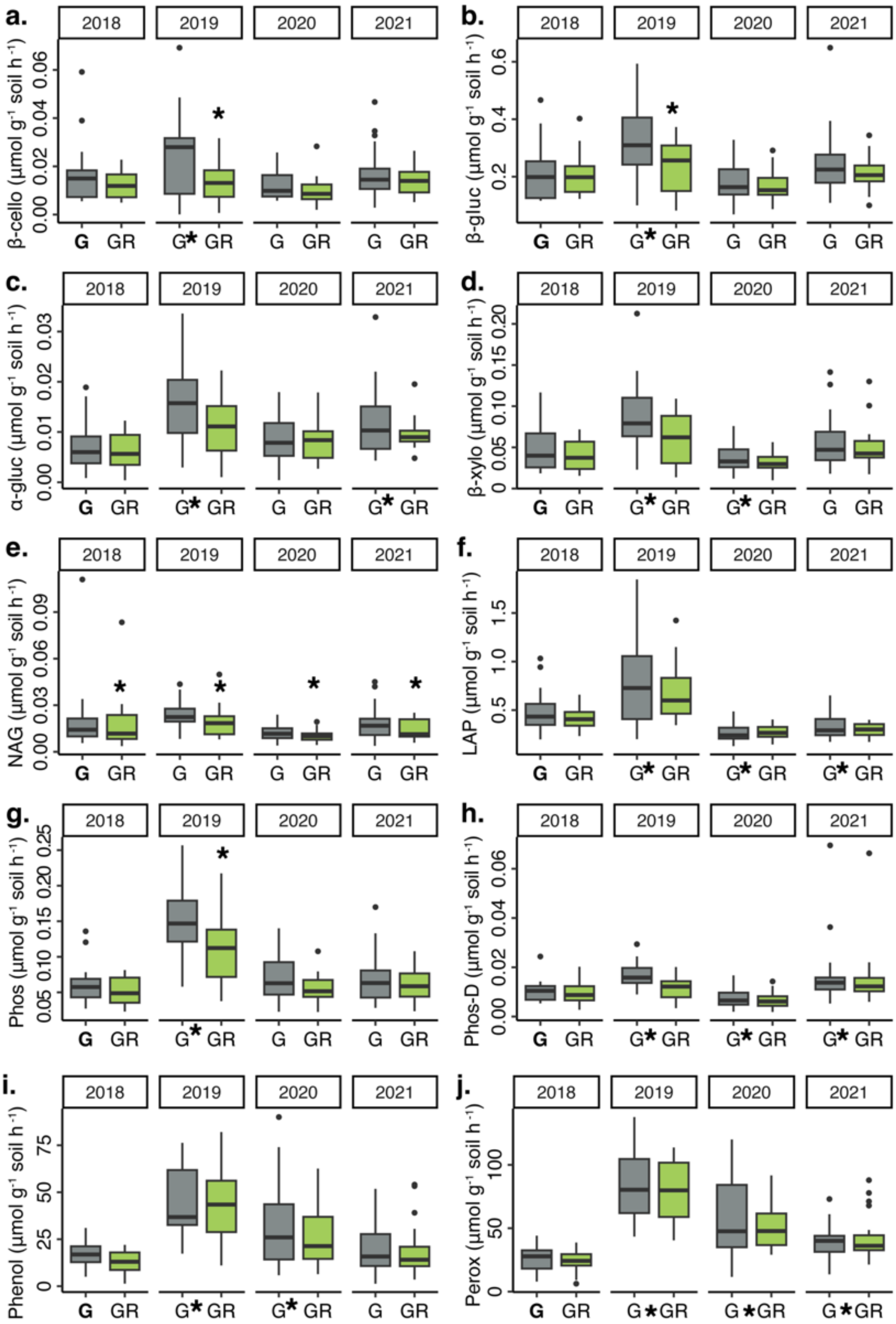

Fig. S5.1. Boxplots of hydrolytic extracellular enzyme potentials β -D-cellobioside (β -cello, a), β-glucosidase (β-gluc, b), α-Glucosidase (α-gluc, c), β-xylosidase (β -xylo, d), N-acetylglucosaminidase (NAG,e), leucyl aminopeptidase (LAP, f), acid phosphatase (Phos, g) and phosphodiesterase (Phos-D, h), and oxidative enzymes peroxidase (Perox, i), and phenol oxidase (Phenol, j) in a 4-year invasive grass removal (2018 and 2019), recovery (2020 and 2021), and N addition manipulation experiment. Removal treatments include *E. lehmanniana* present (G), and *E. lehmanniana* removed (GR). Asterix above GR boxplots indicate significant differences between treatments relative to control in 2018 (first G on the x-axis in bold). Asterix next to G (x-axis) indicates a significant change in control relative to G in 2018.

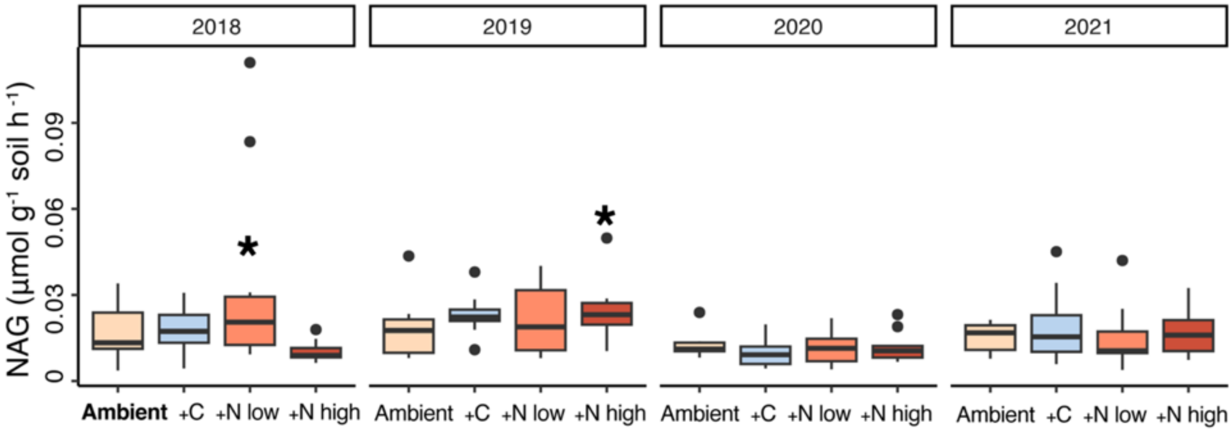

Fig. S5.2. Boxplots of hydrolytic extracellular enzyme N-acetylglucosaminidase (NAG) in a 4-year invasive grass removal (2018 and 2019), recovery (2020 and 2021), and N addition manipulation experiment. N addition treatments include Ambient N (background N deposition, ~4kg N ha^{-1} yr^{-1}), C addition treatment (+C, 6g of sucrose), +N low treatment (+2 kg N ha^{-1} yr^{-1}), and +N high treatment (+4 kg N ha^{-1} yr^{-1}). Asterix above boxplots indicates a significant difference between treatments relative to Ambient in 2018 (first Ambient on the x-axis in bold).

VITA

Jennifer received her Bachelor of Science with a concentration in Ecology and Evolution from the University of Texas at El Paso (UTEP) in the Winter of 2018, where she received the Academic and Research Excellence Award in Ecology and Evolutionary Biology. As an undergraduate research associate and as a National Science Foundation REU, Jennifer led research examining the effects of extreme climate events on below-ground productivity and biogeochemical cycling at the Sevilleta Long-term Ecological Research Station under the mentorship of Dr. Scott L. Collins and Dr. Jennie R. McLaren. As a graduate student, Jennifer was the recipient of the AAUW American Dissertation Fellowship (2022-2023), UTEP Graduate Excellence Fellowship (2022), Real Brown Student Section Travel Award (2022), Ecological Society of America Biogeosciences Travel Award (2022), UTEP Graduate School Travel Grant (2019, 2021, and 2022), Les and Harriet Dodson Research Grant (2019 and 2021), Alan Schwarzschild Poster Award (2018), and the Long Term Ecological Research All Scientist Meeting Travel Award (2018). Jennifer has presented her work at the National Atmospheric Deposition Program Fall Meeting Scientific Symposia (2019, 2020, and 2022), the Ecological Society of America Annual Meeting (2022 and 2018), and the Long-Term Ecological Research All Scientist Meeting (2018). Throughout her 5 years of graduate research, Jennifer has mentored and trained eight undergraduate students in various laboratory and field methods and independent research. She is also an active leader and member of the Ecological Society of America Southwestern Chapter and the National Atmospheric Deposition Program Critical Loads of Atmospheric Deposition Committee. Jennifer has published manuscripts and book chapters on climate change and nitrogen deposition in the *Encyclopedia of Biodiversity* and *Soil Biology and Biochemistry.*