Ecosystem Structure and Function in Semiarid Drylands: Exploring the Effects of Water and Nitrogen Availability in the Great Plains of the Western United States

Christopher Beltz
Yale University Graduate School of Arts and Sciences, christopher.beltz@gmail.com

Follow this and additional works at: https://elischolar.library.yale.edu/gsas_dissertations

Recommended Citation
https://elischolar.library.yale.edu/gsas_dissertations/559
Abstract

Ecosystem Structure and Function in Semiarid Drylands: Exploring the Effects of Water and Nitrogen Availability in the Great Plains of the Western United States

Christopher W. Beltz
2022

The global soil carbon (C) pool holds more than twice the C of the atmosphere, and a large portion of terrestrial C as a whole. Within the global soil C pool, drylands contain 20% of the soil C, in addition to covering 40% of the earth’s surface. Because they are both widespread and a significant reservoir of C, drylands are an important component of the global C cycle. Despite the importance of drylands, ecosystem function can be difficult to predict, with drylands switching from a C source to sink on an interannual basis. This uncertainty in net C flux creates difficulty projecting dryland contributions to atmospheric C and global change, particularly with expected future changes in annual precipitation and seasonality, changes in magnitude of nitrogen (N) deposition, and widespread land use change.

The overall goal of my dissertation research is to improve our understanding of ecosystem structure and ecosystem function for semiarid drylands. In particular, I am interested in asking questions about the response of several dryland ecosystem types to changing water and N additions, similar to those predicted to occur under multiple scenarios of global change. I used field and lab-based methods to explore the structure and function of the shortgrass steppe, mixed-grass prairie, and sagebrush steppe – ecosystem types that are among the most common semiarid drylands in the western U.S. I assessed the composition of and the differences among these dryland ecosystem types in their ‘natural’ state, and under treatments of added water and N, altering the availability of these two components in situ.
In Chapter 1, I investigated multiple facets of ecosystem structure and function across three dryland ecosystem types in the western United States. I conducted this study to better understand patterns in structure and function within semiarid drylands and to understand how those patterns relate to the lesser-studied sagebrush steppe. As expected, plant and soil microbial communities varied by site. Surface soils were largely similar in their soil C content, while subsurface soils (> 5 cm depth) varied widely in their C content; the lower depth soils at the mixed-grass prairie had more than twice the soil C of the other two dryland types in all size fractions studied. I found that each of the sites functioned largely similarly with respect to aboveground net primary production, soil respiration, and soil N mineralization. The data suggest that functional attributes may be similar across dryland ecosystem types, at least when constrained to similar climates and soil textures, as in my study.

In Chapter 2, I explored the effects of increasing water and N availability on ecosystem structure across the shortgrass steppe, mixed-grass prairie, and sagebrush steppe. I was particularly interested in changes within the plant and soil microbial communities. β-diversity within plant and microbial communities were largely resistant to change. However, there were three notable changes within functional types and indicator species: grasses increased in abundance at the mixed-grass prairie under high N additions with and without water, saprotrophic fungi increased in abundance at most locations under combined water and N (of differing levels), and arbuscular mycorrhizae decreased across all sites with greater decreases generally accompanying higher loadings of N. Indicator species within the cyanobacteria and lichenized fungi experienced increases in abundance at the shortgrass steppe. While these results show no major widespread shifts in β-diversity, changes in those plant, fungal, and bacterial
indicator species may portend future changes in biomass production, decomposition, and nutrient cycling.

In Chapter 3, I assessed the effects of changing water and N availability on ecosystem function in the three dryland systems. I measured the responses of aboveground net primary productivity (ANPP), soil respiration, and soil C to these treatments. All dryland systems experienced similar responses to N additions and combined high N with water additions, with increased ANPP and soil respiration. Soil C showed no significant differences after treatment. My results demonstrate co-limitation of ecosystem function by water and N over the course of this study in these three semiarid drylands of the western Great Plains.
Ecosystem Structure and Function in Semiarid Drylands: Exploring the Effects of Water and Nitrogen Availability in the Great Plains of the Western United States

A Dissertation
Presented to the Faculty of the Graduate School
Of
Yale University
In Candidacy for the Degree of
Doctor of Philosophy

By
Christopher W. Beltz

Dissertation Director: Ingrid C. Burke

May 2022
TABLE OF CONTENTS

Abstract .................................................................................................................................................. i
List of Figures ......................................................................................................................................... iv
List of Tables .......................................................................................................................................... vi
Dedication ............................................................................................................................................... vii
Acknowledgements ............................................................................................................................ viii
Author’s Note .......................................................................................................................................... xi

Introduction to the Dissertation ........................................................................................................... 1

Chapter 1 - Ecosystem Structure and Function across Western Dryland Ecosystems: a cross-site comparison of
semiarid ecosystem types in Colorado and Wyoming ........................................................................... 5
  Introduction ........................................................................................................................................... 6
  Methods ............................................................................................................................................... 9
  Results ............................................................................................................................................... 12
  Discussion ........................................................................................................................................... 14
  Literature Cited .................................................................................................................................. 19

Chapter 2 - The effect of changing water and nitrogen availability on plant and microbial communities across
three dryland ecosystem types in the western United States ................................................................ 31
  Introduction ........................................................................................................................................... 32
  Methods ............................................................................................................................................... 35
  Results ............................................................................................................................................... 43
  Discussion ........................................................................................................................................... 49
  Literature Cited .................................................................................................................................. 55
  Supplementary Material ..................................................................................................................... 73

Chapter 3 - The effect of changing water and nitrogen availability on the fluxes of carbon and on soil carbon
pools across three dryland ecosystem types in the western United States ........................................ 79
  Introduction ........................................................................................................................................... 80
  Methods ............................................................................................................................................... 82
  Results ............................................................................................................................................... 89
  Discussion ........................................................................................................................................... 92
  Literature Cited .................................................................................................................................. 98
  Supplementary Material ..................................................................................................................... 108

Conclusion of the Dissertation ............................................................................................................. 112

Appendices .......................................................................................................................................... 114
LIST OF FIGURES

Figure 1-1: Site locations in the western United States along with their dominant species. ................................. 23
Figure 1-2: Summer precipitation and temperature varies across sites using Walter-Lieth diagrams ....................... 24
Figure 1-3: Few significant differences in ecosystem structure and function variables among three semiarid dryland plant communities in Wyoming and Colorado (USA) ................................................................. 24
Figure 1-4: Soil carbon content within various soil fractions is consistent in the 0-5 cm layer but differs in the 5-10 cm layer among three semiarid dryland plant communities in Wyoming and Colorado (USA). .................. 25
Figure 1-5: Soil inorganic carbon content is similar among within both the 0-5 cm layer and the 5-10 cm layer among three semiarid dryland plant communities in Wyoming and Colorado (USA) ........................................ 26
Figure 1-6: Plant communities are distinct, but also have overlap in species composition among three semiarid dryland plant communities in Wyoming and Colorado (USA) ........................................................................ 26
Figure 1-7: Bacterial communities in the upper soil are distinct, but also have overlap in species composition among three semiarid dryland plant communities in Wyoming and Colorado (USA). .......................... 27
Figure 1-8: Fungal communities in the upper soil are distinct, but also have overlap in species composition among three semiarid dryland plant communities in Wyoming and Colorado (USA). ......................... 28

Figure 2-1: Soil mineralized nitrogen (NO$_3^-$ + NH$_4^+$) increased with all high nitrogen addition treatments (with and without water) and some low nitrogen treatments at three semiarid dryland plant communities in Wyoming and Colorado (USA) .............................................................................................................................. 64
Figure 2-2: Plant community composition shifted in response to the water + high N treatment at the mixed-grass prairie in Wyoming (USA), though species richness and evenness remain unchanged. ......................... 65
Figure 2-3: Grasses significantly increased after two years of high N and water + high N treatments at the mixed-grass prairie in Wyoming (USA) ........................................................................................................... 66
Figure 2-4: Bacterial abundance significantly decreased in multiple treatments within the shortgrass steppe community in Colorado (USA) ......................................................................................................................................... 67
Figure 2-5: Fungal abundance showed no significant differences to water and nitrogen treatments at three semiarid dryland communities in Wyoming and Colorado (USA) ...................................................... 68
Figure 2-6: Bacterial indicator species mapped to phylum across both depths within all three semiarid dryland communities in Wyoming and Colorado (USA) .................................................................................. 69
Figure 2-7: Fungal indicator species mapped to trophic mode across both depths within all three semiarid dryland communities in Wyoming and Colorado (USA). ............................................................ 70
Figure 2-8: Fungal indicator species mapped to functional guilds within all three semiarid dryland communities in Wyoming and Colorado (USA) ............................................................................................................... 71
Figure 2-51: Bacterial community composition, species richness and evenness did not show significant differences at the 5 cm depth within all three semiarid dryland communities in Wyoming and Colorado (USA) .................................................. 73
Figure 2-52: Bacterial community composition, species richness and evenness did not show significant differences at the 10 cm depth within all three semiarid dryland communities in Wyoming and Colorado (USA) ........................................................................................................... 74
Figure 2-53: Fungal community composition was affected by treatments at the sagebrush steppe at the 5 cm depth, though no treatments were different from the control. ...................................................... 75
Figure 2-54: Fungal community composition, species richness and evenness did not show significant differences at the 10 cm depth within all three semiarid dryland communities in Wyoming and Colorado (USA) ...... 76

Figure 3-1: Experimental design using a split-plot, nested structure ......................................................... 103
Figure 3-2: Soil water potential increased after the application of a once monthly water treatment (20% mean monthly precipitation) at the 5 cm depth at three semiarid dryland plant communities in Wyoming and Colorado (USA) .................................................................................................................. 104
Figure 3-3: Daily mean soil water potential increased for multiple days after a once-monthly water application (20% mean monthly precipitation) during summer 2018 at three semiarid dryland plant communities in Wyoming and Colorado (USA).

Figure 3-4: ANPP increases in the second year of data collection under high N only or water + high N, depending on the site.

Figure 3-5: Soil carbon at the 0-5 cm depth showed no significant differences between treatments in 2017 and 2018 at three semiarid dryland plant communities in Wyoming and Colorado (USA).

Figure 3-6: Soil carbon at the 5-10 cm depth showed few differences in 2017 and 2018 between treatments at three semiarid dryland plant communities in Wyoming and Colorado (USA).
LIST OF TABLES

Table 1-1: Mean annual precipitation (MAP), mean annual temperature (MAT), and soil texture at each location.
We calculated mean precipitation and temperature values using 30-year Climate Normals from 1980-2010.
Summer precipitation includes precipitation in June, July, and August. .................................................. 29

Table 1-2: Attributes, variables, and methods used for data and sample collection to examine ecosystem
structure (ES) and ecosystem function (EF). .................................................................................................. 30

Table 2-1: Soil water availability increased after water application at the 5 cm depth at all three ecosystem types
in Wyoming and Colorado (USA). .............................................................................................................. 72
Table 2-S1: Linear mixed model regression coefficients for the effect of treatments on plant functional type
abundance within all three semiarid dryland ecosystem types in Wyoming and Colorado (USA). .............. 77
Table 2-S2: Linear mixed model regression coefficients for the effect of treatments on soil bacterial abundance
within all three semiarid dryland ecosystem types in Wyoming and Colorado (USA). ................................ 77
Table 2-S3: Linear mixed model regression coefficients for the effect of treatments on soil fungal abundance
within all three semiarid dryland ecosystem types in Wyoming and Colorado (USA). .............................. 78
Table 2-S4: Taxonomy associated with ASVs in contaminated blank. These were removed using the ‘decontam’
package from associated samples. ........................................................................................................... 78

Table 3-1: Water addition at each site in 2017 & 2018 ................................................................................. 107
Table 3-2: Soil mineralized total N increased with the addition of both low and high nitrogen treatments, with
and without water, at three semiarid dryland plant communities in Wyoming and Colorado (USA). ........ 107
Table 3-S1: ANPP increased with the addition of both low and high nitrogen treatments, with and without
water, at three semiarid dryland plant communities in Wyoming and Colorado (USA). ............................. 110
Table 3-S2: Soil respiration significantly increased with the addition of high nitrogen and high nitrogen with
water at three semiarid dryland plant communities in Wyoming and Colorado (USA). ............................. 110
Table 3-S3: Soil organic carbon showed no significant differences to treatments at three semiarid dryland plant
communities in Wyoming and Colorado (USA). ........................................................................................ 111
DEDICATION

Dedicated to my parents, Barbara and Gerald Beltz, and to my wife, Amy Beltz.

Your support has been unwavering.
ACKNOWLEDGEMENTS

Foremost, I would like to thank my advisor, Indy Burke, for her support and advice throughout my doctoral studies. Her guidance has been particularly helpful in writing up this dissertation and she has my sincere gratitude for reading my many drafts of each chapter. I would also like to acknowledge my other committee members, Bill Lauenroth, Mark Bradford, and Linda van Diepen. Thank you to Bill for his recent comments as I was writing up and for his ongoing support as a Burke-Lauenroth Lab member. Thank you to both Mark and Linda for your mentorship, suggestions, and feedback. This research and my abilities as a scientist are better for all of your input and investment.

Thank you to my many colleagues and friends at Yale University, the University of Wyoming, and beyond. In particular, thank you to Jonathan Reuning-Scherer, Andis Arietta, Daniel Schlaepfer, Marc Brock, Charley Hubbard, Gordon Custer, and Ryan Auster for their advice and willingness to talk about statistics, bioinformatics, and other data-centric issues. Also thank you to Mark Lindquist, Mike Curran, and Michael Smith for sharing their networks, resources, and digging the occasional hole on my behalf.

Many thanks to Richard Walker and Bryan Maitland. You both shared your wonderful Red Buttes home, offered encouragement (and eggs), and were just generally wonderfully supportive colleagues. Also thank you to Lisa Barrett for continuing to stay in touch and always inviting me to every PiE event, even after my move to New Haven. I always felt like I belonged at the BBQs in Laramie because of you.

A special thanks is due to Kennan and Joey Oyen. Your support of me was (and is) too widespread to truly enumerate, but it is why I give you your own paragraph. Thank you both so much.
Thank you to the many current and past members of the Burke-Lauenroth Lab. I am particularly grateful to Cait Rottler, Trace Martyn, Victoria Pennington, and Kyle Palmquist for their early and ongoing support of my work, but also of me as a scientist and person. To my field and lab technicians, Bella Buongiorno, Laura Jardine, and Hannah Yokum – you all are incredibly hardworking, and I am indebted to each of you. Additional thanks to Scott Carpenter, Jessica Swindon, and Adeline Thompson.

Thank you to the USDA-ARS and Justin Derner for access to the High Plains Grassland Research Station (Cheyenne, WY) and the Central Plains Experimental Range (near Nunn, CO). In addition, thank you to Nicole Kaplan for providing access to data and Matt Mortensen for his help with a broken-down vehicle. Additional thanks to The Nature Conservancy, their Wyoming chapter, and Trey Davis for access to their property in Thunder Basin.

Thank you to Brad Erkkila and Jonas Karosas at the Yale Analytical and Stable Isotope Center (YASIC). They hosted and supported me over a multi-year period and went far beyond simply accepting samples for submission. I learned a lot from both of them during my many months in the basement of the Environmental Science Center! I would also like to thank and credit Bayla Arietta for the artwork she generated for Chapter 1. It is beautiful and I am very pleased to incorporate it into this manuscript.

I received two fellowships that were important to my development as a scientist, an Earth Science Information Partners (ESIP) Student Fellowship and a National Center for Ecological Analysis and Synthesis (NCEAS) Data Science Fellowship. I’d like to thank ESIP, along with Erin Robinson, Bill Teng, Nancy Hoebelheinrich, and Annie Burgess. ESIP was incredibly important in driving home the importance of communication, teamwork, and the multifaceted nature of data stewardship. I am very thankful that you all took a chance on a young PhD.
student. I’d also like to thank the NCEAS team, and particularly Amber Budden, Matt Jones, and Jeanette Clark. The experience I had over the 6-months working with you all changed my professional trajectory. I will ever be thankful.

Financial support for this research and my training came from a wide range of sources: Yale University and the Yale School of the Environment, the Yale Center for Research Computing, the University of Wyoming and the Wyoming Excellence Fund, Meter Group (formerly Decagon Devices), the Department of Botany (Univ. of Wyoming), Google, the Federation of Earth Science Information Partners, Wyoming INBRE Bioinformatics and Administrative Core, and the Program in Ecology (Univ. of Wyoming).
AUTHOR’S NOTE

All three chapters in my dissertation come from the same large experiment that I conducted in Wyoming and Colorado from 2016-2019. As they come from the same experiment, there are similarities in descriptions of the experimental design across my three chapters. Particularly relevant for Chapters 2 and 3, my analyses that confirm the effect of our water and nitrogen treatments utilize the same data. These analyses are critical to both studies and are included in figures and tables within each chapter, though we have varied the presentation and/or analyses where possible.
INTRODUCTION TO THE DISSERTATION

Soil carbon (~2000 Gt) comprises a large portion of terrestrial carbon (~5000 Gt), and is more than twice the size of the atmospheric carbon pool (~860 Gt) (Lal 2004, Zimov et al. 2006, Friedlingstein et al. 2020). Carbon (C) is actively cycled between the atmosphere and soils (IPCC 2021). It is sequestered in soil through plant productivity, then released back to the atmosphere through a combination of autotrophic and heterotrophic respiration. Maintaining this balance between C fluxes, inputs from plant production and outputs through ecosystem respiration, is critical to limiting increases in atmospheric C and to countering global change.

Drylands are particularly important with respect to soil C. They comprise 40% of global terrestrial land mass and store 20% of global soil C (Graaff et al. 2014, Huang et al. 2016). However, drylands also represent a major gap in our understanding of C cycling; they are able to switch from being a source of atmospheric C to a C sink on an interannual basis (Biederman et al. 2017), and we currently do not fully understand the mechanisms of this switch. While the long-term direction of C sequestration is more critical, the ability of drylands to modulate atmospheric C is particularly important because of the expected future changes in precipitation, increased deposition of nitrogen (N), and widespread land use change throughout drylands of the world.

The relationship between ecosystem structure and function has long been a focus of ecological research (Bradshaw 1984, Schimel and Gulledge 1998, Cortina et al. 2006). In these studies, we assess ecosystem structure through measurements of plant communities, soil microbial communities, and soil C. Ecosystem function is assessed by estimating rates of aboveground net primary productivity (ANPP) and soil respiration. This relationship is complex
because of the feedback between structure and function, which can reinforce or stabilize trends (Hooper et al. 2005, Maestre et al. 2016).

Precipitation (i.e., water availability) and N deposition (i.e., N availability) are both predicted to change under future global change scenarios (IPCC 2021). With water and N known to directly affect both ecosystem structure and function (Sala et al. 1992, Lauenroth and Burke 1995, Burke et al. 1998, Yahdjian et al. 2011, Maestre et al. 2016), it is important to understand the degree of control each play and the consistency of their effects across drylands.

In this dissertation, my goal was to examine patterns in the response of ecosystem structure and function to changing water and N availability across three semiarid dryland ecosystem types. My objectives were to: 1) investigate the similarity of three different ecosystem types in terms of ecosystem structure and function; 2) explore changes to ecosystem structure under altered water and N availability; and 3) assess changes to C cycling and ecosystem function after water and N additions. I organized my work in three chapters, respectively addressing each of these objectives.

**Literature Cited**


CHAPTER 1

Ecosystem Structure and Function across Western Dryland Ecosystems: a cross-site comparison of semiarid ecosystem types in Colorado and Wyoming
INTRODUCTION

Drylands extend over approximately 40% of the earth’s surface, provide homes for 2.5 billion people, support 44% of the world’s agriculture, while storing 20% of global soil carbon (C) (Millenium Ecosystem Assessment 2005, Reynolds et al. 2007, Feng and Fu 2013, Graaff et al. 2014). The extent of drylands increased by 4-8% through the 20th century; this expansion is expected to continue as global temperature increases in the future (Dai 2013, Feng and Fu 2013, Schlaepfer et al. 2017). Dryland systems are defined by their aridity index (AI) (Budyko 1958, Loik et al. 2004, Feng and Fu 2013), with water often thought to be the major limiter of net primary productivity and ecosystem function more generally (Sala et al. 1992, Hooper and Johnson 1999, Lauenroth et al. 2008). Among drylands (AI <0.65), semiarid drylands (AI = 0.2 to 0.5) currently comprise 15% of the world’s total landmass (Huang et al. 2016, Maestre et al. 2021). Semiarid drylands are predicted to increase their share of global drylands; they are expected to account for approximately half of all dryland expansion under multiple global change scenarios, and are anticipated to contribute almost half of all gross primary productivity within drylands by 2100 (Huang et al. 2016, Yao et al. 2020).

While interest has increased over the past few decades, drylands – including semiarid drylands – remain less studied than their more mesic counterparts, potentially due to their perceived lack of species and productivity (Schimel 2010). Within North America, semiarid drylands are located in the western and southwestern portion of the country, including large portions of Colorado, Wyoming, Montana, Nevada, and Utah (Huang et al. 2016). These North America drylands are comprised largely of semiarid grasslands and semiarid shrublands; the two major semiarid grassland types are short and mixed grass. These two semiarid grassland types have received emphasis through long term research programs. Research focused on the
shortgrass steppe has been driven by the presence of the Central Plains Experimental Range (CPER) research site of the USDA Agricultural Research Service (ARS), which hosted the International Biome Project (IBP) Grasslands Biome and the Shortgrass Steppe Long-Term Ecological Research (SGS LTER) site (Lauenroth et al. 2008). The CPER was established in 1939 and is still active today. The IBP and SGS LTER were active from 1964-1974 and from 1982-2013, respectively. The High Plains Grassland Research Station (HPGRS), another USDA-ARS research site, is located within the mixed-grass prairie and has been active since it was authorized in 1928 (Booth and Derner 2004). In contrast, the sagebrush steppe has not historically had a long-term scientific research station. Recently the National Ecological Observatory Network (NEON) established terrestrial field sites at both the shortgrass steppe (Central Plains Experimental Range, 2014) and sagebrush steppe (Onaqui, 2017) (NEONscience.org 2021).

Big sagebrush (Artemisia tridentata ssp.) ecosystems are the most prevalent form of semiarid drylands in western North America, covering 13 U.S. states, three Canadian provinces, and 50 million hectares (ha) (Connelly et al. 2004). Big sagebrush once covered almost 63 million ha (Knick et al. 2003), however multiple forms of development have impacted much of this area and 50-60% of the historical extent of big sagebrush was transformed to non-native annual grasslands (West 2000, Knick et al. 2003, Connelly et al. 2004). Impacts and development are expected to continue with an additional 2.3-5.5 million ha developed by 2027 (Copeland et al. 2009); this trend predicted by Copeland et al. (2009) has continued to materialize, with new oil and gas leases in big sagebrush increasing from 54% to 67% between 2015 and 2019 (Gardner et al. 2019). In the future, 21-25% of shrub steppe ecosystems in western North America are expected to be impacted by energy development (Pocewicz et al.
2011, Rottler et al. 2018), with renewables occupying an increasing portion of overall energy development (Rehbein et al. 2020).

Despite the lack of a dedicated long-term research station, research into the sagebrush steppe has become more prevalent due to three factors: (1) a decrease in the extent of sagebrush; (2) a predicted increase in development and conversion to other land uses throughout the future; and (3) the direct impacts these two factors have had on loss of biological diversity and sagebrush obligates – particularly the greater sage-grouse. Currently sage grouse are not listed as “threatened” due to the recovery of their population numbers, however declines in the sage-grouse population continue to be predicted in the future (USFWS 2015, Heinrichs et al. 2019, Coates et al. 2021).

**Project Goals & Hypotheses**

The goal of this study was to assess the ecosystem structure and function of three sites that span three semiarid dryland ecosystem types in order to identify similarities and differences. This is an initial step in filling gaps in knowledge about the sagebrush steppe and in promoting regional dryland studies in the western United States.

We hypothesized that trends in precipitation and water availability would explain the majority of differences we might see between sites. As precipitation increases, we expected increases in the more labile forms of soil C and soil respiration, as well as plant and microbial biomass. In addition, we expected plant and soil microbial community structure to differ among ecosystem types with the largest differences between the sites with the greatest difference in mean annual precipitation, as long-term climate is the major determinant of community structure (Ulrich et al. 2014, Bunting et al. 2017, Palmquist et al. 2021).
METHODS

Study area and site selection

We ran this study from 2016 to 2019 in three locations across Wyoming and Colorado – collecting data in 2016 and 2017. Each location corresponded to a different semiarid ecosystem type - shortgrass steppe, mixed-grass prairie, and sagebrush steppe. The first two locations were at long-term USDA-ARS properties: the shortgrass steppe site was located at the Central Plains Experimental Range (CPER) near Nunn, CO (40° 49’N, 104° 43’W) and the mixed-grass prairie location was at the High Plains Grassland Research Station (HPGRS) in Cheyenne, WY (41° 11’N, 104° 54’W). The third location, in the sagebrush steppe, was on property owned by The Nature Conservancy near Thunder Basin National Grassland (TBNG) outside Bill, WY (43° 25’N, 104° 55’W).

We chose the sites because they are representative of common ecosystem types and plant communities. They are also protected areas for research, and are relatively similar to the other sites in terms of mean annual precipitation (MAP), mean annual temperature (MAT), and soil textures (Table 1-1).

Despite controlling for these parameters, some variation exists between sites and expectations are included in our hypotheses. Each location is currently being grazed by cattle during summer and has a history of moderate cattle grazing for the past 50-100 years. The shortgrass steppe is dominated by blue grama (Bouteloua gracilis), a C4 bunchgrass, while the mixed-grass prairie is dominated by western wheatgrass (Pascopyrum smithii) and needle and thread grass (Hesperostipa comata), two C3 bunchgrasses. Big sagebrush (Artemisia tridentata) dominates the sagebrush steppe, with cheatgrass (Bromus tectorum), sixweeks fescue (Vulpia octoflora), and field cottonrose (Logfia arvensis) in the understory. With the exception of big
sagebrush, these three sites share a common species pool with many of the species occurring across all three sites. All of these ecosystems have been shown to demonstrate strong soil organic matter patterns associated with the presence and absence of plants (Burke 1989, Vinton and Burke 1997), but because the dominant plant functional type varies across sites, we focused on interspaces for comparisons among sites.

This design, with each ecosystem type represented by a single site, limits the realm of inference of the results. However, there remains significant value in sampling across a gradient of semiarid dryland ecosystem types with a common, shared methodology and timing of sampling. The multiple plots within each site, spacing, and the individual exclosures around each plot provide confidence in our calculated means and variance at each site.

**Experimental Design**

We established seven 225 m² fenced exclosures (15 m x 15 m) at each of the three sites (Figure 1-1). We evaluated each exclosure location for the following characteristics: upland locations, visually homogenous vegetation, no visible disturbances (ex. tire tracks, cow path, or animal burrow), soil texture, and minimal slope (i.e., flat). Exclosures were necessary, as all three sites are actively grazed by cattle for significant portions of the year. Each exclosure had a 9 m² (3 m x 3 m) un-altered, subplot used to collect data for this study. We used multiple methods for evaluating ecosystem structure and function (Table 1-2).

**Data Analysis**

We used R version 4.1.1 (R Core Team 2021) to conduct all data analysis and data visualization.
We used the following R packages: here, tidyverse, lubridate, data.table, climatol, dada2, phyloseq, vegan, ggsignif, cowplot, and patchwork.

We evaluated climate through Walter-Lieth climate diagrams (\textit{diagwl} function, ‘climatol’ package v 3.1.2), on a monthly time-step across the entire calendar year. We used 30-year climate normals derived from 1981-2010 PRISM Climate Group data that were interpolated specifically for each site.

We used a one-way ANOVA (\textit{aov} function, ‘stats’ package v 4.1.1) with a post-hoc Tukey’s HSD (\textit{TukeyHSD} function, ‘stats’ package v 4.1.1) to examine differences among sites for multiple measured variables including, ANPP, microbial biomass, species richness, soil respiration, soil N mineralization, and soil C content for each pool. We tested normality and equal variance assumptions of our models visually using diagnostic plots. We measured soil gas fluxes four times during late August and early September 2017. We took the mean of these fluxes and then analyzed the seven exclosure averages. We report statistical significance using an $\alpha$ of 0.05 for our statistical analyses.

We assessed plant and microbial community differences in two ways: (1) using a permutational multivariate analysis of variance (PERMANOVA) on the Bray-Curtis dissimilarity profiles using 9999 permutations to assess the significance of site (\textit{adonis} function, ‘Vegan’ package v2.5–7 for R), and (2) using non-metric multi-dimensional scaling (NMDS) in two dimensions (\textit{metaMDS} function, ‘Vegan’ package v2.5–7 for R).

For the plant community comparisons, we used the cover class midpoint (i.e., cover class 5 ranges from 26-40% cover; the midpoint is 33%) for each species to calculate total cover across ten 0.1 m$^2$ Daubenmire quadrats at each site and exclosure. Then we converted the total cover to relative abundance (\textit{decostand} function, ‘Vegan’ package v2.5–7 for R).
For the soil microbial community, we pre-processed sequences for each sample using the standard operating procedure for DADA2 (multiple functions, ‘dada2’ package v1.20.0 for R) (Callahan et al. 2016). We then assigned taxonomy to amplicon sequence variants (ASVs) using the SILVA database (v138.1) for bacteria and the UNITE database (v8.3) for fungi. We removed sequences without an identified phylum (subset_taxa function, ‘phyloseq’ package v1.36.0 for R) and we used the remaining sequences to calculate Bray-Curtis dissimilarity (distance function, ‘phyloseq’ package v1.36.0 for R). Alpha diversity metrics were calculated from rarefied sequence data at 90% of the sequences in the sample with the least number of copies. All other calculations used non-rarefied data.

RESULTS

Climate

Despite similarity in annual precipitation and temperature, the magnitude and duration of the summer season dry period differ among sites (Figure 1-2). The most northerly site, the sagebrush steppe located in Thunder Basin National Grassland, has the largest dry period lasting from July through September, followed by the shortgrass steppe at the Central Plains Experimental Range with similar seasonal timing of the dry period, but to a lesser degree. The mixed-grass prairie at the High Plains Experimental Range typically has no significant dry period.

Ecosystem Structure & Function

Soil respiration was significantly elevated (p=0.0011) in the sagebrush steppe compared to the mixed-grass prairie in late August and early September (Figure 1-3, B). ANPP, microbial abundance, and soil inorganic N were not significantly different among ecosystem types after the
2017 summer season. Means across these five variables indicate that the shortgrass steppe and sagebrush steppe were more similar than the mixed-grass prairie.

**Soil Carbon Content**

Soil C in the upper 0-5 cm layer was largely similar across ecosystem types (Figure 1-4, A-E). The exception is fine particulate organic matter (FPOM) at the mixed-grass prairie, which had significantly high C compared to the sagebrush steppe (p=0.0202).

There were many differences across soil fractions at the 5-10 cm depth (Figure 1-4, F-J). Total soil C varied across each of the ecosystem types (p≤0.0024), as did dissolved organic carbon (DOC) (p≤0.0267), FPOM (p≤0.0413), and mineral associated organic matter (MAOM) (p≤0.0039). We found the highest amounts of C in the mixed-grass prairie, with the least C in the sagebrush steppe (p-values specific to each pairwise comparison are located within Figure 1-4).

Soil inorganic carbon (SIC) was consistent across depths and ecosystem types (Figure 1-5). Regardless of site or depth, SIC was 0.08-0.10% by mass in samples that had total C ranging 0.51-2.84%.

**Plant Community**

The plant community at each site is significantly different from each of the others (p<0.001), though there is also overlap in the species that make up each community (Figure 1-6C). In terms of observed species richness (Figure 1-6A), the mixed-grass prairie has significantly more species than both other ecosystem types (p≤0.0329). The shortgrass steppe is significantly less diverse (p≤0.0380) than either of the other sites, according to the Shannon Index (Figure 1-6B).
Microbial Community

The bacterial communities across the three sites show significant differences in composition from one another (p<0.001), though they shared some species in the makeup of their community membership (Figure 1-7C). Species richness showed no statistically significant differences, either in the number of observed species or the Shannon Index (Figure 1-7A and 1-7B). The fungal communities also differ significantly across the three sites in their community composition (p<0.001), though species richness among sites was similar (Figure 1-8).

Discussion

The goal of this study was to assess the ecosystem structure and function at three sites, each representing a different dryland ecosystem type in order to: (1) fill gaps in knowledge about the sagebrush steppe, and (2) examine the three semiarid drylands sites for similarities and differences to inform future research.

The three dominant semiarid ecosystem types of the U.S. – the shortgrass steppe, the mixed-grass prairie, and the sagebrush steppe – all have distinct plant communities. This is also true for the soil microbial communities across all three ecosystem types. In the case of both aboveground plant communities and soil microbial communities, despite significant differences among ecosystem types, there are large overlaps in species composition. These community composition differences are due, in part, to the specific conditions at each site (i.e., climate, soils), which helps to determine relative species success and abundance. Despite these differences in ecosystem structure among sites, we found only a single significant difference in ecosystem function: soil respiration differed between the sagebrush steppe and mixed-grass prairie.
We found no differences with respect to ANPP, soil N mineralization, or bacterial and fungal abundance. With respect to soil C, there were few differences among sites in the top soil layer, with a single exception in the FPOM pool between a single pair of ecosystem types. In contrast, the 5-10 cm layer had many differences among sites in nearly all pools and fractions. Across both soil layers, SIC showed no significant differences and was consistently 0.08-0.10% by mass, regardless of depth or site. An additional trend is that the shortgrass steppe and the sagebrush steppe, appear to have more similar function than the mixed-grass prairie. Statistical significance aside, the means and ranges at both sites for ANPP, soil respiration, microbial abundance, and soil N mineralization were all more closely aligned than the mixed-grass prairie and the sagebrush steppe.

The lack of significant difference in ecosystem function among and between sites may be driven, at least in part, by the high variability in the measured variables. We expected this high variability, likely due to the heterogeneity in the landscape and within soil more generally. We attempted to moderate this natural variability by focusing on coarse textured soils and sites with similar macroclimatic variables (i.e., MAT and MAP). This expected variability also informed our choice to increase the replicates within each site; our experimental design includes seven replicates per site but only a single site for each ecosystem type.

What is also clear is that despite the lack of differences among examined variables at or near the surface, there were many differences between ecosystem types at the lower soil depth sampled. Within drylands, available water often determines growth and activity. Deeper soil layers are known to have more wet days than surface soils (Sala et al. 1992); it is likely that without the consistent pressure of evaporation at the surface – as well as differences in size and frequency of precipitation events, site differences emerge in lower soil layers based, at least in
part, on the amount of time the soil is wet. It is distinctly possible that the increased variability in soil C content between sites, as opposed to the surface soil layer where evaporation is similarly high everywhere in the summer, is a result of differing degrees of water availability at depth resulting in greater differences between our sites; soil microbial communities are known to respond quickly to pulses of precipitation with effects typically lasting days to a week (Nielsen and Ball 2015).

We hypothesized that labile forms of soil C, soil respiration, as well as plant and microbial biomass would increase as available moisture increases across sites. In addition, we expected that plant and soil microbial community structure would differ among ecosystem types with the largest differences due to different seasonal precipitation regimes; mean summer precipitation is highest in the mixed-grass prairie, with similar values at both the sagebrush steppe and shortgrass steppe. The results of this study only partially confirm our hypotheses.

For ecosystem structure, we saw similar differences in β-diversity among the microbial and plant communities at our various sites. As expected, these differences were greatest between the sagebrush steppe – the location with the lowest MAP – and the other two locations. In addition, the mixed-grass prairie occupies a space of shared species composition at the intersection of the other two ecosystem types, despite having the greatest MAP and mean summer precipitation. Contrary to expectations, soil C was largely similar for the upper soil layers. However, the deeper 5-10 cm soil cores exhibited differences among sites in all forms of organic C, except CPOM; these differences also conform to our predictions with amounts of C decreasing with site precipitation. Bacterial and fungal biomass in the 0-5 cm soil layer was similar across all locations; we did not collect samples for microbial biomass at deeper layers in
2017, so we are unable to know if similar differences in the deeper layers would persist as we observed for soil C.

For ecosystem function, ANPP follows the expected trend of increased biomass with increasing MAP, from the mixed-grass prairie to sagebrush steppe. Mean summer precipitation is lowest in the sagebrush steppe and highest in the mixed-grass prairie; ANPP follows suits with the largest amount of aboveground production in the mixed-grass prairie and smallest ANPP within the sagebrush steppe. However, for soil respiration and soil N mineralization, they did not conform to expectations either wholly or in part – though the mixed-grass prairie had the highest mean CO₂ flux measured.

Our results are in line with other studies that have found high variability in ecosystem function within sites and types of drylands ecosystems (Biederman et al. 2017); individual measurements within the same year at a site were still highly variable. Despite many studies that have found increases in MAP to correspond with ANPP (Whittaker 1970, Sala et al. 1988, Lauenroth et al. 2008), we found that MAP did not directly relate to trends in soil respiration, though it was correlated with the microbial community (Delgado-Baquerizo et al. 2017).

Delgado-Baquerizo et al. (2017) also found that latitude was an important determinant of multifunctionality; in our study we are unable to parse this distinction as we do not have multiple replicates at each latitude.

Our results suggest that literature from both the shortgrass steppe and mixed-grass prairie may be appropriate to infer general processes for the sagebrush steppe. The shortgrass steppe is also likely to be a better proxy than the mixed-grass prairie; the mean value associated with multiple functions were more similar. Similarly, the upper soil layers down to 5cm in depth are
more similar and may provide a better comparison, as long as the comparisons are limited to coarse textured soils, as we did in this study.

There are clear trade-offs to having only a single site within each ecosystem type with respect to the realm of inference. However, as mentioned earlier, there is a long history of gradient studies using only a single site for each level (Lauenroth et al. 1999, Paruelo et al. 1999, Barrett and Burke 2000, McCulley et al. 2009). Additional research can now build on this study, increasing the realm of inference and allowing for generalization of the findings.

Conclusions

We conducted this study to examine the similarities – and differences – in ecosystem structure and function among three sites, each a representative of extensive semiarid dryland plant communities in the western United States. We evaluated structural attributes in the form of plant and microbial community structure, as well as in soil C and microbial biomass. We also examined the functional attributes of ANPP, soil respiration, and soil N mineralization. This study suggests that the sites representing each ecosystem functioned similarly.

In addition, the single area of widespread difference was in the soil C within subsurface soils. These differences were across nearly all fractions. This may be due to similarities in high evaporation in the surface soils or with dissimilar water availability in deeper soils across locations, causing differences in biological activity that is highly sensitive to water availability. This is an interesting avenue for future study.

Given the importance of global drylands, broad and high-powered systematic sampling (Yang et al. 2021) across dryland types will be beneficial to understanding the extent of similarities and ability to extrapolate across and within dryland ecosystem types.
LITERATURE CITED


Whittaker, R. H. 1970. Communities and ecosystems. Communities and ecosystems.


Figure 1-1: Site locations in the western United States along with their dominant species. With the exception of big sagebrush, all of these highlighted species occur at all sites – though they are most prevalent at their identified site.
Figure 1-2: Summer precipitation and temperature varies across sites using Walter-Lieth diagrams. Vertical blue lines indicate increased water availability, while stippled red areas indicate increased dryness. These use 30-year Climate Normals from 1980-2010 and interpolated values for each location using PRISM data from the PRISM Climate Group, Oregon State University, http://prism.oregonstate.edu, created 16 Oct 2017.

Figure 1-3: Few significant differences in ecosystem structure (A-C) and function (D-E) variables among three semiarid dryland plant communities in Wyoming and Colorado (USA). Soil respiration was significantly higher in the sagebrush steppe (B). Horizontal, dashed red line represents the cross-site mean.
Figure 1-4: Soil carbon content within various soil fractions is consistent in the 0-5 cm layer (A-E) but differs in the 5-10 cm layer (F-J) among three semiarid dryland plant communities in Wyoming and Colorado (USA). Horizontal, dashed red line represents the cross-site mean.
Figure 1-5: Soil inorganic carbon content is similar among within both the 0-5 cm layer (A) and the 5-10 cm layer (B) among three semiarid dryland plant communities in Wyoming and Colorado (USA). Horizontal, dashed red line represents the cross-site mean.

Figure 1-6: Plant communities are distinct, but also have overlap in species composition among three semiarid dryland plant communities in Wyoming and Colorado (USA).
Figure 1-7: Bacterial communities in the upper soil are distinct, but also have overlap in species composition among three semiarid dryland plant communities in Wyoming and Colorado (USA).
Figure 1-8: Fungal communities in the upper soil are distinct, but also have overlap in species composition among three semiarid dryland plant communities in Wyoming and Colorado (USA).
Table 1-1: Mean annual precipitation (MAP), mean annual temperature (MAT), and soil texture at each location. We calculated mean precipitation and temperature values using 30-year Climate Normals from 1980-2010. Summer precipitation includes precipitation in June, July, and August. All data are interpolated values for each location using PRISM data from the PRISM Climate Group, Oregon State University.

<table>
<thead>
<tr>
<th>Site</th>
<th>Abbreviation</th>
<th>MAP (mm)</th>
<th>MAT (°C)</th>
<th>Mean Summer Precip (mm)</th>
<th>Summer Precip (mm)</th>
<th>Soil Texture(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central Plains Experimental Range</td>
<td>CPER</td>
<td>339</td>
<td>8.4</td>
<td>129.0</td>
<td>119.0</td>
<td>sandy clay loam, sandy loam, loam</td>
</tr>
<tr>
<td>High Plains Grassland Research Station</td>
<td>HPGRS</td>
<td>392</td>
<td>7.3</td>
<td>153.0</td>
<td>123.0</td>
<td>sandy clay loam, sandy loam, clay loam, loam</td>
</tr>
<tr>
<td>Thunder Basin National Grassland</td>
<td>TBNG</td>
<td>325</td>
<td>8.0</td>
<td>118.0</td>
<td>179.0</td>
<td>sandy clay loam, sandy loam, loamy sand, loam</td>
</tr>
</tbody>
</table>
Table 1-2: Attributes, variables, and methods used for data and sample collection to examine ecosystem structure (ES) and ecosystem function (EF). Detailed methods available in Appendix A.

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Variables measured</th>
<th>Methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weather</td>
<td>Precipitation, air temp, soil temperature, &amp; soil moisture</td>
<td>Continuous site-level measurements via Decagon data loggers</td>
</tr>
<tr>
<td>ES: Soil Carbon &amp; Nitrogen</td>
<td>Labile, intermediate, &amp; recalcitrant pools</td>
<td>Fractionation and dry combustion (Elliott et al. 1999); incubations (Robertson et al. 1999b)</td>
</tr>
<tr>
<td>ES: Plant Community</td>
<td>Percent cover by species</td>
<td>Daubenmire quadrats (Daubenmire 1959)</td>
</tr>
<tr>
<td>ES: Bacterial Community</td>
<td>Community structure</td>
<td>16S Sequencing: 515F &amp; 806rB primers (Caporaso et al. 2012)</td>
</tr>
<tr>
<td>ES: Fungal Community</td>
<td>Community structure</td>
<td>ITS Sequencing: fITS7 &amp; ITS4 primers (Ihrmark et al. 2012)</td>
</tr>
<tr>
<td>ES: Bacterial/Fungal Biomass</td>
<td>Microbial biomass</td>
<td>qPCR: 515F &amp; 806r; ITS3 &amp; ITS4 (White et al. 1990, Kozich et al. 2013)</td>
</tr>
<tr>
<td>EF: N Availability</td>
<td>Mineralized N</td>
<td>Resin probes PRS® (Morrow et al. 2016)</td>
</tr>
<tr>
<td>EF: Soil Respiration</td>
<td>CO₂</td>
<td>LI-COR 8100A</td>
</tr>
<tr>
<td>EF: Productivity</td>
<td>Aboveground biomass (grass, forb, sub-shrub)</td>
<td>Plot-level on experiments (Lauenroth et al. 2008b)</td>
</tr>
</tbody>
</table>
CHAPTER 2

The effect of changing water and nitrogen availability on plant and microbial communities across three dryland ecosystem types in the western United States
INTRODUCTION

Drylands, including both semi-arid and arid ecosystems, comprise 40% of global terrestrial land mass (Graaff et al. 2014, Huang et al. 2016) and 20% within the United States (Sobecki et al. 2001). They are critical to worldwide agriculture, supporting 44% of all land under cultivation (Millenium Ecosystem Assessment 2005). In addition, nearly 2.5 billion people (38% of the global population) live in these water-limited areas (Reynolds et al. 2007).

Drylands are defined by their lack of water and increased aridity, with precipitation insufficient to meet evaporative demand (Budyko 1958, Loik et al. 2004, Feng and Fu 2013). The degree of water limitation varies over the course of the year following seasonal cycles in temperature and precipitation (Schwinning et al. 2004, Sala et al. 2012). Net primary productivity (NPP) responds to increased water availability (Whittaker 1970, Lauenroth and Sala 1992, Hooper and Johnson 1999, Lauenroth et al. 2008) and to nitrogen (N) additions (Yahdjian et al. 2011). The response of NPP indicates some level of limitation of ecosystem function by both water and N.

Precipitation regimes in drylands will most certainly change in the coming years and decades, both globally (Millenium Ecosystem Assessment 2005, IPCC 2014, Stanley 2017) and in the western U.S. (Palmquist et al. 2016). In the Intermountain West of the U.S., mean annual precipitation (MAP) will likely increase (Melillo et al. 2014). However, increases in precipitation are predicted to occur in the winter and spring, followed by warmer, drier summers (Palmquist et al. 2016). Globally, increased aridity is expected to increase the extent of drylands, with likely decreases in temperate drylands as those areas shift to become subtropical (Lkhagya et al. 2017). In addition to precipitation, N will continue to become more available (Galloway et al. 2008a), particularly in areas of energy development, intensive agriculture, or where diesel and fossil
fuels are used heavily – all of which are associated with N deposition (Baron et al. 2000, Burns 2003, Delgado-Baquerizo et al. 2016). It may be difficult to generalize the response of drylands to changes of water and N availability, as the response may be partially determined by other factors, such as those that alter the ability of site-specific organisms to survive or compete. Native species, adapted for the typical limited water and N in drylands, may be outcompeted by those adapted to higher resource environments, thereby altering the dominant taxa in the local community (Cross and Schlesinger 1999, Zak et al. 2003, Lü et al. 2014). For instance, changes to the local plant community can feed back to affect the nutrient availability in soil, particularly with regards to N (Vinton and Burke 1995).

Climate – typically generalized using MAP, mean annual temperature (MAT), and their seasonality – is the single largest determining factor of ecosystem structure and function (Grimm et al. 2013, Maestre et al. 2016). MAP and MAT are critical to determining aridity and water availability, which over the short-term place physiological stresses on plants. Over the long-term, climate, and particularly aridity, provide selection pressure influencing the dominant plant functional types at a given locale. After climate, N availability has the greatest impact in determining presence of plant function types and nutrient use efficiency of ecosystems, a key example of interactions between structure and function (Vitousek et al. 1997, Aerts and Chapin 1999, Zeng et al. 2016). For instance, changes in water availability or N availability in dryland ecosystems are known to affect the composition of plant communities (Lauenroth et al. 1978, Milchunas and Lauenroth 1995, Paschke et al. 2000, Blumenthal et al. 2017) and the composition of soil microbial communities (Drenovsky et al. 2004, Williams and Rice 2007, Fierer et al. 2012a, Zhou et al. 2017, Liu et al. 2017). Plant community changes modify plant litter abundance and chemistry, which in turn alter soil carbon (C), nutrient cycling, and turnover
(Burke et al. 1998). Changes to water and N availability also affect soil microbial communities. The addition of N often affects soil bacterial and fungal communities, through its influence on soil biogeochemical properties (i.e., soil organic carbon, pH, and N availability) (Mueller et al. 2015, Liu et al. 2018). Increased N typically reduces microbial abundance, with effects increasing as the size of addition and length of treatment increase (Knorr et al. 2005, Treseder 2008). In addition, the application of water can interact with N additions to further alter the community structure of soil microbes (Liu et al. 2017).

Further, altered soil bacterial and fungal community structure may affect ecosystem function through changing the dominant microbial taxa present, thereby modifying the occurrence, type, and rate of chemical and enzymatic reactions driving C and N cycling (Schimel and Guldé 1998, Bontti et al. 2009, Nazaries et al. 2015). The relationship between soil microbial community structure and ecosystem function represents a major focus of lab and field studies (Van Der Heijden et al. 2008, Fierer et al. 2012b, Crowther et al. 2015, Maynard et al. 2017, Hubbard et al. 2017). Many of these studies have found important relationships between microbial community structure and ecosystem function (Schimel and Guldé 1998, Maestre et al. 2012, Graham et al. 2016, Delgado-Baquerizo et al. 2017). However, others have not (Marschner et al. 2003, Bowles et al. 2014), suggesting complex interactions that have not yet been adequately defined. We are beginning to understand the climatic, environmental, and site characteristics that determine the composition of these communities (Lozupone and Knight 2007, Lauber et al. 2009, Fierer et al. 2012b), as well as other relationships between microbial structure and function.

Current literature suggests that community structure may influence function at small scales, but is less important as the scale increases (Schimel and Guldé 1998), with regional
variation in climate and soil type dominating. Recent dryland literature indicates that microbial community structure may be important under global change scenarios, particularly as these scenarios relate to water and N availability in soil (Bontti et al. 2011, Delgado-Baquerizo et al. 2017). Significant research has been conducted on the effect of N additions and water availability on microbial communities (Knorr et al. 2005, Treseder 2008, Nielsen and Ball 2015, Martins et al. 2015, Zhou et al. 2017, 2020); however a gap in knowledge remains on the effect of combined changes in water and N \textit{in situ}.

\textit{Project Goals & Hypotheses}

The overall goal of this study was to assess the effects of increasing the availability of water and N on community structure in three dryland ecosystem types. Our specific objectives were (1) to evaluate the effect of water and N additions on the species and functional type composition of plant communities, and (2) to examine the responses of the soil microbial community, both bacterial and fungal, to altered water and N availability. We expected that both plant and microbial community structure would shift, with larger shifts in treatments with greater water and N additions (Mueller et al. 2015, La Pierre et al. 2016, Zhou et al. 2020). We predicted that community shifts would be toward species or functional groups better able to take up labile N.

\textbf{METHODS}

\textit{Study area and site selection}

Our study sites were in Wyoming and Colorado, including locations in the shortgrass steppe, mixed-grass prairie, and sagebrush steppe ecosystem types. The design of using three different
dryland ecosystem types allowed us to examine the response of multiple dryland plant and soil microbial communities to altered water and N availability.

The shortgrass steppe and mixed-grass prairie were located at two separate USDA-ARS properties, the Central Plains Experimental Range (CPER) near Nunn, CO (40° 49’N, 104° 43’W) and the High Plains Grassland Research Station (HPGRS) in Cheyenne, WY (41° 11’N, 104° 54’W), respectively. The sagebrush steppe is located in Wyoming in the Thunder Basin National Grassland (TBNG; 43° 25’N, 104° 55’W) and is on a private in-holding owned by The Nature Conservancy. We used the same three locations as Beltz et al. (2021) [in review], but also added treatments of water and N.

Each site has a MAP between 325-400 mm, a MAT between 7.3-8.5°C, and a coarse soil texture (sandy clay loam, sandy loam, loamy sand, or loam). All three sites have a history of moderate summer grazing by cattle for the past several decades. Soils at our site locations are classified as:

- Shortgrass steppe: Aridic Argiustolls (Hook et al. 1991)
- Mixed-grass prairie: Typic Argiustolls (Munn and Arneson 1998)

*Experimental design*

At each site, we constructed seven exclosures (15 m x 15 m) to prevent disturbance by locally grazed cattle. Nine sub-plots (3 m x 3 m) were located within each exclosure; three sub-plots were reserved as replicate controls and six were randomly assigned a treatment. We left a buffer
zone between each subplot (1.5 m) and the exterior of the plot (1.25 m) to prevent cross contamination among our treatments and to facilitate access for data collection.

We applied water and N monthly during the summer (June – August) in 2017 and 2018. We applied the water treatment in the amount of 20% of the mean monthly precipitation for each site. The effectiveness of water treatments was monitored using soil water potential sensors linked with a Decagon meteorological station.

We added N monthly at the same time as the water application, using pelleted urea (CH₄N₂O) dissolved in water, at a rate of either 10 kg ha⁻¹ yr⁻¹ or 100 kg ha⁻¹ yr⁻¹. We chose rates of N addition reflective of the total N deposition at our sites. The low-level approximates current annual N deposition, while the higher rate of N addition was selected as an experimental treatment approximating longer term, decadal-scale inputs.

We measured soil mineralized N through the summer-long placement of Plant Root Simulator (PRS®) probes (Western Ag Innovations, Inc., Saskatoon, Canada). We log-transformed the soil mineralized N data to conform to assumptions of normality.

**Soil Texture**

We collected soil cores using a custom steel soil core with a 6.5 cm internal diameter. We placed cores in a paper bag in the field and broke them up by hand, so that individual cores were in multiple pieces. All soil samples were placed in a drying oven (45°C) within 48 hours and dried to constant a weight. We then sieved samples to 2 mm and stored them plastic Ziploc bags.

We collected samples in 2016 during the late summer and fall (September to October). Samples were collected from four depths: 0-5 cm, 5-10 cm, 10-20 cm, and 20-30 cm. Soil texture
was assessed by the Soil, Water, and Forage Analytical Laboratory at Oklahoma State University (Klute 1986, Gavlak et al. 2003).

**Plant Community**

We sampled the plant communities at each site in early to mid-June during 2018 and 2019, to best evaluate species richness and community structure; many forbs in this region flower only in the spring. We sampled the control sub-plot within each exclosure using 10 Daubenmire quadrats (0.2 m x 0.5 m) across 5 m², with each individual m² being sampled by two quadrats in different, non-overlapping locations. We estimated the canopy cover of each plant species using a modified Daubenmire (1959) method with the following cover categories: <1%, 1-5%, 6-15%, 16-25%, 26-40%, 41-60%, and 61-100%. Unknown species were given a unique descriptive name in the field and identified later using the Rocky Mountain Herbarium (Univ. of Wyoming).

For plant community comparisons, we use the cover class midpoint (i.e., cover class 5 ranges from 26-40% cover; the midpoint is 33%) for each species to calculate the total cover across the ten Daubenmire quadrats. These cover values were then standardized to relative abundance (decostand function, ‘Vegan’ package v2.5–7 for R).

**Microbial Community & Biomass**

We extracted DNA from our soil samples to characterize the microbial community, due to its importance in regulating decomposition and nutrient cycling. We collected soil cores after two seasons of water and N additions in September/October 2018. In 2018, all treatments were sampled at two depths (0-5 cm and 5-10 cm).
We used a 6.5 cm diameter steel soil core. Cores were rinsed and sterilized in between uses; we used flame sterilization in 2018. We placed soil samples in a sterile 7 oz (207 mL) Whirl-Pak® (Nasco) and homogenized slightly by hand, then placed them in a cooler with ice packs at -2°C. Within 24 hours of collection, we placed all samples in a -20 or -80°C freezer. We isolated DNA from the soil samples using a Qiagen DNeasy PowerSoil Kit following the manufacturer’s protocol. Extracted DNA was stored at -80°C, then submitted to two separate laboratories for qPCR and sequencing.

For microbial biomass, we submitted samples to the Microbial Analysis, Resources, and Services (MARS) lab at the University of Connecticut (UCONN) for qPCR of both the 16S and ITS2 regions. MARS amplified the V4 region using the following primers: 515F (5’-GTGCCAGCMGCCGCGGTAA-3’) and 806R (5’-GGACTACHVGGGTWTCTAAT-3’). (Kozich et al. 2013). The ITS2 region was amplified using ITS3 (5’-GCATCGATGAAGAACGCAGC-3’) and ITS4 (5’-TCCTCCGCTTATTGATATGC-3’) primers (White et al. 1990). Both amplifications used Illumina adapters and dual indices.

We submitted samples to the Roy J. Carver Biotechnology Center (University of Illinois) for amplification and sequencing of the 16S ribosomal RNA gene and the ITS2 region. Library generation and amplification were conducted using the Fluidigm Access Array by the Functional Genomics Unit. Bacterial and fungal amplicons were generated for all DNA samples. The bacterial 16S rRNA V4 hypervariable region was amplified using the primers 515F (5’-GTGYCAGCMGCCGCGGTAA-3’) and 806R (5’-GGACTACNVGGGTWTCTAAT-3’). The fungal ITS2 region was amplified using the fITS7 (5’-GTGAATCATCGAATCTTTG-3’) and ITS4 (5’-TCCTCCGCTTATTGATATGC-3’) primers. Cleaned amplicon pools were quantified using a Qubit. Sequencing was then performed by the DNA Services Lab using standard
operating procedures. Samples were sequenced to generate paired-end reads using the NovaSeq platform (2x250 bp) for the 2018 samples.

For the soil microbial community, we processed sequences for each sample using the standard operating procedure for DADA2 (multiple functions, ‘dada2’ package v1.20.0 for R) (Callahan et al. 2016). We began by trimming primers from our demultiplexed sequences, which we conducted differently for 16S and ITS2 amplicons, as ITS read-lengths are variable. We trimmed ITS primers using cutadapt (v3.4) in Python (v3.8.2) as suggested by the DADA2 ITS protocol. 16S primers were trimmed as part DADA2’s built-in filterAndTrim function for primer removal. 16S sequences were filtered and trimmed using the following parameters: (truncLen=c(250,200), maxN=0, maxEE=c(1,1), truncQ=10, rm.phix=TRUE, compress=TRUE, multithread=TRUE, trimLeft = c(19,20)). ITS filtering used the following parameters: (maxN = 0, maxEE = c(2, 2), truncQ = 2, minLen = 50, rm.phix = TRUE, compress = TRUE, multithread = TRUE). Error learning was performed using one million bases (i.e., nbases=1e8) using enforced monotonicity of quality scores for the weighted loess error model; this is recommended for NovaSeq data as there are so few points at the intermediate scores between the bins (see DADA2’s GitHub for discussion of this approach). We then removed chimeras using DADA2’s function removeBimeraDenovo. We assigned taxonomy to each amplicon sequence variant (ASV) using the SILVA database (v138.1) for bacteria and the UNITE database (v8.3) for fungi.

Prior to statistical analysis, we conducted some post-processing using the Phyloseq package (v1.36.0 for R). We removed reads that were not associated with taxonomic kingdom bacteria (16S) and fungi (ITS). For 16S sequences, we then removed all reads that were identified as chloroplast and mitochondrial in origin. We also removed ASVs that did not have 50 reads in at least 5% of samples within each site. A single batch of extractions was associated
with a contaminated extraction blank. After the post-processing steps above, we removed contaminated ASVs (Table 2-S4) using the ‘prevalence’ method with the default parameters (multiple functions, ‘decontam’ package v1.12.0 for R) (Davis et al. 2018).

**Data Analysis**

We conducted all data analysis and figure creation using R version 4.1.1 (R Core Team 2021), using the following libraries: here, tidyverse, lubridate, data.table, dada2, phyloseq, vegan, ggsignif, DescTools, pairwiseAdonis, MuMIn, lme4, lmeTest, and patchwork.

Observed species were quantified directly for plants (*specnumber* function, ‘Vegan’ package v2.5–7 for R). Microbial sequences were rarefied (Willis 2019) (i.e., normalizing sequence counts across samples) to 90% of the number of sequences in the smallest sample within each site (*rarefy_even_depth* function, ‘phyloseq’ package v2.5–7 for R) prior to calculating species richness (*estimate_richness* function, ‘phyloseq’ package v2.5–7 for R). Species evenness was calculated by dividing the Shannon Index by the log of observed species. We examined the effects of treatments on species richness using a one-way ANOVA (*aov* function, ‘stats’ package v 4.1.1) with a post-hoc Dunnett’s Test (*DunnettTest* function, ‘DescTools’ package v0.99.43) to examine differences between treatments and a pre-determined control within each ecosystem type. We tested normality and equal variance assumptions of our models visually using diagnostic plots. Unless specified otherwise, we evaluate our statistical tests for significance using an alpha of 0.05. For context, we may provide p-values above 0.05 in some situations, but do not consider them significant.

In order to understand differences in species composition between our sites and treatments, we assessed β-diversity of plant, bacterial, and fungal communities separately using
Bray-Curtis pairwise dissimilarities, calculated separately for each of the three ecosystem types. We analyzed plant and soil microbial community using (1) a permutational multivariate analysis of variance (PERMANOVA) with 9999 permutations (adonis function, ‘Vegan’ package v2.5–7 for R), followed by (2) an assessment of contrasts between groups using Bonferroni correction for multiple comparisons (pairwise.adonis function, ‘pairwiseAdonis package v0.4 for R) while controlling for exclosure location (strata=Exclosure), and then visualized using (2) non-metric multi-dimensional scaling (NMDS) in two dimensions (metaMDS function, ‘Vegan’ package v2.5–7 for R).

We also assessed the effect of treatments on plant functional type cover. For the plant community, we examined the response of canopy cover within each of three functional types (shrub, grass, and forb). We then explored treatment effects using a linear mixed-effects (LME) model (lmer function, ‘lmer4’ package v1.1-27.1) that incorporates random effects to account for our split-plot design and for multiple samples within each subplot. We then ran post-hoc t-tests (summary function, ‘lmerTest’ package v3.1-3) and estimated pseudo-R² (r.squaredGLMM function, ‘MuMIn’ package v1.43.17) for each model. We calculated post-hoc standardized coefficients (β) for each parameter (model_parameters function, ‘parameters package v0.16.0). Site, collection year, and treatment were included as factors, with an interaction between plant functional type and treatment (Equation 1).

Equation 1: \( \text{cover}_{perm} \sim \text{Site} + \text{collectionYear} + \text{functionalType} \ast \text{Treatment} + (1|\text{Site:Exclosure}) + (1|\text{Site:Exclosure:Subplot}) \)

We explored the effect of our treatments on multiple response variables (plant cover, soil microbial abundance, and soil N availability) using linear mixed-effects models (lmer function, ‘lmer4’ package v1.1-27.1) and post-hoc t-tests using the Satterthwaite method (summary function, ‘lmerTest’ package v3.1-3). We also explored the effect of our treatments using
reduced models, with treatment as the only fixed effect, in order to explore each of the sites separately in both 2017 and 2018. We utilized multiple models and tests, increasing the potential for a Type 1 error; we focused our analysis and discussion on major trends that are confirmed across all types of models and analyses. We examined the effect of treatments on N availability (Equation 2). Total N was log-transformed to meet assumptions of normality.

$$\text{Equation 2: } \log_{10}(\text{totalN}) \sim \text{Treatment} + (1|\text{Exclosure})$$

We assessed bacterial and fungal abundance similarly to plant functional type cover using LME with site, depth, and treatment included as fixed effects (Equation 3).

$$\text{Equation 3: } \text{abundance} \sim \text{Site} + \text{depth}_\text{cm} + \text{Treatment} + (1|\text{Site}:\text{Exclosure}) + (1|\text{Site}:\text{Exclosure}:\text{Subplot})$$

Last, we examined indicator species for both the bacterial and fungal communities. We first identified differentially abundant taxa within each site (DESeq function, ‘DESeq2 package v1.3.2). We reported differentially abundant taxa at the genus level ($\alpha = 0.001$), along with their phylum. Multiple comparisons were controlled for using the Benjamin-Hochberg correction. Bacteria were reported on at the phylum level for the most frequently occurring phyla. We assigned trophic modes and guilds to all fungal taxa using FUNGuildR (funguild_assign function, ‘FUNGuildR package v0.2.0.90) with single trophic modes reported (i.e., Saprotroph, Pathotroph, Symbiotroph).

**RESULTS**

**Water and Nitrogen Treatments**

Soil water potential increased after the application of water at all sites and times down to 10cm in depth. Increased water availability was evident at all sites by six hours post-treatment.
application (Table 2-1). The maximum increases in water availability within the 48-hour after-treatment window ranged from 1.335 to 2.054 MPa at the shortgrass steppe, to 0.124 to 0.491 MPa at the mixed-grass prairie. The sagebrush steppe had increased water availability, though to a lesser extent, with maximal increases of 0.031 to 0.119 MPa after water application.

Our high N additions significantly increased N availability in 2017 and 2018, both with (β=2.07, p<0.001) and without (β=1.81, p<0.001) the addition of water in combination (Figure 2-1). The high N addition with water, measured using PRS probes, increased inorganic N from a mean ± standard deviation in the control of 37 μg N 10cm⁻² ± 30 (shortgrass steppe), 25 μg N 10cm⁻² ± 11 (mixed-grass prairie), and 29 μg N 10cm⁻² ± 17 (sagebrush steppe) to 397 μg N 10cm⁻² ± 95 (shortgrass steppe), 414 μg N 10cm⁻² ± 145 (mixed-grass prairie), and 264 μg N 10cm⁻² ± 207 (sagebrush steppe). Using reduced models, low N additions similarly increased available N in 2017 (β=0.46 to 0.75, p≤0.048), and more sporadically in 2018 with no effect of low N additions at the mixed-grass prairie. The results in Table 2-1 and Figure 2-1 were also reported in Chapter 3 and were calculated from the same dataset.

Plant Community

We found a total of 70 plant species across all three ecosystem types. This included 50 forbs, 15 grasses, mosses, and 4 shrubs/sub-shrubs. There were 12 forbs identified only to genus and 4 unknown forb species.

Plant species richness and evenness were unaffected by water and N treatments within each of the plant communities (Figure 2-2). Treatments had no effect on changing overall plant community composition in the shortgrass steppe and sagebrush steppe, and there were no visible changes on NMDS plots. However, we saw significant changes in β-diversity within the mixed-
grass prairie \((p=0.0053, F=2.25, R^2=0.238)\). $\beta$-diversity was significantly altered in the water and high N treatment compared to the control \((p=0.03, F=5.97, R^2=0.332)\). This change in community composition is visibly apparent on the NMDS plot (Figure 2-2F) with the water + high N in the upper portions of the plot.

Among functional types, grasses had the greatest cover within all three ecosystem types \((p < 0.001; \text{Figure 2-3})\). Grasses composed 47.4% ±19 of cover at the shortgrass steppe, 47.1% ±12 at the mixed-grass prairie, and 43.2% ±16 at the sagebrush steppe, relative to 2-17% cover in other functional types. Grass cover increased with the addition of high N \((\beta=1.05, p = 0.01)\) and water + high N \((\beta=1.75, p < 0.001)\) at the mixed-grass prairie using reduced models. Within the mixed-grass prairie grass cover was 69.7% ±11 in the water + high N addition and 60.6% ±11 in the high N only, relative to 47.1% ±12 in the control. Using our full models, the water and high N treatment significantly increased total plant cover as a main effect across all sites \((\beta=0.16, p = 0.038; \text{Table 2-S1})\). In addition to treatment, only collection year \((\beta=0.07, p=0.003)\) helped to predict plant functional type cover. Our model explained functional type cover in plants fairly well \((R^2_{\text{marg}} = 0.622, R^2_{\text{cond}} = 0.622)\).

**Microbial Abundance**

Bacterial abundance, measured using qPCR in copies per gram of soil, was unaffected by treatments, though site \((\beta=-0.36 \text{ to } 1.43, p \leq 0.003)\) and depth \((\beta=-0.09, p=0.019)\) were both significant predictors \((\text{Table 2-S2})\). The abundance of fungi increased with our application of water and high N \((\beta=0.40, p = 0.033)\), with site \((\beta=-0.85 \text{ to } 0.45, p < 0.028)\) and depth \((\beta=-0.38, p < 0.001)\) also predictive \((\text{Table 2-S3})\). Our models predicted bacterial abundance well \((R^2_{\text{marg}} = 0.609, R^2_{\text{cond}} = 0.615)\), though less so for fungi \((R^2_{\text{marg}} = 0.308, R^2_{\text{cond}} = 0.340)\). Fine scale
inspection with our reduced models showed slight decreases in bacterial abundance at the 5cm depth at the shortgrass steppe under low N only ($\beta=-1.02$, $p=0.015$), high N only ($\beta=-1.04$, $p=0.013$), and water only treatments ($\beta=-1.29$, $p=0.002$) (Figure 2-4). In these reduced models, bacterial abundance decreased from $2.25 \times 10^7 \pm 7.3 \times 10^6$ copies per gram (control), to $1.72 \times 10^7 \pm 3.6 \times 10^6$ (low N only), $1.71 \times 10^7$ copies per gram $\pm 4.0 \times 10^6$ (high N only), and $1.59 \times 10^7$ copies per gram $\pm 3.6 \times 10^6$ (water only). Reduced models showed no significant pairwise differences among treatments for fungal abundance (Figure 2-5).

_Bacterial Community_

Bacterial samples contained a mean of 710,218 reads per sample prior to beginning sequence processing. After quality filtering, denoising, and removing chimeras the number of sequences was reduced to a mean of 562,722 (79.2% retention). Rarefaction yielded 170,734 to 240,725 sequences, depending on site; these sub-samples were then used for calculating alpha diversity. After post-processing, and using non-rarefied data, the remaining reads were split among 10,174 bacterial ASVs, ranging from 7,309 to 8,517 ASVs at each site.

Bacterial species richness and evenness did not show a significant difference by treatments at all sites and at both depths (Figure 2-S1 and 2-S2). Treatments also had no effect on overall bacterial community composition and $\beta$-diversity. However, depth helps to predict differences in community composition across all sites ($p<0.0001$).

Using differential abundance testing, we identified 4,235 ASVs across all three sites to be significantly different between our five treatments and the control ($\alpha = 0.001$). These ASVs comprise 304 genus-level bacterial taxa. The most common phyla were Actinobacteriota (24%), Proteobacteria (18%), and Acidobacteriota (12%). The response of taxa differed among sites and
ecosystem types (Figure 2-6). Indicator species at the shortgrass steppe saw moderate changes in response to all treatments, including some significant increases in cyanobacteria. Indicator taxa at the mixed-grass prairie largely experienced declines, with the largest declines in the treatments with high N additions (with and without water). The sagebrush steppe also had large declines in abundance of indicator species in the high N treatments with and without water in all phyla examined, however moderate increases were noted in the water only and low N additions.

Fungal Community

Prior to processing sequences, fungal samples contained a mean of 540,009 reads per sample. This was reduced to a mean of 462,544 after quality filtering, denoising, and removing chimeras – retaining 85.7% of the original reads. Rarefaction yielded 142,667 to 179,356 sequences per sample, which we then used to calculate alpha diversity metrics. Of the remaining reads from non-rarefied sequences, they were composed of 1,574 fungal ASVs after post-processing, with individual sites having 708 to 1009 ASVs.

Fungal species richness and evenness were unchanged by treatments at all sites and depths (Figure 2-S3 and 2-S4). However, β-diversity was significantly altered by treatments at the sagebrush steppe (p = 0.008, F=1.187, R²=0.068). Despite this, pairwise contrasts between the control and all treatments did not yield any significant relationship at α = 0.05, however the high N treatment had a p-value of 0.078. Soil depth (p<0.0001) remains an important predictor of the fungal community composition.

Functional type assignment placed 1142 fungal ASVs into guilds (72.6%). This was reduced to 972 ASVs (61.8%) when only the “probable” or “highly probable” confidence rankings were retained for further analysis. Differential abundance testing identified 619 unique
ASVs across all three sites to be significantly different between the five treatments and the control ($\alpha = 0.001$). Of these indicator species, the three most frequently occurring fungal phyla were Ascomycota (61%), Glomeromycota (27%), and Basidiomycota (7%). Trophic modes (i.e., functional group reflecting the major “feeding habits” of fungi) were well-represented across the saprotrophs, symbiotrophs, and pathotrophs (Nguyen et al. 2016). Arbuscular mycorrhizae were the most common functional guild present at the shortgrass steppe and mixed-grass prairie, and third most common at the sagebrush steppe.

The majority of trophic mode-treatment combinations showed a decline in abundance at all sites (Figure 2-7). However, large increases were noted in a small number of trophic modes. Symbiotrophs increased for the mixed-grass prairie (water and low N). The water only and water + high N treatment increased saprotrophs in the sagebrush steppe. The shortgrass steppe had increases in saprotrophs in the water + high N treatment.

Within fungal functional guilds, saprotrophic taxa from multiple guilds increased in relative abundance across all three sites, with larger increases typically being associated with combined treatments or higher treatment levels (Figure 2-8). The largest relative saprotroph increases were at the mixed-grass prairie and shortgrass steppe under combined water and N treatments, though they also increased under water additions alone at the mixed-grass prairie. The sagebrush steppe experienced changes in a reduced number of saprotrophic guilds, however larger increases in abundance occurred in wood saprotrophs. Endophytes also increased at the sagebrush steppe in all but the low N only treatment, while the shortgrass steppe was singular in experiencing widespread increases in lichenized fungi. Arbuscular mycorrhizae generally decreased across all treatments relative to the control, with the greatest decreases with the highest loadings of N and in the absence of water.
DISCUSSION

Our goal was to examine the effects of combinations of water and N additions on plant and microbial community structure in three semiarid dryland ecosystem types common in Colorado and Wyoming over two years of treatment. We evaluated the plant community, as well as the soil bacterial and fungal communities, and specifically evaluated changes in species composition, species richness and evenness, and prevalence of various functional groups.

We found that β-diversity was largely unaffected by treatments across ecosystem types in plant, bacterial, and fungal communities – with only a few exceptions. The plant community was altered by the water + high N treatment in the mixed-grass prairie site, with grasses increasing in abundance. No changes were found in bacterial community β-diversity among treatments, however cyanobacteria in the shortgrass steppe increased under multiple treatments. While our treatments did help to predict fungal community β-diversity within the sagebrush steppe, there were no major differences between treatments and the control. In all cases, α-diversity remained unchanged by treatments. Only total fungal abundance increased with the application of water and high N; bacterial abundance was unchanged. This may indicate a lack of significant water and N limitation in the case of bacteria.

We identified several indicator species from both the bacterial and fungal communities. Among the subset of species that were differentially abundant, we saw different responses to treatment based on bacterial phylum and fungal trophic mode, but also by ecosystem type. N-only additions and water-only treatments tended to decrease abundance of all fungal trophic modes in general, though saprotrophic fungi increased at the sagebrush steppe. Combined water and N additions tended to increase the abundance of saprotrophic fungi indicator species. However, the amount of N caused differing effects among saprotrophs at the mixed-grass prairie
and sagebrush steppe, with increases caused by water + low N (mixed-grass prairie) and water + high N (sagebrush steppe). The increase in fungal biomass and saprotrophic fungi may be associated with an increase in surface plant litter inputs, as fungi were more abundant and experienced larger increases in the upper soil layers – and our N additions help to counteract the expected immobilization (Clocchiatti et al. 2020). These changes in indicator species taxa were found through examination of changes in relative abundance. Few changes in fungal or bacterial abundance were found among treatments, with the only widespread change being a doubling in the abundance of fungi under water and high N. Given the relative stability in the abundance of the microbial communities and the large scale of changes seen among some of the indicator species (measured in log2fold change), the most plausible scenario is that relative changes in indicator species are actual changes in abundance; some fungal and bacterial species changed in abundance.

Bacterial phyla responses ranged from moderate increase to severe declines in abundance across all sites. However, cyanobacteria at the shortgrass steppe increased orders of magnitude more than at either other location. This response of cyanobacteria indicator species was specific to the shortgrass steppe; cyanobacteria were largely unchanged and absent from the indicator species at other sites. At the shortgrass steppe we also saw widespread increases in lichenized fungi. We are likely seeing increases in fungi and bacteria associated with soil crusts within this ecosystem type, though soil crusts are a general feature of most drylands (Mager and Thomas 2011). Soil moisture is the major limiting factor for most soil crusts; the response of cyanobacteria to the water only treatment or lichenized fungi to water and N highlights this limitation by soil moisture. Since soil crusts are typically a component of most dryland sites, it is unknown why there is largely no response in abundance at the mixed-grass prairie and sagebrush...
steppe. Soil crusts can be hugely impacted by disturbance. It may be that the grazing pressure was more impactful at the some locations (Read et al. 2008, Concostrina-Zubiri et al. 2014), that the shortgrass steppe is a leading indicator, or there could be additional explanations. This would be an excellent avenue for future study.

We found that soil depth had a significant effect on both bacterial and fungal community composition. Abundance decreased for both bacteria and fungi, while richness also declined with fungi. Soil depth is correlated with changes in temperature, oxygen, and water availability; all of these factors can contribute to favoring certain species over others. This was expected and conforms to the literature on this subject. While not surprising or novel, this finding gives increased confidence in our field, laboratory, and analytical methods.

Only the mixed-grass prairie showed a response to treatments in terms of plant community composition, with changes in β-diversity from the greatest application of water and N, with increases in the abundance of grasses largely driving this trend. Water is a known limitation in drylands, and is considered the primary limitation of ecosystem function (Hooper and Johnson 1999, Young et al. 2021). The addition of N is also known to be a driver of change, as N limitation is a global phenomenon (Galloway et al. 2008b). Given these limitations, changes to the plant community composition and the increase of grasses under increasing availability of water and N is not surprising. However, this does not explain the lack of effect in the sagebrush steppe and shortgrass steppe. Biomass production at the shortgrass steppe is known to lag for several years, even after water availability has increased (Lauenroth and Sala 1992); it may be that changes to plant community composition lag after additions at both of these semiarid dryland sites.
It is surprising that we saw no changes to β-diversity within bacterial of fungal communities among any of the ecosystem types. Changes in availability of soil water and soil N are well known to be associated with altered community composition (Nielsen and Ball 2015, Zhao et al. 2016). Combined additions would be expected to have an even larger effect (Bi et al. 2012, Zhou et al. 2020). N and soil moisture are also known to affect fungal community composition (Cox et al. 2010, Wehner et al. 2014). Microbial communities are not particularly known for resistance to disturbance – or in this case experimentally applied stressors (Shade et al. 2012), though 18% of studies surveyed reported no effect to disturbance. In addition, Shade et al. (2012) noted that negative results were less likely to be published, so the likelihood of encountering no effect may be greater than their estimate.

Changes to functional groups and indicator species suggest changes are occurring within a large number of taxa in both bacterial and fungal communities. In particular, the widespread decrease of arbuscular mycorrhizae corresponds to findings of other studies (Avio et al. 2013, Jach-Smith and Jackson 2018). It may be that the degree of stress placed by our treatments was not enough to induce community-wide change over the two-year time course of our treatments. N additions are well-documented to cause changes to soil microbial communities (Treseder 2008, Wen et al. 2020), as are changes in precipitation regimes (Clark et al. 2009, Manzoni et al. 2012, Nielsen and Ball 2015). Over a longer period of time, we may have seen larger changes to β-diversity – and those changes may be driven by continued changes within our indicator taxa. While data tend to suggest consequences from water and N additions to bacterial and fungal community composition at the finer scale of indicator species, we have documented greater than expected resistance to β-diversity change across all sites and ecosystem types.
The lack of response of the plant communities in this study is consistent with some literature. Additions of N are known to alter plant community composition (Avolio et al. 2014) and decrease species richness in plant communities (Bobbink et al. 2010, Maestre et al. 2016), but we know that it can take more than two years for plant community shifts to occur to applied stresses (Yahdjian and Sala 2006, Sala et al. 2012). Additionally, the magnitude of outcomes of water and N addition can be variable. Species richness can remain unchanged, even as community composition changes (Avolio et al. 2014), as occurred at the mixed-grass prairie in our study, or large changes to richness and composition can be observed when water and N are changed in combination (Yue et al. 2020).

Our research has several limitations. First, our study was conducted over only two years. It may be that continued additions of water and N would cause additional or increased effect. In addition, the two years of the study were both wetter than average at the sagebrush steppe site. This increased natural precipitation may have had large ramifications for inference from our water additions. Second, we chose sites with limited geographic scope. While we experimentally manipulated seven exclosures for each ecosystem type, the exclosures were located in close proximity to each other. Third, we purposely chose to limit the range of MAP, MAT, and soil texture at our sites. The results should be interpreted with these considerations in mind.

Conclusions
We conducted this study to assess the effects of combined changes to water and N availability on plant and soil microbial communities in three semiarid dryland communities in the western United States. We evaluated α-diversity, β-diversity, and functional group composition. We found varied effects of our treatments across those three communities: (1) only a single
ecosystem type had significantly affected plant communities with grasses increasing in abundance under water and N; (2) soil bacterial β-diversity remained unchanged at all three sites, though indicator species suggested changes in abundance were occurring among multiple phyla; and (3) fungal community β-diversity was generally resistant to change within all ecosystem types, however saprotrophic fungal indicator species increased under application of water and N across all sites.

While the bacterial and fungal communities displayed different responses to water and N treatments, the differing responses for both bacterial and fungal functional groups across the ecosystem types was more striking. Widespread decreases in arbuscular mycorrhizae along with increases in saprotrophic activity seem likely to have an effect on C cycling given their connection to plant productivity and decomposition, respectively. Understanding those effects will be important for understanding changes in a future of changing water and N availability. Our findings demonstrate a need for a greater focus on regional studies where we can better develop generalizable theories of plant and microbial community assembly in drylands.


Whittaker, R. H. 1970. Communities and ecosystems. Communities and ecosystems.


Figure 2-1: Soil mineralized nitrogen ($\text{NO}_3^- + \text{NH}_4^+$) increased with all high nitrogen addition treatments (with and without water) and some low nitrogen treatments at three semiarid dryland plant communities in Wyoming and Colorado (USA). Statistical tests performed on log-transformed data. Reduced models with contrasts were run for each individual site and year.
Figure 2-2: Plant community composition shifted in response to the water + high N treatment at the mixed-grass prairie (F) in Wyoming (USA), though species richness and evenness remain unchanged (D-E). No treatment effects on species richness, evenness, or community composition at the shortgrass steppe (G-I) or at the sagebrush steppe (A-C).
Grasses significantly increased after two years of high N and water + high N treatments at the mixed-grass prairie in Wyoming (USA). Treatments had no significant effects among forbs or shrubs. Large shrubs (i.e., big sagebrush) only occurred at the sagebrush steppe. Sub-shrubs (included in the “shrub” category) occurred at all sites, though in relatively low amounts. Reduced models with contrasts were run for each individual site and functional type.
Figure 2-4: Bacterial abundance significantly decreased in multiple treatments within the shortgrass steppe community in Colorado (USA). Treatments had no affect at the other sites and soil depths. Site and soil depth were both significant predictors of abundance. Reduced models with contrasts were run for each individual site and functional type.
Figure 2-5: Fungal abundance showed no significant differences to water and nitrogen treatments at three semiarid dryland communities in Wyoming and Colorado (USA). Site and soil depth were both significant predictors of abundance. Reduced models with contrasts were run for each individual site and functional type.
Figure 2-6: Bacterial indicator species mapped to phylum across both depths within all three semiarid dryland communities in Wyoming and Colorado (USA). White indicates that there were no differentially abundant taxa present in the indicator species analysis. Guild and species may still be present in the population at stable levels.
Figure 2-7: Fungal indicator species mapped to trophic mode across both depths within all three semiarid dryland communities in Wyoming and Colorado (USA).
Figure 2-8: Fungal indicator species mapped to functional guilds within all three semiarid dryland communities in Wyoming and Colorado (USA). White indicates that there were no differentially abundant taxa present in the indicator species analysis. Guild and species may still be present in the population at stable levels.
Table 2-1: Soil water availability increased after water application at the 5 cm depth at all three ecosystem types in Wyoming and Colorado (USA). Values are the difference in soil water potential (MPa) from the control.

<table>
<thead>
<tr>
<th></th>
<th>hours since water application</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>6</td>
<td>24</td>
<td>48</td>
</tr>
<tr>
<td><strong>Shortgrass steppe</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jun</td>
<td>0.612</td>
<td>0.963</td>
<td>1.211</td>
<td>1.335</td>
</tr>
<tr>
<td>Jul</td>
<td>-0.439</td>
<td>0.938</td>
<td>1.649</td>
<td>2.030</td>
</tr>
<tr>
<td>Aug</td>
<td>-0.163</td>
<td>0.899</td>
<td>2.054</td>
<td>1.491</td>
</tr>
<tr>
<td><strong>Mixed-grass prairie</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jun</td>
<td>0.016</td>
<td>0.030</td>
<td>0.039</td>
<td>0.124</td>
</tr>
<tr>
<td>Jul</td>
<td>-0.039</td>
<td>-0.001</td>
<td>0.084</td>
<td>0.491</td>
</tr>
<tr>
<td>Aug</td>
<td>-0.009</td>
<td>0.022</td>
<td>0.031</td>
<td>0.030</td>
</tr>
<tr>
<td><strong>Sagebrush steppe</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jun</td>
<td>0.000</td>
<td>0.014</td>
<td>0.026</td>
<td>0.031</td>
</tr>
<tr>
<td>Jul</td>
<td>-0.066</td>
<td>-0.040</td>
<td>-0.005</td>
<td>0.118</td>
</tr>
<tr>
<td>Aug</td>
<td>0.001</td>
<td>0.032</td>
<td>0.004</td>
<td>0.006</td>
</tr>
</tbody>
</table>
Figure 2-S1: Bacterial community composition, species richness and evenness did not show significant differences at the 5 cm depth within all three semiarid dryland communities in Wyoming and Colorado (USA).
Figure 2-S2: Bacterial community composition, species richness and evenness did not show significant differences at the 10 cm depth within all three semiarid dryland communities in Wyoming and Colorado (USA).
Figure 2-S3: Fungal community composition was affected by treatments at the sagebrush steppe at the 5 cm depth (C), though no treatments were different from the control. However, species richness and evenness did not show significant differences within the remaining two semiarid dryland communities (D-I) in Wyoming and Colorado (USA).
Figure 2-S4: Fungal community composition, species richness and evenness did not show significant differences at the 10 cm depth within all three semiarid dryland communities in Wyoming and Colorado (USA).
Table 2-S1: Linear mixed model regression coefficients for the effect of treatments on plant functional type abundance within all three semiarid dryland ecosystem types in Wyoming and Colorado (USA). Results based on a full model using data across all sites, years, and functional types. Treatment codes: "CC" = "control", "Cn" = "low N only", "CN" = "high N only", "WC" = "water only", "Wn" = "water + low N", and "WN" = "water + high N".

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>N</th>
<th>B unstandardized</th>
<th>B 95% CI</th>
<th>(\beta) standardized</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site</td>
<td>756</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shortgrass steppe</td>
<td></td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mixed-grass prairie</td>
<td></td>
<td>0.018</td>
<td>0.00, 0.04</td>
<td>0.09</td>
<td>0.7</td>
</tr>
<tr>
<td>Sagebrush steppe</td>
<td></td>
<td>0.014</td>
<td>-0.01, 0.04</td>
<td>0.07</td>
<td>0.2</td>
</tr>
<tr>
<td>collectionYear</td>
<td>756</td>
<td>0.03</td>
<td>0.01, 0.05</td>
<td>0.07</td>
<td>0.003</td>
</tr>
<tr>
<td>functionalType</td>
<td>756</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>forb</td>
<td></td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>grass</td>
<td></td>
<td>0.35</td>
<td>0.33, 0.37</td>
<td>1.74</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>shrub</td>
<td></td>
<td>0.04</td>
<td>0.01, 0.06</td>
<td>0.18</td>
<td>0.001</td>
</tr>
<tr>
<td>Treatment</td>
<td>756</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td></td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cn</td>
<td></td>
<td>0.00</td>
<td>-0.03, 0.04</td>
<td>0.02</td>
<td>0.8</td>
</tr>
<tr>
<td>CN</td>
<td></td>
<td>0.02</td>
<td>-0.01, 0.05</td>
<td>0.10</td>
<td>0.2</td>
</tr>
<tr>
<td>WC</td>
<td></td>
<td>0.01</td>
<td>-0.03, 0.04</td>
<td>0.03</td>
<td>0.7</td>
</tr>
<tr>
<td>Wn</td>
<td></td>
<td>0.01</td>
<td>-0.02, 0.04</td>
<td>0.05</td>
<td>0.5</td>
</tr>
<tr>
<td>WN</td>
<td></td>
<td>0.03</td>
<td>0.00, 0.06</td>
<td>0.16</td>
<td>0.038</td>
</tr>
<tr>
<td>Marg/Cond R(^2)</td>
<td>0.620/0.620</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2-S2: Linear mixed model regression coefficients for the effect of treatments on soil bacterial abundance within all three semiarid dryland ecosystem types in Wyoming and Colorado (USA). Treatment codes: "CC" = "control", "Cn" = "low N only", "CN" = "high N only", "WC" = "water only", "Wn" = "water + low N", and "WN" = "water + high N".

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>N</th>
<th>B unstandardized</th>
<th>B 95% CI</th>
<th>(\beta) standardized</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site</td>
<td>243</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shortgrass steppe</td>
<td></td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mixed-grass prairie</td>
<td></td>
<td>4.37x10(^7)</td>
<td>36,881,687, 50,415,387</td>
<td>1.43</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sagebrush steppe</td>
<td></td>
<td>-1.10x10(^7)</td>
<td>-17,822,423, -4,078,058</td>
<td>-0.36</td>
<td>0.003</td>
</tr>
<tr>
<td>depth_cm</td>
<td>243</td>
<td>-1.15 x10(^6)</td>
<td>-2,111,429, -189,741</td>
<td>-0.09</td>
<td>0.019</td>
</tr>
<tr>
<td>Treatment</td>
<td>243</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td></td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cn</td>
<td></td>
<td>2.63 x10(^6)</td>
<td>-5,842,884, 11,098,542</td>
<td>0.09</td>
<td>0.5</td>
</tr>
<tr>
<td>CN</td>
<td></td>
<td>-5.42 x10(^6)</td>
<td>-13,774,057, 2,944,742</td>
<td>-0.18</td>
<td>0.2</td>
</tr>
<tr>
<td>WC</td>
<td></td>
<td>1.34 x10(^6)</td>
<td>-6,920,000, 9,601,820</td>
<td>0.04</td>
<td>0.8</td>
</tr>
<tr>
<td>Wn</td>
<td></td>
<td>2.60 x10(^6)</td>
<td>-5,708,779, 10,913,381</td>
<td>0.09</td>
<td>0.5</td>
</tr>
<tr>
<td>WN</td>
<td></td>
<td>-4.55 x10(^5)</td>
<td>-8,716,073, 7,805,747</td>
<td>-0.01</td>
<td>&gt;0.9</td>
</tr>
<tr>
<td>Marg/Cond R(^2)</td>
<td>0.609/0.615</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^{1}\)CI = Confidence Interval
Table 2-S3: Linear mixed model regression coefficients for the effect of treatments on soil fungal abundance within all three semiarid dryland ecosystem types in Wyoming and Colorado (USA). Treatment codes: "CC" = "control", "Cn" = "low N only", "CN" = "high N only", "WC" = "water only", "Wn" = "water + low N", and "WN" = "water + high N".

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>N</th>
<th>B unstandardized</th>
<th>B 95% CI</th>
<th>β standardized</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shortgrass steppe</td>
<td>231</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Mixed-grass prairie</td>
<td></td>
<td>-35,002</td>
<td>-49,298 -20,706</td>
<td>-0.85</td>
<td>0.028</td>
</tr>
<tr>
<td>Sagebrush steppe</td>
<td></td>
<td>-18,604</td>
<td>-32,734 -4,474</td>
<td>-0.45</td>
<td>0.013</td>
</tr>
<tr>
<td>depth_cm</td>
<td>231</td>
<td>-6,338</td>
<td>-8,120 -4,556</td>
<td>-0.38</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cn</td>
<td></td>
<td>469</td>
<td>-15,083 16,022</td>
<td>0.01</td>
<td>&gt;0.9</td>
</tr>
<tr>
<td>CN</td>
<td></td>
<td>-7,144</td>
<td>-22,490 8,203</td>
<td>-0.17</td>
<td>0.4</td>
</tr>
<tr>
<td>WC</td>
<td></td>
<td>6,141</td>
<td>-9,020 21,302</td>
<td>0.15</td>
<td>0.4</td>
</tr>
<tr>
<td>Wn</td>
<td></td>
<td>-801</td>
<td>-16,509 14,908</td>
<td>-0.02</td>
<td>&gt;0.9</td>
</tr>
<tr>
<td>WN</td>
<td></td>
<td>16,618</td>
<td>1,366 31,870</td>
<td>0.40</td>
<td>0.033</td>
</tr>
<tr>
<td>Marg/Cond R²</td>
<td></td>
<td>0.308/0.340</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^1\)CI = Confidence Interval

Table 2-S4: Taxonomy associated with ASVs in contaminated blank. These were removed using the ‘decontam’ package from associated samples.

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Phylum</th>
<th>Class</th>
<th>Order</th>
<th>Family</th>
<th>Genus</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria</td>
<td>Cyanobacteria</td>
<td>Cyanobacteria</td>
<td>Cyanobacteriales</td>
<td>Coleofasciculaceae</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Bacteria</td>
<td>Bacteroidota</td>
<td>Bacteroidia</td>
<td>Chitinophagales</td>
<td>Chitinophagaceae</td>
<td>Parafilimonas</td>
<td>NA</td>
</tr>
<tr>
<td>Bacteria</td>
<td>Actinobacteriota</td>
<td>Actinobacteria</td>
<td>Micromonosporales</td>
<td>Micromonosporaceae</td>
<td>Virgisporangium</td>
<td>NA</td>
</tr>
<tr>
<td>Bacteria</td>
<td>Actinobacteriota</td>
<td>Actinobacteria</td>
<td>Micromonosporales</td>
<td>Micromonosporaceae</td>
<td>Salinispora</td>
<td>NA</td>
</tr>
<tr>
<td>Bacteria</td>
<td>Actinobacteriota</td>
<td>Actinobacteria</td>
<td>Micrococcales</td>
<td>Intrasporangiaceae</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Bacteria</td>
<td>Planctomycetota</td>
<td>OMI90</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Bacteria</td>
<td>Chloroflexi</td>
<td>Anaerolineae</td>
<td>SBR1031</td>
<td>A4b</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Bacteria</td>
<td>Actinobacteriota</td>
<td>Actinobacteria</td>
<td>03J19-7L14</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Bacteria</td>
<td>Actinobacteriota</td>
<td>Actinobacteria</td>
<td>Frankiales</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Bacteria</td>
<td>Proteobacteria</td>
<td>Alphaproteobacteria</td>
<td>Rickettsiales</td>
<td>Mitochondria</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Bacteria</td>
<td>Verrucomicrobiota</td>
<td>Verrucomicrobia</td>
<td>Opitutales</td>
<td>Opitutaceae</td>
<td>Opitutus</td>
<td>NA</td>
</tr>
<tr>
<td>Bacteria</td>
<td>Planctomycetota</td>
<td>Planctomyctes</td>
<td>Isosphaerales</td>
<td>Isosphaeraceae</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Bacteria</td>
<td>Armatimonadota</td>
<td>Chthonomonadetes</td>
<td>Chthonomonadales</td>
<td>Chthonomonadaceae</td>
<td>Chthonomonas</td>
<td>NA</td>
</tr>
<tr>
<td>Bacteria</td>
<td>Chloroflexi</td>
<td>Chloroflexia</td>
<td>Chloroflexales</td>
<td>Roseiflexaceae</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Bacteria</td>
<td>Acidobacteriota</td>
<td>Holophagae</td>
<td>Subgroup 7</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Bacteria</td>
<td>Proteobacteria</td>
<td>Alphaproteobacteria</td>
<td>Rhizobiales</td>
<td>Beijerinikiaeae</td>
<td>Psychroglacieola</td>
<td>NA</td>
</tr>
<tr>
<td>Bacteria</td>
<td>Gemmatimonadota</td>
<td>Gemmatimonadetes</td>
<td>Gemmatimonadales</td>
<td>Gemmatimonadaceae</td>
<td>Gemmatimonas</td>
<td>NA</td>
</tr>
<tr>
<td>Bacteria</td>
<td>Chloroflexi</td>
<td>Chloroflexia</td>
<td>Thermomicrobiales</td>
<td>JG30-KF-CM45</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Bacteria</td>
<td>Actinobacteriota</td>
<td>Actinobacteria</td>
<td>Micromonosporales</td>
<td>Micromonosporaceae</td>
<td>Dactylosporangium</td>
<td>NA</td>
</tr>
<tr>
<td>Bacteria</td>
<td>Chloroflexi</td>
<td>Chloroflexia</td>
<td>Chloroflexales</td>
<td>Roseiflexaceae</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Bacteria</td>
<td>Gemmatimonadota</td>
<td>Longimicrobia</td>
<td>Longimicrobiales</td>
<td>Longimicrobiae</td>
<td>YC-ZSS-LKJ147</td>
<td>NA</td>
</tr>
<tr>
<td>Bacteria</td>
<td>Planctomycetota</td>
<td>Planctomyctes</td>
<td>Isosphaerales</td>
<td>Isosphaeraceae</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Bacteria</td>
<td>Verrucomicrobiota</td>
<td>Verrucomicrobia</td>
<td>Opitutales</td>
<td>Opitutaceae</td>
<td>Opitutus</td>
<td>NA</td>
</tr>
<tr>
<td>Bacteria</td>
<td>Actinobacteriota</td>
<td>MB-A2-108</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>
CHAPTER 3

The effect of changing water and nitrogen availability on the fluxes of carbon and on soil carbon pools across three dryland ecosystem types in the western United States
INTRODUCTION

Atmospheric carbon dioxide (CO$_2$) has increased approximately 30% since pre-industrial times, from 560 gigatons (Gt) to 860 Gt today (Zimov et al. 2006, Friedlingstein et al. 2020). These increases in atmospheric CO$_2$ are driving – and will continue to drive – changes to global climate (IPCC 2021). Despite our understanding of the influences of global climate change, our capability to fully predict future climate is hampered by our limited ability to understand feedbacks within the terrestrial carbon (C) cycle (Schuur et al. 2015, Mystakidis et al. 2016, Biederman et al. 2017). This uncertainty is particularly pronounced in water-limited areas (henceforth ‘drylands’) because of complex co-limitation by both water and nitrogen (N) of ecosystem processes (Hooper and Johnson 1999, Delgado-Baquerizo et al. 2016, 2017).

The terrestrial C pool (~5000 Gt) is estimated to be four times the size of the entire atmospheric C pool, with soil C (~2000 Gt) comprising a large majority of the terrestrial pool (Lal 2004, Zimov et al. 2006, Friedlingstein et al. 2020). After geologic C and oceanic C, soil C is the largest single pool of C, with 32% of global soil C found within drylands (Plaza et al. 2018). Semiarid drylands, such as those found in much of Wyoming and Colorado, contain approximately $\frac{1}{3}$ of the dryland soil carbon or about 260 Gt globally in the top 2 m of soil. While the size of the soil C pool is dwarfed by the amount of C in marine and geological pools, the terrestrial soil C pool turns over more rapidly and is one of the greatest sources of uncertainty when predicting the effects of future climate change. This uncertainty is due to our lack of understanding about the interactions between the C cycle and future changes in both water availability and increased N availability (Poulter et al. 2014, Delgado-Baquerizo et al. 2016).

By definition, drylands are characterized by water deficits, defined as having evaporative demand that exceeds the water available through precipitation (Budyko 1958, Loik et al. 2004,
This limitation by water availability can vary throughout the year and be affected by variation in precipitation in both the intra- and inter-annual regimes (Schwinning et al. 2004, Sala et al. 2012). Drylands are clearly limited by water; net primary productivity (NPP) is well known to increase with annual precipitation (Whittaker 1970, Lauenroth and Sala 1992, Hooper and Johnson 1999, Lauenroth et al. 2008). However, most dryland systems also show a response to increases in N availability, representing some degree of N limitation (Yahdjian et al. 2011).

These constraints on ecosystem function are often described as the result of co-limitation by water and N (Hooper and Johnson 1999), which create complex and often synergistic effects (Lauenroth et al. 1978, Harpole et al. 2007). In part due to these complexities, drylands are one of the major sources of uncertainty in C cycle outcomes and are the cause of much of the inter-annual variability of C fluxes (Poulter et al. 2014). Drylands may switch between being a global source of C and a sink (Biederman et al. 2017). As a result, understanding the contributions of CO₂ to the atmosphere from drylands and the effects of limitation by water and N are critically important for predicting CO₂ and future climate. Thus, research that aims to understand how water and N control ecosystem structure and function is essential; this challenge motivates this study.

Our central hypothesis is that resource availability (i.e., water and N) provides direct and proximal control of ecosystem function; changes to the availability of these key resources will alter the functioning of ecosystems. This hypothesis predicts that large alterations in the availability of water and N will both influence the function of drylands systems, with effects further determined by the long-term climate and vegetation characteristics of the dryland ecosystem.
We are interested in the response of three variables to altered water and N availability: aboveground net primary productivity (ANPP), soil respiration, and soil C. We hypothesize that different dryland ecosystem types – including the sagebrush steppe, mixed-grass prairie, and shortgrass steppe – will differ in their responses to water and N based upon the season and the timing of N additions relative to their water limitation status; the system with the largest precipitation deficit is likely to have the largest response. Increasing additions of water and N will increase ANPP, soil respiration, and labile soil C pools in all three dryland ecosystem types. N additions without water will show minimal or no response, as these systems are primarily limited by water.

**METHODS**

*Study and site location*

We ran this study for four years, beginning in summer 2016 with active data collection for C fluxes and soil C occurring in 2017 and 2018. We applied our treatments at three locations in the western United States: the Central Plains Experimental Range (CPER) north of Greeley, CO (40° 49’N, 104° 43’W); the High Plains Grassland Research Station (HPGRS) in Cheyenne, WY (41° 11’N, 104° 54’W); and an in-holding near Thunder Basin National Grassland (TBNG) east of Wright, WY (43° 25’N, 104° 55’W). We chose each site because it represented a common dryland ecosystem type in the western U.S., was similar in terms of climate and soil texture, and had a similar history of moderate grazing. Soils at our site locations were coarse texture soils (sandy clay loam, sandy loam, loamy sand, or loam). They are classified as Aridic Argiustolls (shortgrass steppe), Typic Agrustolls (mixed-grass prairie), and Ustic Haplargids, Ustic Haplocalcids and Aridic Haplustolls (sagebrush steppe) (Hook et al. 1991, Munn and Arneson...
This experiment used the same sites as Beltz et al. (2021) [in review], but included additions of water and N.

The CPER is classified as a shortgrass steppe, with blue grama (*Bouteloua gracilis*) as the predominant and most widespread species. The HPGRS hosts a mixed-grass prairie community dominated by two grass species, needle and thread (*Hesperostipa comata*) and western wheatgrass (*Pascopyrum smithii*). TBNG is classified as the *sagebrush steppe* and is a shrubland dominated by big sagebrush (*Artemisia tridentata*).

**Experimental Design & Treatment Application**

We built seven exclosures (15m x 15m) at each of the three sites to prevent grazing and other disturbance by cattle during the study (Figure 3-1). Each exclosure contained six subplots (3m x 3m) with randomly assigned treatment combinations, which included varying levels of water and N addition. We created buffer zones between each subplot (1.5m), as well as between subplots and the fence surrounding the exterior of the exclosure (1.25m).

We applied water and N each month, from June through August, at each site for two years (2017 and 2018). We added water in the amount of 20% of the mean monthly precipitation (MMP) that was specific to the site and month of application (Table 3-1). We also added N monthly, in the form of urea (CH$_4$N$_2$O), at two levels, 10 kg ha$^{-1}$ yr$^{-1}$ and 100 kg ha$^{-1}$ yr$^{-1}$, with $1/3$ of the annual amount added during each of the three months. The 10 kg ha$^{-1}$ yr$^{-1}$ and 100 kg ha$^{-1}$ yr$^{-1}$ were roughly 1x and 10x, respectively, the annual deposition across our three sites. These levels were chosen in order to examine the effects of low-level additions in addition to higher levels where a significant response was expected, and which may better approximate long-term deposition.
**Weather & Site Conditions**

We installed one meteorological tower at each site using equipment from Meter Group (formerly Decagon Devices). Towers were first installed in early June 2017 and fully functional at all sites by June 21. Each data logger had three sets of sensors: (1) air temperature, barometric pressure, and relative humidity, (2) precipitation, and (3) soil temperature and water potential (5 & 10 cm).

**Soil Collection & Characteristics**

We collected soil cores that were 6.5 cm in diameter. Within 24-48 hours of collection, we placed all soil samples in a drying oven at 45°C to achieve constant weight. We sieved soil samples using a 2mm sieve and placed the sieved samples inside a sealed plastic bag for storage.

We used soil from these collection methods for estimating soil texture, bulk density, pH, and electrical conductivity (EC). We measured these characteristics because they affect water availability, nutrient availability, diffusion of gases, as well as plant and microbial community composition (Appendix A).

**Plant-Available Inorganic Nitrogen**

To estimate inorganic N availability (i.e., NO$_3^-$ and NH$_4^+$) and confirm the effect of our N treatments, we used Plant Root Simulator (PRS®) probes in 2017 and 2018 placed in situ during both years. PRS® probes are ion exchange resin membranes (Western Ag Innovations, Inc., Saskatoon, Canada) that provide an integrated estimate of N availability over the time period the probes are in place.

During 2017 and 2018, we placed probes in the field during the last week of May and then retrieved them in late August/early September to provide an estimate of inorganic N
availability during the summer season. We placed additional probes in the field in early May 2018 and removed in late May 2018 to estimate N availability during the peak growing season when water is typically most available.

We placed four pairs of probes within each sub-plot. Probes were oriented vertically in the soil and placed to a depth of 6-7cm, keeping the resin membrane fully covered by soil but within 1cm of the surface. When we recovered the PRS probes, we placed all four sets of cation/anion probes together in a cooler within a sealed and labelled plastic bag. Upon returning from the field, we rinsed the probes with DI water following the manufacturer’s protocol, and placed them in a refrigerator until shipped to Western Ag Innovations, Inc., (Saskatoon, Canada) for analysis.

A PRS® probe’s ability to adsorb NH$_4^+$ and NO$_3^-$ is dependent on soil temperature and moisture, and absorption may have occurred as much because of ion transport as because of other biological processes. As such, we have not compared measurements between sampling years when conditions were not identical. However, the probes represent an estimate of what a plant root experiences.

**Aboveground Net Primary Productivity**

To estimate ANPP, we collected aboveground biomass in late summer during 2017 and 2018. We clipped all vegetation at the soil surface using manual, hand-operated grass shears. Once clipped, we placed vegetation in brown paper lunch bags. In 2017, we clipped all aboveground biomass within two 0.1 m$^2$ quadrats (0.2 m x 0.5 m). In 2018, we harvested all biomass within four 0.1 m$^2$ quadrats, twice as many as in 2017, and separated into functional groups (grasses, forbs, shrubs/sub-shrubs) in the field. Sub-shrubs rarely comprised more than one species within
any sub-plot, so they were bagged at the species level by default. We assessed forb, graminoid, and sub-shrub biomass in the interspace between sagebrush; we did not collect annual growth of big sagebrush (*Artemisia tridentata*). We focused on the interspace between shrubs due to the similarity of the interspace vegetation with grassland plant communities and the already known differences in function under sagebrush canopies (Burke 1989).

All clipped and bagged biomass was dried; we placed bags in a drying oven at 45°C until it reached constant weight. Drying typically took approximately four days, but did take up to seven days in some instances. We emptied individual bags into a large bowl, sorted by functional group (if needed), and then weighed each on a balance to the nearest 0.01 g. All dried biomass was then used to estimate ANPP (Sims et al. 1978, Sala et al. 1988).

*Soil Respiration*

We measured soil respiration in chambers to estimate combined respiration from plant roots, soil fauna, and soil microbes. We measured soil CO₂ efflux at discrete time points during two years of the experiment, 2017 (August – September) and 2018 (May – September). We measured soil respiration at a single time point in the late morning (STP).

To measure CO₂ efflux from soils *in situ*, we used a LI-COR LI-8100a Automated Soil Gas Flux System (LI-COR Biosciences, Lincoln, NE) combined with polyvinyl chloride pipe (PVC) collars as chambers. The LI-8100a is an infrared gas analyzer (IRGA) with an attached survey chamber with a 20 cm diameter. PVC collars were custom manufactured to LI-COR specifications using 20 cm PVC pipe cut to 10 cm in length with a 45° bevel at the end to ease insertion into the soil. We placed collars 5 cm into the soil (±3 cm) in July 2017. Prior to collecting measurements in May 2018, we reset all PVC collars in the exact same locations to 6
cm (±1 cm) when the soil was wet and soft. We measured the height of the individual PVC collars in the field every day that sampling occurred; we took three measurements from the interior of the collar from the ground’s surface to the top of the collar using a ruler. We averaged these three measurements to obtain a single average value for height. Then we recorded the height and entered into it the LI-8100a to calculate the volume of air within the interior of the PVC collar-survey chamber space.

**Soil Organic Carbon & Nitrogen**

To evaluate the size and accessibility of the C and N pools, we fractionated 2 mm sieved, dry soil into four pools using particle size fractionation (Cambardella and Elliott 1992, Robertson et al. 1999): dissolved organic carbon (DOC), coarse particulate organic matter (CPOM), fine particulate organic matter (FPOM), and mineral associated organic matter (MAOM).

We obtained DOC through shaking a soil sample with DI water, placing the wetted sample in a centrifuge, and then pouring the DOC supernatant onto a 20 μm nylon filter, and using vacuum filtration to extract only the liquid fraction. We passed the soil remaining after the DOC extraction through multiple sieves to separate the physical soil in size-based pools; we washed the soil off the top of a particular sized sieve with DI water into an aluminum loaf pan and then placed the tin in an oven at 60°C until dry (typically 4-7 days). We then ground non-liquid fractions by hand with a mortar and pestle. Particle sizes for each fraction are:

CPOM > 500 μm > FPOM >53 μm > MAOM

We conducted dry combustion of both the sieved bulk soil and non-liquid fractions for each sample on a Costech ECS 4010 Elemental Analyzer at the Yale Analytical and Stable Isotope
Center (New Haven, CT). We measured DOC through acidification and combustion on a Shimadzu Total Organic Carbon Analyzer (TOC-V).

Data Analysis

We conducted all data analysis and figure creation using R version 4.1.1 (R Core Team 2021), using the following libraries: here, tidyverse, lubridate, data.table, rstatix, ggdist, lmer, lmerTest, MuMIn, ggsignif, ggpubr, and patchwork.

We used linear mixed-effects models (lmer function, ‘lmer4’ package v1.1-27.1) with post-hoc t-tests using the Satterthwaite method (summary function, ‘lmerTest’ package v3.1-3) to assess the response to treatment for each of our variables. We evaluated statistical significance using α=0.05. We report some values above p=0.05 if the trend could be biologically noteworthy, however we continue to consider those results statistically insignificant. Pseudo-R²s (marginal and conditional) were estimated for each full model (r.squaredGLMM function, ‘MuMIn’ package v1.43.17). Standardized coefficients (β) were calculated post-hoc (model_parameters function, ‘parameters package v0.16.0). Treatment, site, and collection year were included as factors for all full models. To account for the split-plot design, full models for all response variables included the shared exclosure, nested within site, as a random effect. Site-treatment interactions were explored, but not included in the final models as they did not increase our ability to explain the trends in our data.

Soil mineralized N was transformed using log₁₀ to conform to assumptions of normality (Equation 1). ANPP included the fixed effects of percent sand in the soil (Equation 2). Soil respiration included a fixed interaction between soil temperature and soil water potential (Equation 3). Soil C included the fixed effects of sand percentage, depth, and soil fraction.
(Equation 4). For soil respiration (multiple samplings each year) and soil C (multiple soil fractions), where each subplot was sampled multiple times, the subplot location was also included as a nested random effect within exclosure. To better understand the specific differences within each time and site combination, reduced models were also run for each of the six site-year pairings using only treatment as a fixed effect, for soil mineralized N and ANPP. Our use of multiple tests increases the chance of obtaining an erroneous significant p-value. As such, we will look at widespread trends using all test and models in those cases.

\[
\text{Equation 1: } \log_{10}(\text{totalN}) \sim \text{Site} + \text{collectionYear} + \text{Treatment} + (1|\text{Site: Exclosure})
\]

\[
\text{Equation 2: } \text{mass}_{\text{gm2}} \sim \text{Site} + \text{collectionYear} + \text{Sand}_{\text{per}} + \text{Treatment} + (1|\text{Site: Exclosure})
\]

\[
\text{Equation 3: } \text{ExpFlux} \sim \text{Site} + \text{collectionYear} + \text{Treatment} + \text{soilWater}_{\text{MPa}} \times \text{soilTemp}_{\text{C}} + (1|\text{Site: Exclosure}) + (1|\text{Site: Exclosure: Subplot})
\]

\[
\text{Equation 4: } \text{carbon}_{\text{gCm2}} \sim \text{Site} + \text{collectionYear} + \text{depth}_{\text{cm}} + \text{Sand}_{\text{per}} + \text{soilFraction} + \text{Treatment} + (1|\text{Site: Exclosure}) + (1|\text{Site: Exclosure: Subplot})
\]

Soil water potential was evaluated using a direct comparison of values in the control and water addition treatment; only a single meteorological station and soil moisture sensor were located at each site.

**RESULTS**

**Soil water potential**

Soil water potential was elevated six hours after the application of water at all sites at a 5cm depth, except at the mixed-grass prairie and sagebrush steppe in July 2018 (Figure 3-2). At those two sites in July, the water treatment did take effect by 24 and 48 hours, respectively. The magnitude of difference in water potential varied by site, with the largest changes approaching 2MPa in June and July at the shortgrass steppe; the mixed-grass prairie experienced increases in water availability of 0.30 to 0.491 MPa, while the sagebrush steppe saw increased water
potential of 0.032 to 0.118 MPa in the 48 hours after treatment. While water treatments increased availability of water in the near-term after our water additions, there were also periods of increased dryness in the water-treated areas at both the mixed-grass prairie and the shortgrass steppe (Figure 3-3). When this phenomenon occurred, it was typically mid- to late-month at the 5cm depth, though was less apparent at the sagebrush steppe. The results in Figure 3-2 were also reported in Chapter 2 and were calculated from the same dataset.

Mineralized soil nitrogen

Mineralized soil N was significantly higher in the high N only (β= 1.81, p<0.001) and low N only (β= 0.55, p<0.001) additions, as well as water + high N (β= 2.07, p<0.001) and water + low N (β= 0.33, p=0.001) in the full model (Table 3-2). Soil mineralized N increased 411-1131% depending on the specific treatment, year, and location – from a mean of 25-37 μg N 10cm⁻² in the controls for each site to 264-414 μg N 10cm⁻². These differences generally held true even when each of the three plant communities and years of data collection were examined separately using reduced models (Appendix B), however some low N additions did not cause significantly increased N availability. The results in Table 3-2 were also reported in Chapter 2 and were calculated from the same dataset.

Aboveground Net Primary Productivity

ANPP significantly increased with the addition of high N (β= 0.64, p<0.001) and high N with water (β= 0.59, p=0.002) (Table 3-S1). Based on our reduced models (Appendix B), ANPP showed no significant differences among treatments during the first year of treatment application (Figure 3-4). However, in 2018, the combined addition of water and high N significantly
increased ANPP at two of the three plant communities, the shortgrass steppe (β=1.42, p=0.0026) and mixed-grass prairie (β=2.16, p<0.001). The high N treatment (without water) also increased ANPP at the mixed-grass prairie (β=1.38, p<0.001) and sagebrush steppe (β=1.17, p=0.014). In 2018, ANPP increased at the shortgrass steppe from 58.6 g biomass m\(^{-2}\) ± 24.8 (mean ± standard deviation) in the controls to 84.5 g biomass m\(^{-2}\) ± 23.3 under water and high N. At the mixed-grass prairie ANPP increased from 64.0 g biomass m\(^{-2}\) ± 15.5 to 103.0 g biomass m\(^{-2}\) ± 11.8 (high N only) and 125.0 g biomass m\(^{-2}\) ± 26.7 (high N + water). At the sagebrush steppe ANPP increased at the shortgrass steppe from 58.6 g biomass m\(^{-2}\) ± 24.8 in the controls to 87.1 g biomass m\(^{-2}\) ± 29.7 under high N only. Note that in 2018 at the sagebrush steppe, the high N + water treatment had a p-value of 0.059 (β=0.88), and in 2017 (but not in 2018) the shortgrass steppe had a p-value of 0.0572 (β=1.03) in the high N only.

**Soil respiration**

Soil respiration significantly increased with the application of high N alone (β=0.32, p<0.001) and with water (β=0.45, p<0.001) (Table 3-S2). In addition to treatment, site (β=-0.45 to -0.35, p<0.001), year of sampling (β=0.62, p<0.001), soil water potential (β=-1.12, p<0.001), and soil temperature (β=0.4, p<0.001) were all significant factors and covariates in explaining soil respiration (marginal R\(^2\) = 0.50, conditional R\(^2\) =0.52). Soil respiration increased by a maximum of 66-478% for individual samplings depending on the combination of treatment, year, and location – from a mean of 2.6-4.6 g C m\(^{-2}\) day\(^{-1}\) in the controls for each site to 6.9-12.8 g C m\(^{-2}\) day\(^{-1}\).
Soil Organic Carbon

There were no significant differences among treatments for any of the soil C pools using our full models (Table 3-S3; Figure 3-S1 & Figure 3-S2). Significant predictors of soil C included sites ($\beta=-0.17$ to $0.35$, $p<0.001$), years collected ($\beta=0.11$, $p<0.001$), soil texture ($\beta=-0.04$, $p<0.001$), depth ($\beta=-0.09$, $p<0.001$), and soil fraction ($\beta=-2.43$ to $-1.51$, $p<0.001$), but not treatment. The full model predicting soil C fit very well regardless of the lack of significance of our treatments (marginal $R^2 = 0.78$, conditional $R^2 =0.79$).

Discussion

Our goal was to examine the effects of changes to water and N availability on C cycle functioning across three dryland ecosystem types. Our treatments of additional water and N (low and high) increased the availability of water and N. The fluxes of C into and out of our semiarid dryland sites – ANPP and soil respiration, respectively – responded consistently to the addition of high N with and without the addition of water. However, despite significant changes to the fluxes of C with our treatments, soil organic C showed no widespread significant differences to our treatments across all three ecosystem types – regardless of the pool.

Soil water potential increased within 48 hours of our water treatments, and typically within six hours. This increase in water availability is intuitive and what we would expect. Interestingly, we also measured six instances at the mixed-grass prairie and sagebrush steppe when our water treatment subplots had lower soil water potential and increased dryness compared to their un-watered controls. One potential explanation is that our once-monthly water additions increased plant activity and transpiration, which altered soil water potential in the surrounding soils (Reynolds et al. 2004, Feldman et al. 2021).
ANPP responded to both the addition of N and N with water, though only in the second year of the study and only to the high level of N addition. While the mixed-grass prairie saw increases in ANPP in response to N addition with and without water, the remaining two sites were only affected by one or the other. The site with the least amount of summer precipitation (i.e., the shortgrass steppe) was affected only by the N treatment in combination with water, while the site with the greatest amount of summer precipitation (i.e., sagebrush steppe) responded to the high N addition without water. One potential explanation is that the site with the least precipitation was still limited by water. The wettest site in summer 2018, the sagebrush steppe, may have had the N leach through the soil column. While this effect would be similar in both N-only and N-water combined treatments, it may be that our combined water and N caused the N to be more mobile in the soil and/or effectively added at a greater depth.

Soil respiration increased under both the addition of high N, as well as the high N with water. Fungal abundance increased under water and high N, though not under high N alone (Chapter 2; Table 2-S3). Soil respiration is a combination of both heterotrophic and autotrophic respiration, and the increases seen under these treatments likely comes from both sources. It is possible that increases in some fungal saprotroph indicator species associated with decomposition contributed to increases in soil respiration (Liu et al. 2018). Further, increases in some saprotrophic taxa and decreases in others is expected as fewer taxa become the dominant players under altered conditions – leading to increased decomposition (Querejeta et al. 2021). In addition, increases in fungal abundance noted in the water + high N treatment may have also contributed to increased respiration in that treatment (Mack et al. 2004, Allison et al. 2010). Many studies have found decreasing rates of soil respiration with N amendments (Fog 1988,
Janssens et al. 2010), however systems with low lignin litter, such as ours, often respond with increased decomposition (Knorr et al. 2005).

While soil respiration and ANPP significantly increased with the addition of N with and without water, it is not surprising that soil organic C showed no significant differences. The simultaneous changes to inputs and outputs, with both increasing, apparently resulted in no net effect to soil C. In addition, due to the turnover times of soil C pools, it is not surprising that we saw no effect to our treatments. If we were to have seen an effect, it likely would have been in one of our two most labile pools, DOC and CPOM. However, given the size of the soil C pools and the measured variability within our sites, it may take more than two years of treatment to see a significant effect. Recent studies have similar results showing changes to C fluxes, but a lack of change to soil C (Yan et al. 2020, Stoner et al. 2021).

Our study is consistent in some respects with other studies in drylands. Other studies generally show a mixed response to N fertilizer alone – with some clearly showing effects to ecosystem function (Yahdjian et al. 2011, You et al. 2017, Bai et al. 2010). However, other studies show a reduced or total lack of response (Beltz et al. 2019, Phillips et al. 2021). We saw a lack of response from the low N addition (10 kg N ha⁻¹ yr⁻¹), but some significant increases in function from the high N addition depending on locality and timing. This generally agrees with the effect noted by Phillips et al. (2021) using 0-8 kg N ha⁻¹ yr⁻¹ over five years.

The lack of a response from water addition is surprising and is not consistent with most other studies. It is well documented that NPP increases with annual PPT (Whittaker 1970, Lauenroth and Sala 1992, Hooper and Johnson 1999, Lauenroth et al. 2008). This is particularly surprising given that we have measured increases in soil water availability after our water treatments. However, another study showed that the major response of plant biomass to water
was delayed 2-3 years after the beginning of additions at the shortgrass steppe (Lauenroth and Dodd 1979, Broderick et al. n.d.). It may be that our additions simply did not alter water availability enough or it could be that the experiment was not conducted long enough to overcome any delay due to legacies of low water availability.

Studies have shown variable outcomes of combined changes in soil water availability and soil N availability in drylands; changes can be both synergistic (Lauenroth et al. 1978) or non-additive (Niu et al. 2009), but can be inconsistent (Hooper and Johnson 1999). Recent studies have shown that water and N can interact to modify productivity, in addition to altering biodiversity and plant community composition (DeMalach et al. 2017, Kazanski et al. 2021, Wheeler et al. 2021).

Our study has limitations in terms of improving our understanding of combined changes to water and N availability on the C cycle in drylands. These limitations include (1) the relatively short duration of the study, (2) the limited number of sites, (3) increased natural precipitation at the sagebrush steppe during the summer of 2018, which limited the effectiveness of our water treatments, and (4) climate was purposely standardized across sites as much as possible, making findings less applicable to drylands with drastically different MAP and MAT.

Increasing study duration would allow for multiple improvements, including more likely detection of effects from low levels of N addition, increasing the variability of weather conditions experienced to help surmount the issue of atypical weather in small numbers of years, and an increased ability to detect the changes in soil organic C content. Increasing the replication at the site level would allow for additional comparisons and improve generalizability of findings. The sagebrush steppe had high natural summer precipitation in 2018, limiting the effectiveness of our water treatments; we often see the high N addition functioning similarly to the high N +
water. Despite these limitations, the findings of this study – and others from this project – will add important novel findings to the literature on drylands.

Our results indicate that water and N co-limited ecosystem function during our study. N elicited more consistent responses, however the addition of water along with additional N increased the magnitude of the response. Most surprising was the lack of a response to water alone. While the short duration of the study presents limitations, it is nevertheless important to note the similarity of response – and lack of response – among the plant communities to various treatments; high amounts of N and water were needed over a minimum of two years to see a response to ANPP and soil respiration. Our results have important practical considerations for studying water and N additions in the future. In a future with changing water and N availability, ecosystem C dynamics are likely to be altered if co-limitation by water and N continue to play a significant role.

Conclusions

We conducted this study to help clarify the effects of changing water and N availability on ecosystem function and soil C in semiarid drylands plant communities of the western U.S. We evaluated ANPP, soil respiration, and labile to recalcitrant pools of soil C in response to N and water treatments. We showed similar responses to N additions and combined high N and water additions across all three plant communities, with increased ANPP and soil respiration as a result. Our results demonstrate co-limitation by water and N of dryland ecosystem function, particularly given that there was no response to water additions. Further replication across a wider variety of climates, as well as studies of longer duration, are needed to better understand the generalizability of these findings.
Understanding these effects are important (1) to better understand the limitations and constraints on ecosystem function in a future that is likely to include changing availability to both water and N, and (2) to examine the consistency and relative effect size across common plant communities in the semiarid drylands of the western United States.


Whittaker, R. H. 1970. Communities and ecosystems. Communities and ecosystems.


Figure 3-1: Experimental design using a split-plot, nested structure.
Figure 3-2: Soil water potential increased after the application of a once monthly water treatment (20% mean monthly precipitation) at the 5 cm depth at three semiarid dryland plant communities in Wyoming and Colorado (USA).
Figure 3-3: Daily mean soil water potential increased for multiple days after a once-monthly water application (20% mean monthly precipitation) during summer 2018 at three semiarid dryland plant communities in Wyoming and Colorado (USA).
Figure 3-4: ANPP increases in the second year of data collection under high N only or water + high N, depending on the site. Inset figures show an additional 5 data points that fall above the limits on the current y-axis. Reduced models with contrasts were run for each individual site and year.
Table 3-1: Water addition at each site in 2017 & 2018

<table>
<thead>
<tr>
<th>HPGRS</th>
<th>June</th>
<th>July</th>
<th>August</th>
</tr>
</thead>
<tbody>
<tr>
<td>precip (mm)</td>
<td>59</td>
<td>57</td>
<td>50</td>
</tr>
<tr>
<td>20% MMP (mm)</td>
<td>12</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>pre-N (L/subplot)</td>
<td>86</td>
<td>83</td>
<td>71</td>
</tr>
<tr>
<td>post-N (L/subplot)</td>
<td>21</td>
<td>21</td>
<td>18</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CPER</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>precip (mm)</td>
<td>52</td>
<td>56</td>
<td>48</td>
</tr>
<tr>
<td>20% MMP (mm)</td>
<td>10</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>pre-N (L/subplot)</td>
<td>75</td>
<td>81</td>
<td>69</td>
</tr>
<tr>
<td>post-N (L/subplot)</td>
<td>19</td>
<td>20</td>
<td>17</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TBNG</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>precip (mm)</td>
<td>49</td>
<td>38</td>
<td>22</td>
</tr>
<tr>
<td>20% MMP (mm)</td>
<td>10</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>pre-N (L/subplot)</td>
<td>71</td>
<td>54</td>
<td>32</td>
</tr>
<tr>
<td>post-N (L/subplot)</td>
<td>18</td>
<td>14</td>
<td>8</td>
</tr>
</tbody>
</table>

Table 3-2: Soil mineralized total N increased with the addition of both low and high nitrogen treatments, with and without water, at three semiarid dryland plant communities in Wyoming and Colorado (USA). Soil mineralized N (log_{10} [ug N 10 cm^{-2}]) was log-transformed with results based on a full model using data across all sites and years.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>N</th>
<th>B unstandardized</th>
<th>B 95% CI</th>
<th>β standardized</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site</td>
<td>252</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shortgrass steppe</td>
<td></td>
<td>-0.24</td>
<td>-0.38, -0.09</td>
<td>-0.49</td>
<td>0.003</td>
</tr>
<tr>
<td>Mixed-grass prairie</td>
<td></td>
<td>-0.14</td>
<td>-0.29, 0.01</td>
<td>-0.29</td>
<td>0.059</td>
</tr>
<tr>
<td>Sagebrush steppe</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>collectionYear</td>
<td>252</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2017</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2018</td>
<td></td>
<td>-0.08</td>
<td>-0.14, -0.02</td>
<td>-0.16</td>
<td>0.005</td>
</tr>
<tr>
<td>Treatment</td>
<td>252</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CN</td>
<td></td>
<td>0.27</td>
<td>0.17, 0.36</td>
<td>0.55</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CN</td>
<td></td>
<td>0.88</td>
<td>0.79, 1.0</td>
<td>1.81</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>WC</td>
<td></td>
<td>0.00</td>
<td>-0.10, 0.10</td>
<td>4.5x10^{-3}</td>
<td>&gt;0.9</td>
</tr>
<tr>
<td>WN</td>
<td></td>
<td>0.16</td>
<td>0.06, 0.26</td>
<td>0.33</td>
<td>0.001</td>
</tr>
<tr>
<td>WN</td>
<td></td>
<td>1.0</td>
<td>0.91, 1.1</td>
<td>2.07</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Marg/Cond R²</td>
<td>0.73/0.79</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1CI = Confidence Interval
Figure 3-S1: Soil carbon at the 0-5 cm depth showed no significant differences between treatments in 2017 and 2018 at three semiarid dryland plant communities in Wyoming and Colorado (USA).
Figure 3-S2: Soil carbon at the 5-10 cm depth showed few differences in 2017 and 2018 between treatments at three semiarid dryland plant communities in Wyoming and Colorado (USA).
Table 3-S1: ANPP increased with the addition of both low and high nitrogen treatments, with and without water, at three semiarid dryland plant communities in Wyoming and Colorado (USA). ANPP was measured through measuring dried fall biomass (g biomass m$^{-2}$). Table results based on a full model using data across all sites and years.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>N</th>
<th>unstandardized B</th>
<th>95% CI $^1$</th>
<th>standardized $\beta$</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site</td>
<td>251</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shortgrass steppe</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Mixed-grass prairie</td>
<td>16</td>
<td>-2.7, 35</td>
<td>0.33</td>
<td>0.090</td>
<td></td>
</tr>
<tr>
<td>Sagebrush steppe</td>
<td>-16</td>
<td>-35, 2.4</td>
<td>-0.34</td>
<td>0.083</td>
<td></td>
</tr>
<tr>
<td>collectionYear</td>
<td>251</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2017</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>2018</td>
<td>-25</td>
<td>-35, -15</td>
<td>-.53</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Sand_per</td>
<td>251</td>
<td>-0.17</td>
<td>-1.0, 0.66</td>
<td>-0.03</td>
<td>0.7</td>
</tr>
<tr>
<td>Treatment</td>
<td>251</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Cn</td>
<td>-0.80</td>
<td>-19, 17</td>
<td>-0.02</td>
<td>&gt;0.9</td>
<td></td>
</tr>
<tr>
<td>CN</td>
<td>30</td>
<td>12, 48</td>
<td>0.64</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>WC</td>
<td>2.3</td>
<td>-15, 20</td>
<td>0.05</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>Wn</td>
<td>5.0</td>
<td>-13, 23</td>
<td>0.11</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>WN</td>
<td>28</td>
<td>10, 46</td>
<td>0.59</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td>Marg/Cond $R^2$</td>
<td>0.22/0.27</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^1$CI = Confidence Interval

Table 3-S2: Soil respiration significantly increased with the addition of high nitrogen and high nitrogen with water at three semiarid dryland plant communities in Wyoming and Colorado (USA). Soil respiration (g C m$^{-2}$ day$^{-1}$) was measured using an infrared gas analyzer. Table results based on a full model using data across all sites and years.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>N</th>
<th>unstandardized B</th>
<th>95% CI $^1$</th>
<th>standardized $\beta$</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site</td>
<td>1,511</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sagebrush steppe</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Mixed-grass prairie</td>
<td>-1.0</td>
<td>-1.3, -0.64</td>
<td>-0.35</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Shortgrass steppe</td>
<td>-1.2</td>
<td>-1.6, -0.92</td>
<td>-0.45</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>collectionYear</td>
<td>1,511</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2017</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>2018</td>
<td>1.7</td>
<td>1.5, 2.0</td>
<td>0.62</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>1,511</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Cn</td>
<td>0.29</td>
<td>-0.12, 0.70</td>
<td>0.11</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>CN</td>
<td>0.88</td>
<td>0.47, 1.3</td>
<td>0.32</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>WC</td>
<td>-0.06</td>
<td>-0.49, 0.37</td>
<td>-0.02</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>Wn</td>
<td>0.24</td>
<td>-0.19, 0.67</td>
<td>0.09</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>WN</td>
<td>1.2</td>
<td>0.82, 1.7</td>
<td>0.45</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>soilWater_MPa</td>
<td>1,511</td>
<td>-3.3</td>
<td>-4.3, -2.4</td>
<td>-1.12</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>soilTemp_C</td>
<td>1,511</td>
<td>0.21</td>
<td>0.18, 0.25</td>
<td>0.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>soilWater_MPa * soilTemp_C</td>
<td>1,511</td>
<td>0.19</td>
<td>0.15, 0.23</td>
<td>0.35</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Marg/Cond $R^2$</td>
<td>0.50/0.52</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^1$CI = Confidence Interval
Table 3-S3: Soil organic carbon showed no significant differences to treatments at three semiarid dryland plant communities in Wyoming and Colorado (USA). Soil organic carbon (g C m$^{-2}$) was measured using through combustion in an elemental analyzer. Table results based on a full model using data across all sites and years.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>N</th>
<th>B unstandardized</th>
<th>B 95% CI$^1$</th>
<th>$\beta$ standardized</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Site</strong></td>
<td>2,520</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Shortgrass steppe</td>
<td></td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Mixed-grass prairie</td>
<td>159</td>
<td>131, 187</td>
<td>0.35</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Sagebrush steppe</td>
<td>-79</td>
<td>-107, -51</td>
<td>-0.17</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td><strong>collectionYear</strong></td>
<td>2,520</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>2017</td>
<td></td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>2018</td>
<td>50</td>
<td>34, 66</td>
<td>0.11</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td><strong>depth_cm</strong></td>
<td>2,520</td>
<td>-16</td>
<td>-20, -13</td>
<td>-0.09</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Sand_per</strong></td>
<td>2,520</td>
<td>-2.4</td>
<td>-3.8, -1.0</td>
<td>-0.04</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>soilFraction</strong></td>
<td>2,520</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>total</td>
<td></td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>CPOM</td>
<td>-983</td>
<td>-1,008, -958</td>
<td>-2.18</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>FPOM</td>
<td>-748</td>
<td>-773, -722</td>
<td>-1.66</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>MAOM</td>
<td>-679</td>
<td>-704, -654</td>
<td>-1.51</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>DOC</td>
<td>-1,094</td>
<td>-1,119, -1,069</td>
<td>-2.43</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td><strong>Treatment</strong></td>
<td>2,520</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>CC</td>
<td></td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Cn</td>
<td>23</td>
<td>-17, 62</td>
<td>0.05</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>CN</td>
<td>-11</td>
<td>-50, 29</td>
<td>-0.02</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>WC</td>
<td>0.05</td>
<td>-39, 40</td>
<td>1.17 x 10^{-4}</td>
<td>&gt;0.9</td>
<td></td>
</tr>
<tr>
<td>Wn</td>
<td>2.5</td>
<td>-37, 42</td>
<td>5.51 x 10^{-3}</td>
<td>&gt;0.9</td>
<td></td>
</tr>
<tr>
<td>WN</td>
<td>-1.2</td>
<td>-41, 38</td>
<td>-2.60 x 10^{-3}</td>
<td>&gt;0.9</td>
<td></td>
</tr>
<tr>
<td><strong>Marg/Cond R$^2$</strong></td>
<td>0.78/0.79</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

$^1$CI = Confidence Interval
CONCLUSION OF THE DISSERTATION

My goal was to examine ecosystem structure and function across three semiarid dryland ecosystem types, assessing similarities among ecosystem types and patterns in the response to changing soil water and soil nitrogen (N) availability. I found that the shortgrass steppe, mixed-grass prairie, and sagebrush steppe had distinct soil microbial communities, in addition to their distinct plant communities. Additional measurements of ecosystem structure and function were largely similar. This is particularly encouraging for the sagebrush steppe, since long-term research programs have historically focused on the shortgrass steppe and mixed-grass prairie.

Under treatment with combined water and N, all ecosystem types experienced increases in aboveground net primary production and soil respiration. While β-diversity of the plant and soil microbial communities was largely unchanged, regardless of treatment, increases and decreases in abundance within indicator species suggested that alterations to key processes within the carbon and N cycles were occurring. These changes occurred over just two years of applications, with the largest changes typically occurring in combined high N and water treatments – although some of the bacterial and fungal species changed under low N or water only additions. However, changes to ecosystem function never occurred under water additions alone, suggesting that function was predominantly co-limited by water and N during the two years of my experiment.

My hope is that my research will spur additional long-term research that incorporates multiple drivers of global change. In addition, I believe these chapters are an important contribution to examining the region of the semiarid West across ecosystem type boundaries. Three important foci for future research would be to:
1. Advance theory that can better address the complexity of ecosystem structure-function relationships, including feedbacks between the environmental characteristics and soil microbiota, as well as relationships among taxa within the soil microbial community.

2. Conduct long term experiments that incorporate changes to water, N, and potentially additional global change drivers. Longer term trends, in excess of two years, are more important scientifically and policy-wise – and trends can be altered after the first few years of additions.

3. Improve our ability to ask (and answer) a wide variety of broad scale questions about soils across ecosystem types, regions, and continents by integrating soils data from across studies.
Appendix A: Detailed Field & Laboratory Methods

Experimental Design, Weather, & Site Conditions

We created seven fenced plots (15m x 15m) at each site to prevent cattle from accessing the active experimental area. We monitored weather and site conditions to generate site-level information on water availability and temperature. We installed one meteorological tower at each site using equipment from Meter Group (formerly Decagon Devices). We first installed towers in early June 2017 and were fully functional at all sites by June 21, 2017. Each tower contained an EM50G data logger set to collect data on a 5-minute time step. Data were automatically backed up via a cellular network twice daily, except at the Thunder Basin site where no signal was available. Each EM50G data logger had three sets of sensors: (1) air temperature, barometric pressure, and relative humidity via VP-4; (2) precipitation via ECRN-100; and (3) soil temperature and water potential at the 5 cm and 10 cm depth via MPS-6. We added an additional data logger and soil water sensors in May 2018 to monitor soil water potential in the water treatment subplots.

Soil Collection & Characteristics

We collected soil cores using a custom steel soil core with a 6.5cm internal diameter. We placed cores in a paper bag in the field and broken up by hand, so that individual cores were in multiple pieces. Within 24-48 hours of collection, we placed all soil samples in a drying oven at 45°C and then dried until samples achieved constant weight. We then sieved all soil samples using a 2mm sieve and placed the sieved samples inside a sealed plastic bag.

We used soil from these collection methods for estimating soil texture, bulk density, pH, and electrical conductivity (EC). We measured these characteristics because they affect water
availability, nutrient availability, diffusion of gases, as well as plant and microbial community composition. Note that the soil samples used for microbial community analyses and DNA extraction were collected with similar, though different sterile methods described in the microbial community section.

For soil texture, we collected samples in September and October 2016 at four depths: 0-5cm, 5-10cm, 10-20cm, and 20-30cm. We then shipped sieved soil to the Soil, Water, and Forage Analytical Laboratory at Oklahoma State University for particle-size analysis using a protocol based on Klute (1986) and Galvak et al. (2003), which includes an 8-hour soak in sodium hexametaphosphate then separation by settling time.

We measured bulk density using samples collected in September 2018 at two depths: 0-5cm and 5-10cm. We calculated total bulk density (g cm\(^{-3}\)) by dividing the mass of the dried soil sample by the volume of the soil core (Elliott et al. 1999).

For soil pH, we collected samples in September 2018 at two depths: 0-5cm and 5-10cm. We used an Orion Star A111 Benchtop pH meter (Thermo Scientific) and 20g soil with 20mL DI water based on Miller et al. (2013).

For soil EC, we used the same samples as those for soil pH. We measured EC using an EcoTestr EC Meter (Eutech/Oakton Instruments) following the manufacturers protocol using the same 1:1 soil to DI water mixture as for pH.

Soil Carbon & Nitrogen

In ordered to evaluate the size and accessibility of the carbon and nitrogen pools, we fractionated 2mm sieved, dry soil into four pools using particle size fractionation (Cambardella and Elliott 1992, Robertson et al. 1999a): dissolved organic carbon (DOC), coarse particulate organic matter
(CPOM), fine particulate organic matter (FPOM), and mineral associated organic matter fraction (MAOM).

We obtained DOC through shaking a soil sample with DI water, placing the wetted sample in a centrifuge, and then pouring the DOC supernatant onto a 20 μm nylon filter, and using vacuum filtration to extract only the liquid fraction. We passed the soil remaining after the DOC extraction through multiple sieves to separate the physical soil in size-based pools; we washed the soil off the top of a particular sized sieve with DI water into an aluminum loaf pan and then placed the tin in an oven at 60°C until dry (typically 4-7 days). We then ground non-liquid fractions by hand with a mortar and pestle. Particle sizes for each fraction are:

\[
\text{CPOM > 500 μm > FPOM >53 μm > MAOM}
\]

We conducted dry combustion of both the sieved bulk soil and non-liquid fractions for each sample on a Costech ECS 4010 Elemental Analyzer at the Yale Analytical and Stable Isotope Center (New Haven, CT). We measured DOC through acidification and combustion on a Shimadzu Total Organic Carbon Analyzer (TOC-V).

Plant-Available Inorganic Nitrogen

To estimate inorganic nitrogen availability (i.e., NO₃⁻ and NH₄⁺), we used Plant Root Simulator (PRS®) probes in 2017 and 2018 placed in situ during both years. PRS® probes are ion exchange resin membranes (Western Ag Innovations, Inc., Saskatoon, Canada) that provide an integrated estimate of N availability over the time period the probes are in place.

During 2017 and 2018, we placed probes in the field during the last week of May and then retrieved them in late August/early September to provide an estimate of inorganic N
availability during the summer season. We placed additional probes in the field in early May 2018 and removed in late May 2018 to estimate N availability during the peak growing season when water is typically most available.

We placed four pairs of probes within the corners of each sub-plot. Probes were oriented vertically in the soil and placed to a depth of 6-7cm, keeping the resin membrane fully covered by soil but within 1cm of the surface. When we recovered the PRS probes, we placed all four sets of cation/anion probes together in a sealed and labelled plastic bag, specific to that sub-plot. After bagging, we placed probes in a cooler with three ice packs rated to -2°C. Upon returning from the field, we rinsed the probes with DI water and then placed the probes in a refrigerator until shipped. Cleaned PRS probes were analyzed by Western Ag Innovations, Inc., (Saskatoon, Canada).

A PRS® probe’s ability to adsorb NH$_4^+$ and NO$_3^-$ is dependent on soil temperature and moisture and absorption may have occurred as much because of ion transport as because of other biological processes. As such, we have not compared measurements between sampling years where conditions were not identical.

Aboveground Biomass

We collected aboveground biomass in late summer during 2017 and 2018 in order to estimate aboveground net primary productivity. We clipped all vegetation at the soil surface using manual, hand-operated grass shears. Once clipped, we placed vegetation in brown paper lunch bags. In 2017, we clipped all aboveground biomass within two 0.1 m$^2$ quadrats (0.2 m x 0.5 m). In 2018, we harvested all biomass within four 0.1 m$^2$ quadrats, twice as many as in 2017, and separated into functional groups (grasses, forbs, woody sub-shrubs) in the field. Sub-shrubs
rarely comprised more than one species within any sub-plot, so they were bagged at the species level by default. Note that we assessed only forb, graminoid, and sub-shrub biomass in the interspace between sagebrush; we did not collect annual growth of shrub biomass via big sagebrush (*Artemisia tridentata*).

All clipped and bagged biomass was dried; we placed bags in a drying oven at 45°C until it reached constant weight. Drying typically took approximately four days, but did take up to seven days in some instances. We emptied individual bags into a large bowl, sorted by functional group (if needed), and then weighed on a balance to the nearest 0.01 g.

**Plant Community**

We surveyed the plant communities in our three sites in early to mid-June during 2018 and 2019, in order to best evaluate species richness and community structure; many forbs in this region flower in the spring. We sampled the control sub-plot within each exclosure using 10 Daubenmire quadrats (0.2m x 0.5m) across 5 m² of the 9 m² subplot; we sampled each individual m² using two quadrats in different, non-overlapping locations. Canopy cover was estimated for all plant species following a variation of the Daubenmire (1959) method that used these specific cover categories: <1%, 1-5%, 6-15%, 16-25%, 26-40%, 41-60%, and 61-100%. When recording canopy cover, we recorded plant species using the 4-letter symbol from the USDA PLANTS database (www.plants.sc.egov.usda.gov). We gave unknown species a unique descriptive name during data collection in the field and then identified a collected sample or picture later using the Rocky Mountain Herbarium (University of Wyoming).
Microbial Community & Biomass

We examined soil environmental DNA (eDNA) to characterize the microbial community, due to its importance in regulating decomposition and nutrient cycling. We collected soil cores at two timepoints, September 2017 and September/October 2018. In 2017, all samples were from the 0-5cm depth, and we sampled only two treatments, the control and water+high N addition. In 2018 we collected samples from all treatments at two depths, 0-5cm and 5-10cm.

We used a custom steel soil core with a 6.5cm internal diameter. We rinsed the sterilized core in between uses; we used bleach in 2017 and flame sterilization in 2018. We placed soil samples in a sterile 7oz (207 mL) Whirl-Pak® (Nasco), homogenized them slightly by hand, and then placed samples in a cooler on ice at -2ºC. Within 24 hours of collection, we placed all samples in a -20 or -80 ºC freezer.

We isolated DNA from the soil samples using a Qiagen DNeasy PowerSoil Kit following the manufacturers protocol. We stored extracted DNA at -80ºC. We then submitted extracted DNA to two separate laboratories for qPCR and sequencing.

We submitted samples to the Microbial Analysis, Resources, and Services (MARS) lab at the University of Connecticut (UCONN) for qPCR of both the 16S and ITS2 regions. MARS amplified the V4 region using the following primers: 515F (5’-GTGCCAGCMGCCGCGGTAA-3’) and 806R (5’-GGACTACHVGGGTWTCTAAT-3’). (Kozich et al. 2013). The ITS2 region was amplified using ITS3 (5’-GCATCGATGAAGAACGCAGC-3’) and ITS4 (5’-TCCTCGCTTATTGATATGC-3’) primers (White et al. 1990). Both amplifications used Illumina adapters and dual indices.

We amplified and sequenced the 16S ribosomal RNA gene and the ITS2 region at the Roy J. Carver Biotechnology Center (University of Illinois). Library generation and
amplification were conducted using the Fluidigm Access Array by the Functional Genomics Unit. Bacterial and fungal amplicons were generated for all DNA samples. The bacterial 16S rRNA V4 hypervariable region was amplified using the primers 515F (5’-GTGYCAGCMGGCTGCTAA-3’) and 806R (5’-GGACTACNVGGGTWTCTAAT-3’). The fungal ITS2 region was amplified using the fITS7 (5’-GTGAATCATCGAATCTTTG-3’) and ITS4 (5’-TCCTCCGCTTATGGATATGC-3’) primers. Cleaned amplicon pools were quantified using a Qubit. Sequencing was then performed by the DNA Services Lab using standard operating procedures. Samples were sequenced to generate paired-end reads using the MiSeq v2 platform (2x250bp) for the 2017 samples and the NovaSeq platform (2x250 bp) for the 2018 samples.

Soil Respiration

We measured soil respiration in chambers in order to estimate combined plant root respiration and microbial respiration. We measured soil carbon dioxide (CO₂) efflux at discrete time points during two years of the experiment, 2017 (August – September) and 2018 (May – September). We measured soil respiration in two ways, either collecting measurements at a single time-point in the late morning (STP) or collecting six measurements throughout a single day from 4am to 10pm (i.e., diurnal).

To measure CO₂ efflux from soils in situ, we used a LI-COR LI-8100a Automated Soil Gas Flux System (LI-COR Biosciences, Lincoln, NE) combined with polyvinyl chloride pipe (PVC) collars as chambers. The LI-8100a is an infrared gas analyzer (IRGA) with an attached survey chamber with a 20 cm diameter. PVC collars were custom manufactured to LI-COR specifications using 20 cm PVC pipe cut to 10 cm in length with a 45° bevel at the end to ease
insertion into the soil. We placed collars 5 cm into the soil (±3 cm) in July 2017. Prior to collecting measurements in May 2018, we reset all PVC collars in the exact same locations to 6 cm (±1 cm) when the soil was wet and soft. We measured the height of the individual PVC collars in the field every day that sampling occurred; we took three measurements from the interior of the collar from the ground’s surface to the top of the collar using a ruler. We averaged these three measurements to obtain a single average value for height. Then we recorded the height and entered into the LI-8100a to calculate the volume of air within the interior of the PVC collar-survey chamber space.
**APPENDIX B: ADDITIONAL MODEL OUTPUT**

Reduced model output for soil total inorganic N (log$_{10}$[ug N 10 cm$^{-2}$]). Individual models were run for each site and year by treatment, with random effects to describe the nested structure for each exclosure. Post-hoc contrasts and degrees of freedom calculated using the Satterthwaite method.

<table>
<thead>
<tr>
<th>Site</th>
<th>Year</th>
<th>Treatment</th>
<th>Estimate</th>
<th>StdError</th>
<th>df</th>
<th>t_value</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBNG</td>
<td>2018</td>
<td>Cn</td>
<td>0.3097</td>
<td>0.1280</td>
<td>30</td>
<td>2.4190</td>
<td>0.0218</td>
</tr>
<tr>
<td>TBNG</td>
<td>2018</td>
<td>CN</td>
<td>0.8990</td>
<td>0.1280</td>
<td>30</td>
<td>7.0217</td>
<td>0.0000</td>
</tr>
<tr>
<td>TBNG</td>
<td>2018</td>
<td>WC</td>
<td>-0.0216</td>
<td>0.1280</td>
<td>30</td>
<td>-0.1686</td>
<td>0.8672</td>
</tr>
<tr>
<td>TBNG</td>
<td>2018</td>
<td>Wn</td>
<td>0.0870</td>
<td>0.1280</td>
<td>30</td>
<td>0.6797</td>
<td>0.5019</td>
</tr>
<tr>
<td>TBNG</td>
<td>2018</td>
<td>WN</td>
<td>0.8966</td>
<td>0.1280</td>
<td>30</td>
<td>7.0027</td>
<td>0.0000</td>
</tr>
<tr>
<td>TBNG</td>
<td>2017</td>
<td>Cn</td>
<td>0.2319</td>
<td>0.0935</td>
<td>30</td>
<td>2.4812</td>
<td>0.0189</td>
</tr>
<tr>
<td>TBNG</td>
<td>2017</td>
<td>CN</td>
<td>1.0108</td>
<td>0.0935</td>
<td>30</td>
<td>10.8123</td>
<td>0.0000</td>
</tr>
<tr>
<td>TBNG</td>
<td>2017</td>
<td>WC</td>
<td>-0.0216</td>
<td>0.0935</td>
<td>30</td>
<td>-0.1686</td>
<td>0.8672</td>
</tr>
<tr>
<td>TBNG</td>
<td>2017</td>
<td>Wn</td>
<td>0.0870</td>
<td>0.0935</td>
<td>30</td>
<td>0.6797</td>
<td>0.5019</td>
</tr>
<tr>
<td>TBNG</td>
<td>2017</td>
<td>WN</td>
<td>0.8966</td>
<td>0.0935</td>
<td>30</td>
<td>7.0027</td>
<td>0.0000</td>
</tr>
<tr>
<td>HPGRS</td>
<td>2018</td>
<td>Cn</td>
<td>0.2038</td>
<td>0.1059</td>
<td>30</td>
<td>1.9250</td>
<td>0.0638</td>
</tr>
<tr>
<td>HPGRS</td>
<td>2018</td>
<td>CN</td>
<td>1.0334</td>
<td>0.1059</td>
<td>30</td>
<td>9.7601</td>
<td>0.0000</td>
</tr>
<tr>
<td>HPGRS</td>
<td>2018</td>
<td>WC</td>
<td>-0.1547</td>
<td>0.1059</td>
<td>30</td>
<td>-1.4607</td>
<td>0.1545</td>
</tr>
<tr>
<td>HPGRS</td>
<td>2018</td>
<td>Wn</td>
<td>-0.1473</td>
<td>0.1059</td>
<td>30</td>
<td>-1.3914</td>
<td>0.1743</td>
</tr>
<tr>
<td>HPGRS</td>
<td>2018</td>
<td>WN</td>
<td>1.2357</td>
<td>0.1059</td>
<td>30</td>
<td>11.6705</td>
<td>0.0000</td>
</tr>
<tr>
<td>HPGRS</td>
<td>2017</td>
<td>Cn</td>
<td>0.2059</td>
<td>0.0789</td>
<td>30</td>
<td>2.6097</td>
<td>0.0140</td>
</tr>
<tr>
<td>HPGRS</td>
<td>2017</td>
<td>CN</td>
<td>0.7239</td>
<td>0.0789</td>
<td>30</td>
<td>9.1765</td>
<td>0.0000</td>
</tr>
<tr>
<td>HPGRS</td>
<td>2017</td>
<td>WC</td>
<td>-0.0378</td>
<td>0.0789</td>
<td>30</td>
<td>-0.4795</td>
<td>0.6351</td>
</tr>
<tr>
<td>HPGRS</td>
<td>2017</td>
<td>Wn</td>
<td>0.1931</td>
<td>0.0789</td>
<td>30</td>
<td>2.4477</td>
<td>0.0204</td>
</tr>
<tr>
<td>HPGRS</td>
<td>2017</td>
<td>WN</td>
<td>1.0554</td>
<td>0.0789</td>
<td>30</td>
<td>13.3789</td>
<td>0.0000</td>
</tr>
<tr>
<td>CPER</td>
<td>2018</td>
<td>Cn</td>
<td>0.3270</td>
<td>0.1014</td>
<td>30</td>
<td>3.2250</td>
<td>0.0030</td>
</tr>
<tr>
<td>CPER</td>
<td>2018</td>
<td>CN</td>
<td>0.8031</td>
<td>0.1014</td>
<td>30</td>
<td>7.9219</td>
<td>0.0000</td>
</tr>
<tr>
<td>CPER</td>
<td>2018</td>
<td>WC</td>
<td>0.1553</td>
<td>0.1014</td>
<td>30</td>
<td>1.5323</td>
<td>0.1359</td>
</tr>
<tr>
<td>CPER</td>
<td>2018</td>
<td>Wn</td>
<td>0.4172</td>
<td>0.1014</td>
<td>30</td>
<td>4.1148</td>
<td>0.0003</td>
</tr>
</tbody>
</table>
Reduced model output for ANPP (g m\(^{-2}\)). Individual models were run for each site and year by treatment, with random effects to describe the nested structure for each exclosure. Post-hoc contrasts and degrees of freedom calculated using the Satterthwaite method.

<table>
<thead>
<tr>
<th>Site</th>
<th>Year</th>
<th>Trtment</th>
<th>Estimate</th>
<th>StdError</th>
<th>df</th>
<th>t_value</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPER</td>
<td>2018</td>
<td>WN</td>
<td>1.1062</td>
<td>0.1014</td>
<td>30</td>
<td>10.9111</td>
<td>0.0000</td>
</tr>
<tr>
<td>CPER</td>
<td>2017</td>
<td>Cn</td>
<td>0.3263</td>
<td>0.1044</td>
<td>30</td>
<td>3.1258</td>
<td>0.0039</td>
</tr>
<tr>
<td>CPER</td>
<td>2017</td>
<td>CN</td>
<td>0.8285</td>
<td>0.1044</td>
<td>30</td>
<td>7.9362</td>
<td>0.0000</td>
</tr>
<tr>
<td>CPER</td>
<td>2017</td>
<td>WC</td>
<td>0.0449</td>
<td>0.1044</td>
<td>30</td>
<td>0.4297</td>
<td>0.6705</td>
</tr>
<tr>
<td>CPER</td>
<td>2017</td>
<td>Wn</td>
<td>0.2148</td>
<td>0.1044</td>
<td>30</td>
<td>2.0574</td>
<td>0.0484</td>
</tr>
<tr>
<td>CPER</td>
<td>2017</td>
<td>WN</td>
<td>0.9513</td>
<td>0.1044</td>
<td>30</td>
<td>9.1130</td>
<td>0.0000</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Site</th>
<th>Year</th>
<th>Trtment</th>
<th>Estimate</th>
<th>StdError</th>
<th>df</th>
<th>t_value</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBNG</td>
<td>2018</td>
<td>Cn</td>
<td>8.3400</td>
<td>11.2873</td>
<td>30.0000</td>
<td>0.7389</td>
<td>0.4657</td>
</tr>
<tr>
<td>TBNG</td>
<td>2018</td>
<td>CN</td>
<td>29.4714</td>
<td>11.2873</td>
<td>30.0000</td>
<td>2.6110</td>
<td>0.0140</td>
</tr>
<tr>
<td>TBNG</td>
<td>2018</td>
<td>WC</td>
<td>6.0086</td>
<td>11.2873</td>
<td>30.0000</td>
<td>0.5323</td>
<td>0.5984</td>
</tr>
<tr>
<td>TBNG</td>
<td>2018</td>
<td>Wn</td>
<td>-1.1571</td>
<td>11.2873</td>
<td>30.0000</td>
<td>-0.1025</td>
<td>0.9190</td>
</tr>
<tr>
<td>TBNG</td>
<td>2018</td>
<td>WN</td>
<td>22.1657</td>
<td>11.2873</td>
<td>30.0000</td>
<td>1.9638</td>
<td>0.0589</td>
</tr>
<tr>
<td>TBNG</td>
<td>2017</td>
<td>Cn</td>
<td>0.2286</td>
<td>19.0720</td>
<td>29.1611</td>
<td>0.0120</td>
<td>0.9905</td>
</tr>
<tr>
<td>TBNG</td>
<td>2017</td>
<td>CN</td>
<td>16.3571</td>
<td>19.0720</td>
<td>29.1611</td>
<td>0.8577</td>
<td>0.3981</td>
</tr>
<tr>
<td>TBNG</td>
<td>2017</td>
<td>WC</td>
<td>14.4429</td>
<td>19.0720</td>
<td>29.1611</td>
<td>0.7573</td>
<td>0.4550</td>
</tr>
<tr>
<td>TBNG</td>
<td>2017</td>
<td>Wn</td>
<td>1.3429</td>
<td>19.0720</td>
<td>29.1611</td>
<td>0.0704</td>
<td>0.9443</td>
</tr>
<tr>
<td>TBNG</td>
<td>2017</td>
<td>WN</td>
<td>5.2315</td>
<td>19.8971</td>
<td>29.7009</td>
<td>0.2629</td>
<td>0.7944</td>
</tr>
<tr>
<td>Site</td>
<td>Year</td>
<td>Trtment</td>
<td>Estimate</td>
<td>StdError</td>
<td>df</td>
<td>t_value</td>
<td>p</td>
</tr>
<tr>
<td>----------</td>
<td>------</td>
<td>---------</td>
<td>----------</td>
<td>----------</td>
<td>----</td>
<td>---------</td>
<td>------</td>
</tr>
<tr>
<td>HPGRS</td>
<td>2018</td>
<td>Cn</td>
<td>-2.6000</td>
<td>7.4452</td>
<td>30.000</td>
<td>-0.3492</td>
<td>0.7294</td>
</tr>
<tr>
<td>HPGRS</td>
<td>2018</td>
<td>CN</td>
<td>39.2186</td>
<td>7.4452</td>
<td>30.000</td>
<td>5.2676</td>
<td>0.0000</td>
</tr>
<tr>
<td>HPGRS</td>
<td>2018</td>
<td>WC</td>
<td>2.4971</td>
<td>7.4452</td>
<td>30.000</td>
<td>0.3354</td>
<td>0.7397</td>
</tr>
<tr>
<td>HPGRS</td>
<td>2018</td>
<td>Wn</td>
<td>4.0300</td>
<td>7.4452</td>
<td>30.000</td>
<td>0.5413</td>
<td>0.5923</td>
</tr>
<tr>
<td>HPGRS</td>
<td>2018</td>
<td>WN</td>
<td>61.2171</td>
<td>7.4452</td>
<td>30.000</td>
<td>8.2223</td>
<td>0.0000</td>
</tr>
<tr>
<td>HPGRS</td>
<td>2017</td>
<td>Cn</td>
<td>3.3571</td>
<td>25.1062</td>
<td>30.000</td>
<td>0.1337</td>
<td>0.8945</td>
</tr>
<tr>
<td>HPGRS</td>
<td>2017</td>
<td>CN</td>
<td>24.0286</td>
<td>25.1062</td>
<td>30.000</td>
<td>0.9571</td>
<td>0.3462</td>
</tr>
<tr>
<td>HPGRS</td>
<td>2017</td>
<td>WC</td>
<td>-28.1143</td>
<td>25.1062</td>
<td>30.000</td>
<td>-1.1198</td>
<td>0.2717</td>
</tr>
<tr>
<td>HPGRS</td>
<td>2017</td>
<td>Wn</td>
<td>9.5286</td>
<td>25.1062</td>
<td>30.000</td>
<td>0.3795</td>
<td>0.7070</td>
</tr>
<tr>
<td>HPGRS</td>
<td>2017</td>
<td>WN</td>
<td>13.8000</td>
<td>25.1062</td>
<td>30.000</td>
<td>0.5497</td>
<td>0.5866</td>
</tr>
<tr>
<td>CPER</td>
<td>2018</td>
<td>Cn</td>
<td>-5.0414</td>
<td>7.8796</td>
<td>30.000</td>
<td>-0.6398</td>
<td>0.5272</td>
</tr>
<tr>
<td>CPER</td>
<td>2018</td>
<td>CN</td>
<td>1.9100</td>
<td>7.8796</td>
<td>30.000</td>
<td>0.2424</td>
<td>0.8101</td>
</tr>
<tr>
<td>CPER</td>
<td>2018</td>
<td>WC</td>
<td>6.8443</td>
<td>7.8796</td>
<td>30.000</td>
<td>0.8686</td>
<td>0.3920</td>
</tr>
<tr>
<td>CPER</td>
<td>2018</td>
<td>Wn</td>
<td>-1.1129</td>
<td>7.8796</td>
<td>30.000</td>
<td>-0.1412</td>
<td>0.8886</td>
</tr>
<tr>
<td>CPER</td>
<td>2018</td>
<td>WN</td>
<td>25.9057</td>
<td>7.8796</td>
<td>30.000</td>
<td>3.2877</td>
<td>0.0026</td>
</tr>
<tr>
<td>CPER</td>
<td>2017</td>
<td>Cn</td>
<td>-8.4429</td>
<td>35.7521</td>
<td>36.000</td>
<td>-0.2361</td>
<td>0.8147</td>
</tr>
<tr>
<td>CPER</td>
<td>2017</td>
<td>CN</td>
<td>70.2571</td>
<td>35.7521</td>
<td>36.000</td>
<td>1.9651</td>
<td>0.0572</td>
</tr>
<tr>
<td>Site</td>
<td>Year</td>
<td>Trtment</td>
<td>Estimate</td>
<td>StdError</td>
<td>df</td>
<td>t_value</td>
<td>p</td>
</tr>
<tr>
<td>------</td>
<td>------</td>
<td>---------</td>
<td>----------</td>
<td>----------</td>
<td>-------</td>
<td>---------</td>
<td>--------</td>
</tr>
<tr>
<td>CPER</td>
<td>2017</td>
<td>WC</td>
<td>13.6429</td>
<td>35.7521</td>
<td>36.0000</td>
<td>0.3816</td>
<td>0.7050</td>
</tr>
<tr>
<td>CPER</td>
<td>2017</td>
<td>Wn</td>
<td>18.0000</td>
<td>35.7521</td>
<td>36.0000</td>
<td>0.5035</td>
<td>0.6177</td>
</tr>
<tr>
<td>CPER</td>
<td>2017</td>
<td>WN</td>
<td>36.6286</td>
<td>35.7521</td>
<td>36.0000</td>
<td>1.0245</td>
<td>0.3124</td>
</tr>
</tbody>
</table>