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Wetland Biogeochemical Responses to Predicted Climate Change Scenarios

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WETLAND BIOGEOCHEMICAL RESPONSES TO PREDICTED CLIMATE CHANGE SCENARIOS

by

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ABSTRACT

Wetlands are one of the world's largest known carbon sinks while comprising only a small amount of the Earth's surface. However, the amount of carbon sequestered by wetlands is shrinking as droughts and human disturbance increases. Carbon in wetlands is stored through the contrast of decomposition and sedimentation of organic matter and absorption of CO_2 from the atmosphere by soil microbes. Understanding how changing hydrological regimes and increased wildfires will affect wetland soil and microbial processes is important in the face of predicted climate change for future wetland conservation practices. Specifically, I seek to understand the response of southeastern coastal plain wetland soils to the interaction of prescribed burns and variable hydrological conditions through the use of large-scale experimental ponds. By manipulating wetland flood duration, I was able to compare wetland soils that 1) were continuously dry, 2) were continuously flooded, or 3) were flooded and allowed to gradually recede in combination with prescribed burns prior to all flooding. I predicted that wetland soils and soil microbial biomass would respond positively to recede treatments compared to dry and flooded treatments and burned wetlands would have higher microbial biomass than not burned. Immediately following the burn and prior to flooding, I recorded a reduction in soil microbial biomass nitrogen, soil pH, and soil C:N. When assessed, soil microbial biomass carbon was found to be higher in both flooded and receding treatments compared to dry with the prescribed burn having no effect for the duration of the study. Results suggest that the prescribed burn was not intense enough to have lasting effects on wetland soils, though the addition of nutrients post-burn can take time to process through the system. Soil microbial biomass estimates were opposite to my predictions, suggesting that the amount of disturbance the soils

experience is a more important driver of microbial biomass than optimal conditions for microbes (i.e., warm/wet). The results of my thesis address knowledge gaps that will help guide future studies examining the response of wetland soils to climate change.

INDEX WORDS: Wetlands, Soil microbial biomass, Fire, Prescribed burns, Climate change, Hydrological, Drought, Coastal plains.

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DEDICATION

My thesis is dedicated to my parents Chris and Bruce who have supported me through all my crazy endeavors that lead me to this point and to Jewel, without whom, I would have never finished my degree.

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TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER 1

INTRODUCTION

Climate Change

Greenhouse gas emissions and their build up in the atmosphere have increased drastically since the beginning of the Industrial Era, which is the cause of the current and predicted rise in global temperature (Ciais et al., 2013; Collins et al., 2013; Gougoulias et al., 2014). This rise in temperature causes an increase in atmospheric water holding capacity, which allows for more intense storms to form (Karl and Knight, 1998; Trenberth, 2011). Studies have also led to a general consensus on the positive feedback scenario for future precipitation, where wet areas will become wetter and dry areas will become drier leading to increased chances of wildfires in drought-prone regions (Kirtman et al., 2013; Osborne et al., 2013). For example, the coastal southeast is predicted to have more frequent intense storms but also more consecutive days with less than 0.1 inches of rain (Kunkel et al., 2013). Therefore, it can be inferred that the southeastern region of the US will likely experience more droughts in the future interspersed with more intense flooding events. As precipitation events upstream are additive, i.e. flood events often become larger as the storm moves downstream, there is reason to suspect that river floodplains and wetlands of the southeast may be the most susceptible to these increased precipitation fluctuations. By the time the flood waters from the mountain and piedmont regions reach the coastal plains, they may have a significant disturbance effect, making the study of this region's wetlands imperative.

Wetlands and Carbon Storage

Wetlands may arguably be one of the most important ecosystems in the world, both now and in the future. Not only do wetlands provide habitats for numerous plant and animal species, they also purify water, replenish the water table, and their soils are crucial sites for carbon storage (Mitsch and Gosselink, 2007; Batzer and Baldwin, 2012). There are two mid to long-term storage routes that greenhouse gases like carbon dioxide (CO_2) can take: assimilation of carbon by trees or assimilation into soil carbon by soil

microbes and detritus accumulation (Lloyd et al., 2013). Soil carbon storage is the third largest global carbon reservoir, behind only the deep ocean and geologic storage, holding an estimated 1400-2300Pg of carbon (Mitsch and Gosselink, 2007). Wetlands globally store an estimated 20-30% of all soil carbon while only occupying 5-8% of the world's land surface (Nahlik and Fennessy, 2016). Within the U.S., inland freshwater wetlands account for 95% of wetland area within the lower 48 states, with coastal plain wetlands storing up to 198 ± 21 tons of carbon per hectare (Bridgham et al., 2006; Nahlik and Fennessy, 2016). Coastal plain wetlands store less carbon than intermountain and northern wetlands, but due to the vast number of wetlands in this region they are the second highest wetland carbon storage location within the U.S., making their study and preservation vital (Nahlik and Fennessy, 2016).

Wetlands can store more carbon than forests, plains, and other ecosystem types owing to their unique biological, physical, and chemical properties. A wetland is described as a carbon sink if more organic carbon enters the system than leaves, usually in the form of organic matter accumulating in the anaerobic layer and is considered a carbon source if more organic carbon leaves the system than is accumulated, usually through CO_2 release from respiration (Villa and Mitsch, 2015). When wetlands are carbon sources the carbon being released is CO_2 from respiration of aquatic organisms; predominantly soil microbes. Anoxic soils (i.e., soils with low or no oxygen) are common in wetlands due to standing water for much of the year. Anoxic soils often lead to a shift in the microbial community towards anaerobic microbes, which can function under these conditions by using electron acceptors other than oxygen, such as carbon dioxide (Baldwin and Mitchell, 2000; Mettrop et al., 2014). Though some organic matter break-down can occur under anaerobic conditions, lower microbial growth and activity leads to reduced decomposition of the organic matter and greater biomass accumulation on the bottom of the wetland, allowing for increased carbon storage compared to most terrestrial and aerobic areas (Villa and Mitsch, 2015).

Soil Microbial Processes

Soil processes have been linked to approximately 80% of all ecosystem services, with carbon storage perhaps being the most important (Gonzalez-Quinones et al., 2011). Soil is made up of two components: a mineral and organic non-living portion and a living portion consisting of belowground plant tissues and soil microbes. The microbial portion, which is less than 5% of all soil organic matter, is comprised of fungi and bacteria that convert the dead organic carbon in soil into bioavailable nutrients that can be readily taken up by plants (Dalal, 1998; Brookes, 2001; Gougoulias et al., 2014). Through this process of decomposition, soil microbes release carbon dioxide when oxygen is readily available as an electron acceptor and methane when oxygen is not available, making the study of microbial decomposition rates and soil conditions (i.e., soil moisture and temperature) a key area of concern for future climate change predictions since carbon dioxide and methane are the two greenhouse gases of most concern (Brookes, 2001; Mettrop et al., 2014).

There have been many different approaches to studying soil microbes and their processes, including measurements of microbial activity and biomass, soil nutrient changes, and soil gas release. There are several methods used to conduct soil microbial biomass assessments with some being tedious and expensive, such as direct microscopy and culturing, while others have been found to be more time efficient and economically feasible, such as the chloroform fumigation extraction method (Dalal, 1998). While there are pros and cons to each method, quantifying total microbial biomass rather than by taxonomic group, has been one of the most frequently used and assessed methods (Brookes, 2001; Gonzalez-Quinones et al., 2011). Having a quick and cost-effective way to measure microbial biomass allows for the rapid assessment of the health and quality of the soil, leading to improved management techniques and more accurate *in situ* studies (Dalal, 1998; Brookes, 2001). Previously, quantifying soil percent of organic carbon in the soil was the preferred approach to assessing soil health, but studies have found that changes in soil microbial biomass can respond an order of magnitude faster to treatments than soil organic carbon leading to the use of soil microbial biomass as an early indicator of soil health (Dalal,

1998; Brookes, 2001; Gonzalez-Quinones et al., 2011). Changes in soil microbial biomass allow for the early predictions of soil carbon storage, nutrient processing, and greenhouse gas flux.

Fire in Wetlands

Though seemingly counterintuitive, fires regularly occur in wetlands due to both human and natural causes (Zhao et al., 2012; Medvedeff et al., 2013; Osborne et al., 2013). An example is the 2007 Okefenokee blaze that lasted for three months and burned an estimated 330,000 acres of wetlands (Beganyi and Batzer, 2011). There are various reasons for the use of controlled burns in wetlands, but this practice is generally applied to prevent encroaching vegetation such as trees in grassy wetlands and weeds in cultivated wetlands (Osborne et al., 2013; Sutfin et al., 2016). Prescribed wetland burns are also used to maintain native vegetation though little scientific evidence confirms the efficacy of this practice (Osborne et al., 2013). Wetland wildfires often burn only the emergent vegetation but as summer droughts persist, there is a higher likelihood of vegetation burning to soil levels (Venne et al., 2016). When a fire is hot enough and reaches the soil layer, it will combust both above-ground vegetation and soil organic matter, may remove bio-available organic carbon from the ecosystem through nutrient volatilization (Holden and Treseder, 2013). The role of fire in wetlands has been vastly understudied; specifically, its effects on microbial processes (Osborne et al., 2013).

Response to Disturbances

The effect of various hydrological disturbances on soil microbial biomass has been divided in the literature, with studies finding both increases and decreases in soil microbial biomass post-disturbance. Most studies carried out on soil microbial response to drought have seen decreased microbial biomass as a result, though the magnitude of the reduction is highly variable (Gonzalez-Quinones et al., 2011; Wu et al., 2015; Urbanová and Bárta, 2016; Ren et al., 2017). The decrease in soil microbial biomass due to drought has largely been attributed to decreased soil organic carbon substrate quality, reduced soil connectivity providing less microbial habitat, increased temperature stress, and death by desiccation of

microbes (Baldwin and Mitchell, 2000; Holden and Treseder, 2013; Ren et al., 2017; Urbanová et al., 2018). Conversely, when a dry or drying wetland is flooded, many studies have found increased microbial biomass due to a surge in available nutrients and carbon from increased litter decomposition (Baldwin and Mitchell, 2000; Mamilov and Dilly, 2002; Weaver et al., 2012; Moche et al., 2015). However, studies have found that during desiccation, microbes will store extra osmolytes to increase cell water retention and upon rewetting will lyse as water potentials change quicker than cells can regulate, though some groups such as gram-positive bacteria are more resistant to this due to the presence of thick cell walls (Baldwin and Mitchell, 2000; Schimel et al., 2007). Repeated flooding and drying experiments have found a flush of nutrients in flood waters (i.e. from nutrient rich flood waters or lysed microbial cells) followed by optimal moist aerobic conditions as the drying period starts, both of which lead to increased microbial biomass as long as neither the flood nor the drought are too extensive (Baldwin and Mitchell, 2000; Mamilov and Dilly, 2002; Gonzalez-Quinones et al., 2011; Jiang et al., 2013; Mettrop et al., 2014).

Fire associated wetland disturbances are predicted to increase which could either positively or negatively affect wetland microbes (Dooley and Treseder, 2012; Osborne et al., 2013; Sutfin et al., 2016). Though several studies have been conducted on microbial response to fire, there are not enough to conclusively determine if fires have a positive, negative, or neutral effect. When fires occur in wetlands that have high moisture and low fuel loading, effects on microbial biomass have been found to be negligible and attributed to low heat levels reaching the soil (Kara and Bolat, 2009; Dooley and Treseder, 2012; Medvedeff et al., 2013). However, Zhao et al. (2012) conducted a low intensity wetland burn and found that soil microbial biomass increased for one-year post-burn but then tapered back to non-burned levels. Differences in microbial response to fire can be attributed to several factors. If the fire was intense and burned soil organic matter, there would be a large release of nutrients which could be readily used by colonizing and surviving microbes, leading to an increase in biomass (Kara and Bolat, 2009; Medvedeff et al., 2013; Venne et al., 2016). However, if few microbes survive the fire, or none are in close proximity

to recolonize, the fire will lead to a decrease in microbial biomass (Palese et al., 2004; Dooley and Treseder, 2012; Holden and Treseder, 2013).

Though many aspects of soil microbial biomass are under studied, one research area that is most lacking is the response of microbial biomass to fire in wetlands (Osborne et al., 2013). As frequency of summer droughts and severe storms are predicted to escalate, wildfires are also predicted to increase leading to a surge in the frequency and intensity of disturbances over many wetland areas (Kirtman et al., 2013; Osborne et al., 2013). Drought stricken wetlands are far more likely to experience higher intensity fires compared to wetlands still holding water, and therefore soil microbial impacts are predicted to be more severe in response (Urbanová et al., 2018). Even lower intensity fires that occur during droughts may lead to decreased microbial biomass due to increased moisture-stress and temperature-stress post fire (Holden and Treseder, 2013). A study by Kara and Bolat (2009) found that high moisture conditions after fire disturbance lead to increased microbial recovery, though fires occurring during drought conditions will have low humidity compared to non-drought periods. Wetland fires that remove all or most of the standing vegetation and litter have been found to intensify soil moisture loss and soil temperatures, both of which could lead to decreased soil microbial biomass (Holden and Treseder, 2013).

Study Objectives

The objective of this study was to assess the effects of wetland hydrologic fluctuations (i.e., flooding and drying), fire, and their interaction on soil microbial biomass in experimental wetlands to further increase knowledge of the carbon storage processes occurring in these ecosystems. Through this, my project also aimed to fill the knowledge gap in wetland soil microbial biomass response to changes in hydrological regimes and fire *in situ* when many studies are being conducted *in vitro*. Not only do I hope to determine microbial response to these changes but also the soil properties responsible for inciting these changes. The information gathered from this project will help to better inform wetland stakeholders using prescribed burns to control encroaching and undesirable vegetation or employing techniques to improve

water retention or limit water losses in wetlands. Since soil microbes predominately account for large amounts of wetland gas emissions, studying the effects of changing hydrological regimes on soil microbes should help climate modelers to be better able to predict carbon budgets for future periods affected by climate change.

Predictions

Predictions were made based on current literature addressing microbial response to fire and flooding treatments (Table 1). For each treatment, the hypothesis labeled **H_{A1}** is considered the more likely response of soil microbes to treatment conditions.

Within the hydrological experimental setting (dry vs. flooded vs. receding), I predict that:

H_0: Soil microbial biomass will not differ between hydrological treatments. The dry aerobic, flooded anaerobic, and receding alternating anaerobic and aerobic treatments will all have equal amounts of soil microbial biomass.

H_{A1}: Soil microbial biomass will differ between hydrological treatments, with the receding treatment having the highest amount of soil microbial biomass. The dry treatment will have the lowest amount of soil microbial biomass due to low moisture limiting microbe growth. The flood treatment will have more soil microbial biomass than the dry treatment but will have a modest amount due to only some microbes being anaerobic tolerant. The alternating aerobic and anaerobic conditions will have the highest amount of soil microbial biomass due to more frequent aerobic moist conditions allowing for microbial proliferation and short anaerobic periods preventing complete die off of aerobic microbes.

H_{A2}: Soil microbial biomass will differ between hydrological treatments, with the flood treatment having the highest amount of soil microbial biomass. The dry treatment will have the lowest amount of soil microbial biomass due to low moisture limiting microbe growth. The flood treatment will have more soil microbial biomass than the dry treatment due to optimal moisture levels and stable

hydrological conditions. The recede treatment will have a low amount of microbial biomass due to high stress on microbes from rapidly changing conditions that lead to high amounts of microbial die off.

Within the fire experimental setting (burned vs. not burned), I predict that:

H_0: Wetlands that have experienced a prescribed burn will exhibit no difference in soil microbial biomass compared to those that have not been burned.

H_{A1}: Wetlands that have experienced a prescribed burn will have more soil microbial biomass than those that have not been burned. This is predicted due to the surge in nutrient and organic carbon following the burn which will allow the microbes to readily absorb these nutrients and therefore proliferate.

H_{A2}: Wetlands that have experienced prescribed burn will have less soil microbial biomass than those that have not been burned. This is predicted as a result of the burn killing a large portion of the soil microbes in the shallow soils of the experimental wetlands. Fire also alters the vegetation structure and soil characteristics of an area; which soil microbes are reliant on. This may affect their ability to thrive in an area where the typical conditions they are used to are no longer present.

For the interaction of the two treatments, I predict that:

H_0: The interaction between the fire and hydrological treatments will have no effects on soil microbial biomass. The interaction will not be different than the treatments applied separately.

H_{A1}: The interaction between the fire and hydrological treatments will have a positive effect on soil microbial biomass. The burned ponds will have higher nutrient availability after the burn and this, combined with increased soil moisture due to the flood and recede treatments, will provide suitable habitat for microbial proliferation.

H_{A2}: The interaction between the fire and hydrological treatments will have a negative effect on soil microbial biomass. The burned ponds that experience flood or recede treatments will be further stressed than either burn or flood alone and will experience microbial die-off and overall reduction in microbial biomass.

Table 1. Predictions of Microbial Biomass Response by disturbance event supported by the literature.

Parameter	Microbial Biomass Response (1)	Citation	Microbial Biomass Response (2)	Citation	Microbial Biomass Response (3)	Citation
Fire	**Decreased Microbial Biomass:** Microbial biomass may be reduced due to decreased litter and nutrient availability or death of microbes due to high temperatures experienced during the fire.	Brookes, 2001; Zhao et al., 2012; Holden and Treseder, 2013	**Temporarily Increased Microbial Biomass:** Lower intensity burns do not destroy organic matter but leave behind a nutrient rich char that will temporarily promote microbial growth.	Zhao et al., 2012	**No Effect:** If the fire doesn't burn hot enough or passes over soil quickly, it will have little to no effect on microbial biomass, however any amendments to the soil, like ash, may have an impact.	Kara and Bolat, 2009; Medvedeff et al., 2013
Receding Water	**Increased Microbial Biomass**: Wetting and drying cycles allow for more litter decomposition and nutrient release and puts the microbes in more direct contact with these nutrients. Also, upon receding, microbes now have optimal moisture with plenty of O2 and the stress of flooding has been removed.	Baldwin and Mitchell, 2000; Mamilov and Dilly; 2002; Mettrop et al., 2014; Moche et al., 2015; Wu et al., 2015; Sutfin et al., 2016	**Decreased Microbial Biomass:** As water recedes, microbes may face competition for nutrients from plants. Wetland drainage may also lead to accelerated litter decomposition which limits available organic matter available to microbes.	Ladd et al., 1995; Baldwin and Mitchell, 2000; Schimel et al., 2007; Moche et al., 2015; Urbanová and Bárta, 2016; Urbanová et al., 2018		
Dry	**Low Microbial Biomass:** No or low moisture conditions are suboptimal for microbial growth and lead to less biomass production. Persistent drought is stressful to microbes.	Schimel et al., 2007; Mettrop et al., 2014	**High Microbial Biomass:** This is only in the specific case of our treatments since the dry treatment will be the least disturbed.	Urbanová et al., 2018		
Flood	**Decreased Microbial Biomass:** As floods persist, soil conditions become anaerobic leading to community shift or microbial die-off.	Baldwin and Mitchell, 2000; Gonzalez-Quinones et al., 2011; Nahlik, 2016; Sutfin et al., 2016	**Increased Microbial Biomass:** Over time, the microbial community will shift to anaerobic obligate microbes.	Baldwin and Mitchell, 2000		
pH	**Positively Correlated:** Decreased pH changes community composition leading to decreased microbial biomass and some microbes don't function well at low pH.	Baum et al., 2002; Gonzalez-Quinones et al., 2011; Urbanová and Bárta, 2016; Urbanová et al., 2018	**Negatively Correlated:** Several studies have found a large proportion of microbes do not function well above a pH of 7.5 and many have adapted to the acidity of bogs and swamps.	Dalal, 1998; Ma et al., 2017; Weaver et al., 2012; Urbanová et al., 2018		

Table 1. Continued

Parameter	Microbial Biomass Response (1)	Citation	Microbial Biomass Response (2)	Citation
Disturbance	**Negatively Correlated:** More disturbed ecosystems have lower microbial biomass fluctuation but also have much lower biomass values.	Schimel et al.; 2007; Holden and Treseder, 2013; Jiang et al.; 2013; Nahlik; 2016; Urbanová and Bárta, 2016; Urbanová et al., 2018	**Intermediate Disturbance Hypothesis:** Initial disturbances often lead to increased availability of nutrients for microbes, but prolonged disturbances lead to depleted resources and increasing stress.	Baldwin and Mitchell, 2000; Weaver et al., 2012; Wu et al., 2015; Urbanová et al., 2018
Temperature	**Positively Correlated**: Warmer temperatures allow for increased microbial activity and biomass growth.	Baum et al., 2002; Devi and Yadava, 2006; Gougoulias et al., 2014; Nahlik; 2016	**Mesophilic Range:** Very hot temperatures lead to microbial stress and death while very low temperatures can cause stupor and death.	Gonzalez-Quinones et al., 2011; Jiang et al., 2013
Variability	**Fluctuate Together:** Regardless of treatment, microbial biomass fluctuates in similar trends though not necessarily in the same proportion.	Brooks et al., 1998; Tscherko and Kandeler, 1999; Ruan, 2004; Jiang et al., 2013		
Time of Year	**Varies by Season:** Microbial biomass fluctuates throughout the season, likely tied to temperature, moisture, soil moisture, and litter decomposition.	Baum et al., 2002; Gonzalez-Quinones et al., 2011; Moche et al., 2015		
Soil Moisture	**Mesophilic Range:** Low and very high soil moisture cause microbial stress.	Gonzalez-Quinones et al., 2011; Jiang et al., 2013; Ren et al., 2017		
Soil Organic Carbon	**Positively Correlated:** Soil organic carbon provides nutrients and substrate for soil microbes.	Sparling, 1992; Brookes, 2001; Zhao et al., 2012; Urbanová and Bárta, 2016; Urbanová et al., 2018		
Vegetation	**Positively Correlated:** Increased wetland vegetation leads to increased litter and root exudates entering the system providing more nutrients.	Medvedeff et al., 2013; Ma et al., 2017		

CHAPTER 2

MATERIALS AND METHODS

Field Site

My study was conducted in twelve ponds at the former Bo Ginn National Fish Hatchery (USFWS), near Millen, GA, which were used as experimental wetlands. It is important to note that at the start of the project, all of the experimental wetlands were dry and had been unused for more than ten years. Since I wanted to quantify the effects of hydrologic changes and fire on wetland soil microbial biomass, I assigned each of the twelve wetlands one of three hydrological treatments and one of two fire treatments giving me a total of six treatments of two experimental wetlands each (n=2). The hydrological treatments were as follows: 1) continuously flooded, simulating a wetland having anaerobic conditions for much of the year (hereafter flooded), 2) continuously dry, simulating a fully aerobic wetland experiencing an extended drought (hereafter dry), and 3) alternated flooding and drying, simulating a wetland with alternating anaerobic and aerobic conditions (hereafter receding). These three hydrological treatments were also combined with either a fire treatment: Burned or Not Burned (Figure 1).

Prior to sample collection each wetland was divided into four quarters and sampling points (i.e., locations) were randomly selected within each quarter. This assured that soil sampling would be random, but in the same area each sampling interval to reduce confounding factors often associated with the heterogeneous nature of soils (Lloyd et al., 2013). Metal poles were driven into the soil at each point to denote the sampling location and to allow for attachment of sampling devices (e.g., temperature loggers). Soil samples were collected within a one-meter radius of the sampling point, using a handheld 1" soil sampler (LaMotte Company®, Chestertown, MD) in dry or drying wetlands and a 5' piece of 1" PVC pole with a 3/4" dowel rod inside to push the collected sample out in flooded wetlands. All soil samples collected were approximately 100g and consisted of several shallow samples combined to achieve this weight, with four soil samples per pond and twelve ponds for a grand total of 48 samples collected at each

sampling period. Soil cores were not collected deeper than 10cm due to the compacted earth and clay that functions as a liner for water retention, which was an artificial addition to the landscape and therefore would not be an accurate representation of the soil profile. An initial sampling set (i.e., pre-treatment) was collected prior to the start of the study to be used as the baseline for all subsequent sampling and to determine any outliers in soil characteristics between the wetlands.

On the same day that the initial baseline soil samples were collected, a prescribed burn of six of the twelve ponds was conducted with the assistance of the Jenkins-Screven County unit of the Georgia Forestry Commission. Once the fire residence time had ended and soil temperatures were back to ambient, soil samples were collected in the same manner as before to assess for any immediate post fire effects on the soil microbial biomass. After collection of soil samples was complete, eight of the wetlands were inundated with water from Magnolia Spring in the adjacent Magnolia Springs State Park. Four wetlands were filled over the span of a day and another four were filled the following day, due to the flow limitation of the pumps to fill all eight at once. Once full, all eight wetlands received a continuous input of water for 14 days to make up for water lost to evaporation. Four additional wetlands (two burned, two unburned) remained unfilled to serve as a control for seasonal changes in soil microbial biomass. After 14 days, water input to four of the eight inundated wetlands was discontinued to commence the drying period of the Recede treatment.

Sample Collection and Processing

Soil samples continued to be collected twice per week for two weeks after the prescribed burn to capture any initial peaks in soil microbial biomass that might occur due to fire induced nutrient and soil organic carbon release. After the first two weeks, soil samples were collected once a week for the next six weeks. At the end of this two-month collection period, the Recede treatment wetlands were dry and had been so for approximately 14 days, presumably allowing for the full range of aerobic and anaerobic conditions. Along with soil sample collection, HOBO loggers (Onset Corporation®, Bourne, MA)

continuously recorded water and air (in dry or drying wetlands) temperatures at 15-minute intervals. This allowed me to asses if any wetlands had significantly different temperatures throughout the experiment that might affect soil microbial biomass.

Collected soil samples were stored on ice until reaching the laboratory, where they were stored at 4°C until processed. Samples were processed as soon as possible and were kept on ice when not directly being processed to match samples that could not all be processed in one day. Each soil sample was weighed in an aluminum tin, sieved through a 2mm sieve to remove coarse debris (i.e., materials over 2mm, organic and inorganic, were considered coarse debris and discarded), and reweighed to assess how much of the sample was coarse debris. If samples were excessively waterlogged, the water was carefully poured off the top of the sample prior to sieving. Once sieved and weighed, the sample was divided into two subsamples. The first, consisting of roughly 45g of field moist soil, was returned to the original sample cup to await soil microbial processing. The second subsample, made up of the remaining soil, was returned to the aluminum tin, weighed, and dried at 55℃ for 48 hours. The second subsample was used to assess soil moisture content, organic matter content, pH, total carbon, and total nitrogen (i.e., soil characteristics).

Soil Microbial Biomass: Fumigation-Extraction

The following procedure was modified from Brookes et al., 1985 and the Hofmockel Laboratory (2011) chloroform fumigation direct extraction protocol for soil microbial biomass carbon and soil microbial biomass nitrogen. The first soil subsamples were weighed into two parts and used for soil microbial biomass assessment through direct fumigation-extraction. A 12g oven dried equivalent (ODE) of first part of the subsample was placed into a labeled 50mL conical tube, capped, and stored in the dark at room temperature for the duration of the fumigation period. Another 12g ODE of soil was placed into a 50mL glass beaker. The beakers were then placed in a desiccator (Labconco Corporation™, Kansas City, MO), located in a fume hood, along with moist paper towels to prevent the samples from drying out

during fumigation. Once all 48 samples for a single sampling period were in the desiccator, an empty 50mL beaker was added as a control.

In order to estimate the amount of microbial biomass within a soil sample, all the microbe cells must be killed to release their organic carbon. To achieve this, soil samples were fumigated with ethanol-free chloroform. This released carbon was compared to the corresponding subsamples that were not fumigated to determine how much microbial biomass was present (Zhao et al., 2012). A 40mL addition of chloroform was poured into a 100mL beaker with one spoonful of boiling chips and the desiccator was evacuated, causing the chloroform to boil. Once it had boiled, the vacuum was released into the fume hood and this step was repeated three times. During the final repetition, the chloroform was boiled for two minutes and the vacuum was not vented. A dark cover was placed over the desiccator to prevent breakdown of the chloroform (Hofmockel, 2011) and the samples were fumigated for 48-72 hours. Once fumigation was completed, the vacuum was released into the fume hood and the chloroform beaker removed. The desiccator was vacuumed for three minutes then released into the hood; this step was repeated eight times to ensure that all chloroform was removed from the samples and desiccator.

Using 0.5M K_2SO_4, the fumigated samples were rinsed into a labeled 50mL conical tube and filled to the 50mL mark with 0.5M K_2SO_4. All of the non-fumigated samples, previously put into conical tubes, were also filled to the 50mL mark with 0.5M K_2SO_4. All samples were then placed on a shaker for 1 hour at 200rpm at room temperature. The addition of 0.5M K_2SO_4 extracts carbon from the soil so that it can be read on a total organic carbon (TOC, Shimadzu Corporation) analyzer. After extraction, samples were filtered through glass filters (Whatman No. 42) that were pre-leached with 0.5M K_2SO_4. While filtering was in process, the conical tubes were rinsed so the filtrate could be placed back in the same tube after completion. Samples were then frozen until carbon content could be analyzed.

Soil Microbial Biomass: Carbon and Nitrogen

Owing to various densities and moisture retention properties of the soils, not all samples had 40mL of extractant to be analyzed. Samples were diluted into a 10:1 deionized water:sample ratio to allow the machine to register more accurate results. Once samples were thawed and diluted appropriately, they were transferred to 40mL volatile organic analysis (VOA) vials compatible with the TOC analyzer. Once samples were run through the TOC analyzer, results were translated into amount of soil microbial biomass using the following formula:

Microbial Biomass Carbon = $(OC_F - OC_{NF})/0.38$

Where:

OC_F = the amount of organic carbon from the fumigated subsample

OC_{NF} = the amount of organic carbon from the non-fumigated subsample

0.38 = the efficiency of extraction constant (Mclean, 1982; Vance et al, 1987; Joergensen, 1996)

Microbial Biomass Nitrogen = $(ON_F - ON_{NF})/0.54$

Where:

ON_F = the amount of nitrogen from the fumigated subsample

ON_{NF} = the amount of nitrogen from the non-fumigated subsample

0.54 = the efficiency of extraction constant (Brooks et al., 1998)

Soil Physical and Chemical Characteristics

The soil moisture procedure was adapted from the Kellogg Biological Station gravimetric soil moisture protocol. Soil moisture was calculated using the second subsample of soils. After soils had dried

for 48 hours at 55°C, samples were cooled to a constant weight and this was recorded. Percent soil moisture was calculated using the wet and dry weight with the following formula:

%Soil moisture = [(fresh weight) - (dry weight) / (dry weight)]*100

Soil moisture content was calculated since soil microbe growth and proliferation is closely tied to the amount of moisture available.

The soil pH procedure was adapted from the Kellogg Biological Station soil pH protocol and Mclean (1982). Soil samples were dried at 55°C for 48 hours then allowed to cool to a constant weight. Once cooled, 5g of soil were added to a 20mL vial along with 10mL of deionized water. The sample was capped, shaken, then uncapped and allowed to rest for 30 minutes. Using a pH meter, samples were gently stirred until the pH reading stabilized; this value was recorded along with the temperature. The pH meter was checked against calibration solution every ten samples to ensure accuracy and re-calibrated if not within 0.05 of the solutions true value.

The soil organic matter procedure was adapted from Martínez et al. (2018). From the dry soil samples, 5g of soil were placed into a small, pre-weighed tin. These samples were then ashed in a muffle furnace for 4 hours at 550°C. After cooling to a constant weight, calcinate samples were weighed. Using the loss of mass on ignition, we used the following formula to calculate organic matter found in each soil sample:

OM (%) = [(Dry Weight - Calcinate Weight) / (Dry Weight)]*100

Organic matter provides soil microbes with an energy source, therefore more organic matter in the soil often equates to more soil microbial biomass (Martínez et al., 2018).

Soil organic carbon was estimated from %OM using a conversion factor of 2 according to Mitsch and Gosselink (2007) and Pribyl (2010).

$$\%OrgC = \%OM/2$$

The soil total C:N analysis procedure was adapted from the Kellogg Biological Station Costech Elemental Combustion System protocol (VanderWulp, 2004) and the Costech support system. Approximately 3-5g of dried soil sample were placed into 20mL vials. Mixing balls were added to the sample and the capped vial was shaken for 10 minutes in a ball mill (SPEX Sample Prep 8000Mixer/Mill). After samples were thoroughly homogenized, 13mg of sample were placed into tins and folded to ensure no material is lost. Once all samples were tinned, they were run through a Costech elemental combustion system to determine total carbon and nitrogen amounts within each soil sample.

Statistical Analysis

All analyses were performed in R (R Development Core Team 2017), with all figures generated using the ggplot2 (Wickham, 2016) and cowplot (Wilke, 2019) packages, using means and standard errors. Analysis was started by comparing mean values from before the burn and immediately post-burn using a paired two-tailed t-test for the following soil parameters: microbial biomass carbon, microbial biomass nitrogen, soil moisture percent, soil organic carbon percent, soil pH, and soil total C:N. Normality and equal variance were assessed using the Shapiro-Wilk test (shapiro.test function) and Levene test (leveneTest function), respectively. Differences in these parameters means were visualized in boxplots. Next, differences in means for soil microbial biomass carbon, microbial biomass nitrogen, soil moisture percent, soil organic carbon percent, soil pH, and temperature between the fire treatments, hydrological treatments, and through time were tested with a repeated measures ANOVA using the lme function in the nlme package (Pinheiro et al. 2018) to fit linear mixed effects models including both treatments as fixed effects and pond as a random effect. To find the models that best explained variation within soil microbial biomass carbon and soil microbial biomass nitrogen, correlations between all soil

characteristic variables for sample days 16-58 were performed using the cor function with Pearson method and the rcorr function from the Hmisc package (Harrell, 2019). Samples from days 0-15 were not included in model building since the flood treatment and recede treatment were not different at this point. Soil characteristic variables with correlation r values > 0.7 were not used in subsequent model building. Model selection was performed with a multiple regression analysis of all non-collinear soil characteristic variables using the aictab function for small sample sizes in the AICcmodavg package (Mazerolle, 2019) and the lowest AICc value model was selected as the most explanatory model. Using the best fit model for both microbial biomass carbon and microbial biomass nitrogen, t-tests were run using the lme function to determine differences between mean values of microbial biomass carbon and microbial biomass nitrogen for all treatments compared to the control (not burned dry) while accounting for variation due to soil characteristics. Variance between fixed and random effects was assessed using the get_variance function from the Insight package (Lüdecke et al., 2019).

After selecting the best models, the median values were found for the model soil characteristic variables (i.e. soil organic carbon, soil pH, soil moisture, and temperature for microbial biomass carbon and soil pH; soil organic carbon for microbial biomass nitrogen) and these values were used to predict the effect of treatments on microbial biomass carbon and microbial biomass nitrogen responses if the soil characteristic variables were held constant. This was accomplished using the predict function from the doBy package (Højsgaard and Halekoh, 2018), which resulted in a single mean predicted value for each of the six treatments for both microbial biomass carbon and microbial biomass nitrogen and was then used to create a bar plot to express the data.

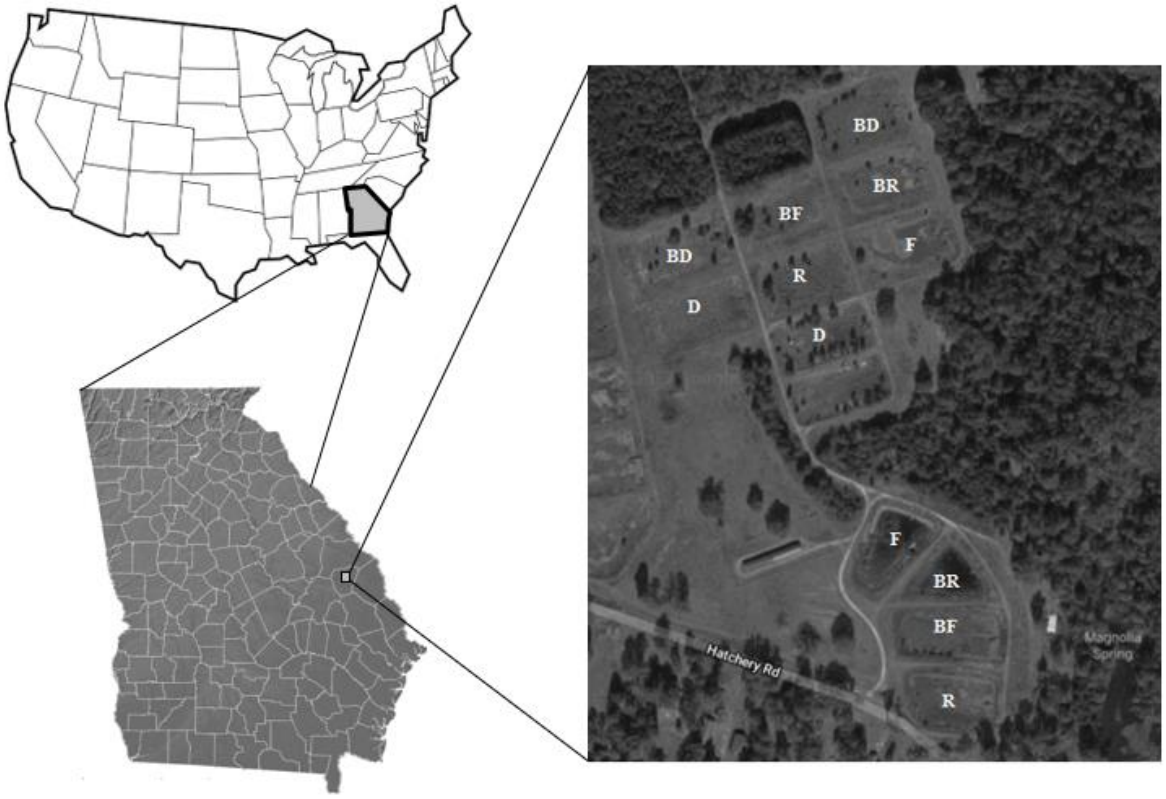

Figure 1. Reference map showing location of Georgia within the USA along with a reference map of Georgia showing approximate location of the Bo Ginn Hatchery in Screven County. Aerial view of Bo Ginn Hatchery, including water source Magnolia Springs. 12 experimental wetlands were used. Wetlands with a **B** were burnt prior to flooding treatment. Flooding treatments were as follows: **D** dry treatment, **R** wetland flooded and allowed to recede, **F** wetland continuously flooded.

CHAPTER 3

RESULTS

Soil and Microbial Response to Disturbances

Mean soil microbial biomass carbon did not differ from before or immediately after the fire treatment (t_5 = -1.462, p = 0.204). However, mean soil microbial biomass nitrogen experienced a significant decrease following the fire treatment (t_5 = 2.827, p = 0.037; Figure 2A, B). Other significant outcomes immediately post burn included an increase in soil C:N (t_5 = -2.813, p = 0.037) and a decrease in pH (t_5 = 3.782, p = 0.013; Figure 2D, F). Soil moisture and soil organic carbon were not significantly impacted by the fire treatment (t_5 = -1.664, p = 0.157; t_5 = -0.508, p = 0.633, respectively; Figure 2C, E; Appendix A). No significant differences were found for means for microbial biomass carbon and microbial biomass in the fire treatments, hydrological treatments, or the interaction of the treatments, over time (Figure 3A, B; Figure 4A, B; Appendix B). The soil characteristic variables, composed of the soil physical and chemical characteristics, soil moisture, soil pH, and soil organic carbon percent, also did not significantly differ across time and treatment (Figure 3C-E; Figure 4C-E; Appendix B). Temperature was not affected by treatment and there was a steady mean temperature increase throughout the study as mean daily temperature increased, with a large increase between days 44 and 58 of the study (Figure 3F; 4F). Although not significantly different, all of the soil physical and chemical characteristics and microbial biomass elements that were measured had higher mean values in the burned wetlands compared to the unburned, with those differences becoming larger as the study progressed (Figure 4; Appendix B). The interaction effect between the fire and hydrological treatments did not yield significant responses for microbial biomass carbon or microbial biomass nitrogen (Appendix B).

Since trends in microbial biomass carbon and soil microbial biomass nitrogen, had some similarities in peaks and depressions in the line graphs (Figures 3 & 4), model selection was used to help determine which of the soil characteristics might be driving microbial biomass variability. Model

selection analyses determined that changes in microbial biomass carbon were best explained by a model that included soil moisture, soil organic carbon, soil pH, and temperature, while microbial biomass nitrogen was best explained by the predictor variables soil organic carbon and soil pH (Table 2). Model selection multicollinearity was avoided by using parameters that had Pearson correlation r values > 0.7 (Table 3). Using the soil characteristic median values, predicted mean responses were calculated for each treatment for both microbial biomass carbon and microbial biomass nitrogen (Figures 5 and 6). When t-tests were performed on the selected models to account for variation due to soil characteristics, soil microbial biomass carbon had a negative response in both the flooding and recede treatments compared to the dry treatment (t_6 = -3.67, p = 0.011; t_6 = -2.98, p = 0.025, respectively; Figure 5; Table 4). While soil microbial biomass nitrogen showed no significant differences between hydrological treatments (flood: t_6 = -0.783, p = 0.464; recede: t_6 = 0.016, p = 0.987), there was a general trend of decreased microbial biomass with increased flood duration (Figure 6; Table 4). Using soil characteristic median values, neither soil microbial biomass carbon nor soil microbial biomass nitrogen, were significantly different between fire treatments outside the first day of burning (Figures 5 and 6; Table 4), however fixed effects were found to account for a substantial portion of the variance compared to the random effect of ponds (Table 5). Though the microbial biomass nitrogen did not have a significant response to the burn treatments, there is a trend towards lower microbial biomass in all burned treatments compared to unburned when accounting for soil organic carbon and pH (Figure 6).

Soil Microbial Biomass Variability

Day to day microbial biomass, independent of treatment, varied much more than expected and not evenly across ponds (Figures 7 & 8, Appendix C). Within the dry treatments alone, microbial biomass carbon ranged from less than 300 mg/kg to more than 1,000 mg/kg within a single pond over the course of the study (Figure 7 B). Large mean differences between ponds in a single treatment were also observed, which accounts for the large variance see in the mean estimates for treatments (Figure 3A, B; Figure 4A, B; Figure 7B, F; Figure 8B, F). Although there was a large amount of variation seen between

ponds as a random effect (microbial biomass carbon = 2,530.61; microbial biomass nitrogen = 202.78), the amount of variation due to fixed effects was substantially more (microbial biomass carbon = 35,283.84; microbial biomass nitrogen = 800.11; Table 5).

Table 2. Model selection using AICc for small sample size. Top two models and all null models shown for comparison.

Only the top model for both microbial biomass carbon and microbial biomass nitrogen were selected and used (bold).

	DF	AICc	Δ AICc	AICcWt	Residuals
Microbial Biomass Carbon					
Temperature*Soil Moisture*Soil Organic Carbon*pH	**12**	**674.47**	**0**	**0.44**	**-321.87**
Temperature*Soil Moisture*pH*Total Carbon	12	675.58	1.20	0.24	-320.48
Cmic~Fire*Hydro	8	743.52	69.15	0	-332.47
Cmic~Hydro	5	773.01	98.64	0	-333.21
Cmic~Fire	4	782.42	108.04	0	-338.24
Cmic	3	791.67	117.29	0	-340.73
Microbial Biomass Nitrogen					
Soil Organic Carbon*pH	**10**	**523.09**	**0**	**0.28**	**-249.30**
Soil Moisture*Soil Organic Carbon*pH	11	523.30	0.21	0.25	-247.90
Nmic~Fire*Hydro	9	561.13	38.04	0	-269.77
Nmic~Hydro	5	579.90	56.81	0	-284.39
Nmic~Fire	4	583.93	60.84	0	-287.60
Nmic	3	590.66	67.58	0	-292.12

Table 3. Pearson correlation matrix for predictor variables, with r values on the top half of the table and p-values below. Multicollinearity was considered for values of r > 0.7 and these were not used for model building.

	Soil Moisture (%)	Soil Organic Carbon (%)	Temperature (°C)	pH	Total Nitrogen (%)	Total Carbon (%)	C:N (%)
Soil Moisture (%)	--	0.545	-0.194	0.575	0.670	0.648	-0.376
Soil Organic Carbon (%)		--	-0.171	0.482	**0.848**	**0.896**	-0.394
Temperature (°C)			--	-0.086	-0.086	-0.180	0.063
pH				--	0.423	0.482	-0.342
Total Nitrogen (%)					--	**0.929**	-0.531
Total Carbon (%)						--	-0.423
C:N (%)							--

Table 4. Summary statistics for microbial biomass carbon and microbial biomass nitrogen lowest AICc linear mixed models. Intercept is the control treatment (Dry.Not Burned) to which all other treatments are compared. $P < 0.05$ considered significant and denoted with *.

	Effect Value	Std Error	DF	t-value	p-value
Microbial Biomass Carbon					
(Intercept)	-547.720	366.251	44	-1.375	0.142
Control*Flood	-357.271	97.471	6	-3.665	0.011*
Control*Recede	-279.525	93.677	6	-2.984	0.025*
Control*Burned	15.356	69.752	6	0.220	0.833
Soil Moisture	6.304	2.225	44	2.833	0.007*
Soil Organic Carbon	75.658	23.899	44	3.166	0.003*
pH	85.454	56.369	44	1.516	0.137
Temperature	0.017	2.354	44	0.007	0.994
Control*Flood.Burned	36.819	100.427	6	0.367	0.727
Control*Recede.Burned	-41.555	97.513	6	-0.426	0.685
Microbial Biomass Nitrogen					
(Intercept)	-25.035	82.035	46	-0.305	0.762
Control*Flood	-14.804	18.918	6	-0.783	0.464
Control*Recede	0.310	18.800	6	0.016	0.987
Control*Burned	22.012	17.720	6	1.242	0.261
pH	1.421	13.302	46	0.107	0.915
Soil Organic Carbon	22.354	3.676	46	6.081	< 0.001*
Control*Flood.Burned	-14.373	25.561	6	-0.562	0.594
Control*Recede.Burned	-14.689	24.970	6	-0.588	0.578

Table 5. Amount of variance observed from fixed and random effects. Calculated from the lowest AICc linear mixed models for microbial biomass carbon and microbial biomass nitrogen. The fixed effects include soil moisture, soil organic carbon, soil pH, and temperature for soil microbial biomass carbon and include soil organic carbon and soil pH for soil microbial biomass nitrogen. The random effect is the effect that ponds might have on soil microbial biomass.

Variance Component	Value
Microbial Biomass Carbon	
Fixed Effects	35283.84
Random Effects	2530.61
Residual	10733.87
Microbial Biomass Nitrogen	
Fixed Effects	800.11
Random Effects	202.78
Residual	526.91

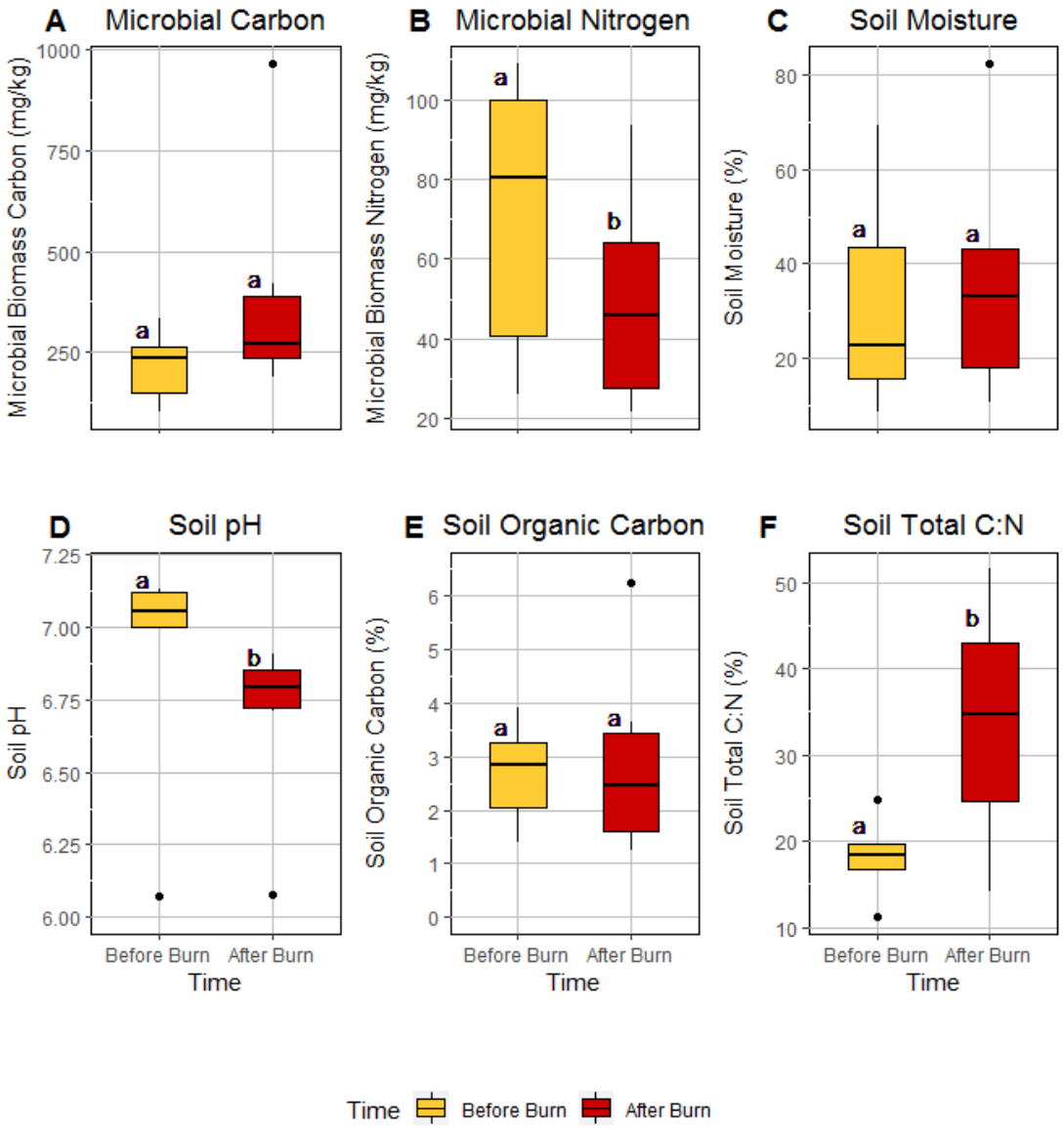

Figure 2. Shows response of: A) soil microbial biomass carbon (mg/kg), B) soil microbial biomass nitrogen (mg/kg), C) soil moisture (%), D) soil pH, E) soil organic carbon (%), and F) soil Carbon to Nitrogen ratio (%) to the prescribed burn. Samples were collected directly before and after the burn on the same day. The line within the box denotes the response median, the box denotes the upper third quartile and the lower first quartile, and the lines extending from either end denote the range of the response variable with the exception of outliers. Before the burn is represented by the yellow boxes whereas after the burn is represented by the red boxes. Different letters denote significant differences in a paired t-test ($p < 0.05$).

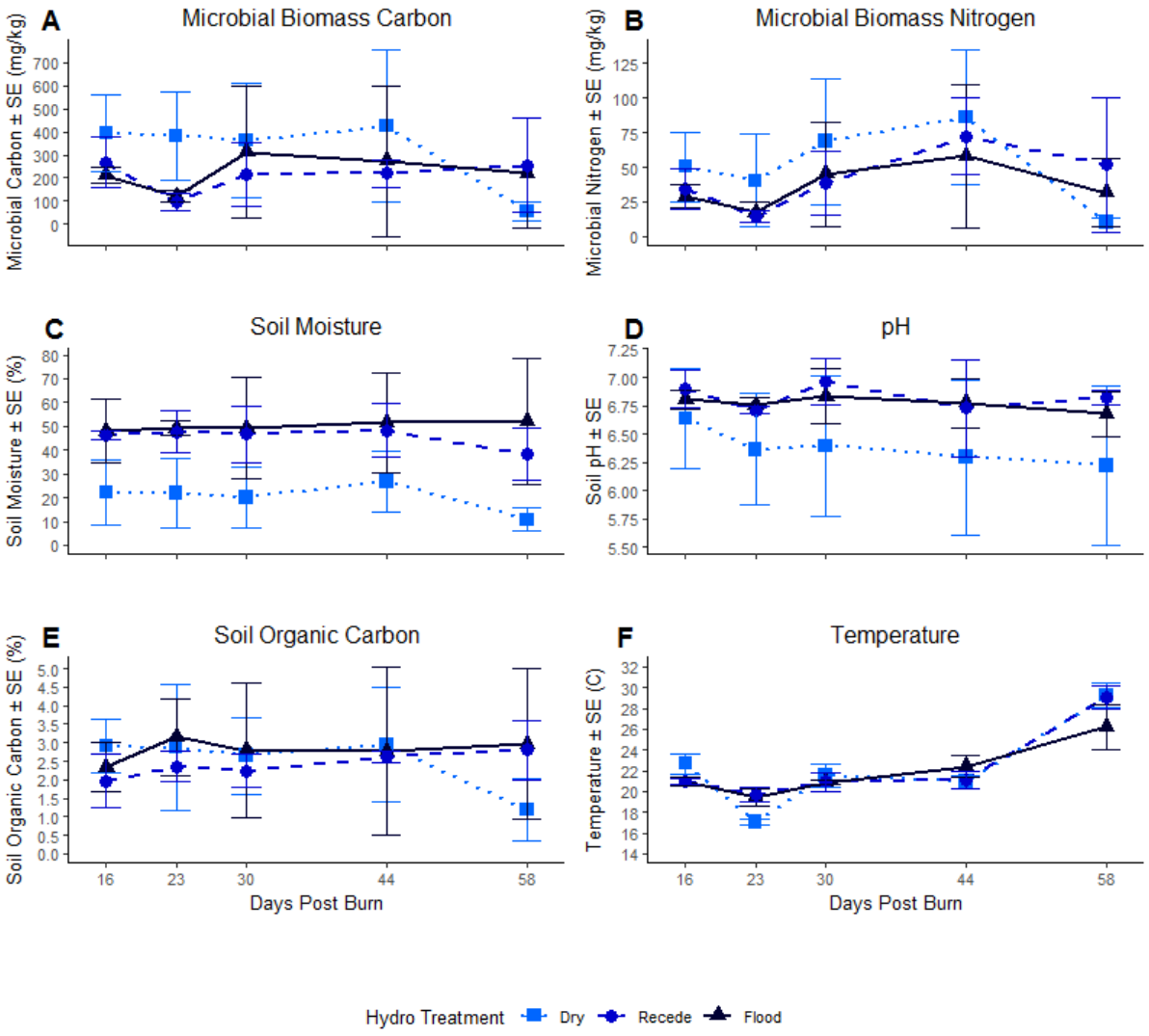

Figure 3. Shows response of: A) soil microbial biomass carbon (mg/kg), B) soil microbial biomass nitrogen (mg/kg), C) soil moisture (%), D) soil pH, E) soil organic carbon (%), and F) temperature (°C) taken in air or water to the hydrological treatment over time, starting with 16 days after the burn. Analysis starts 16 days after the burn since that is the start of the hydrological treatment separation, prior to that date there was no difference between flooded and receding treatments. Estimates are means with error bars depicting ± standard error. No significant differences between treatments or time as detected using a repeated measures ANOVA. The Dry treatment is represented by dotted lines, the Recede treatment by dashed lines, and the Flood treatment by solid lines.

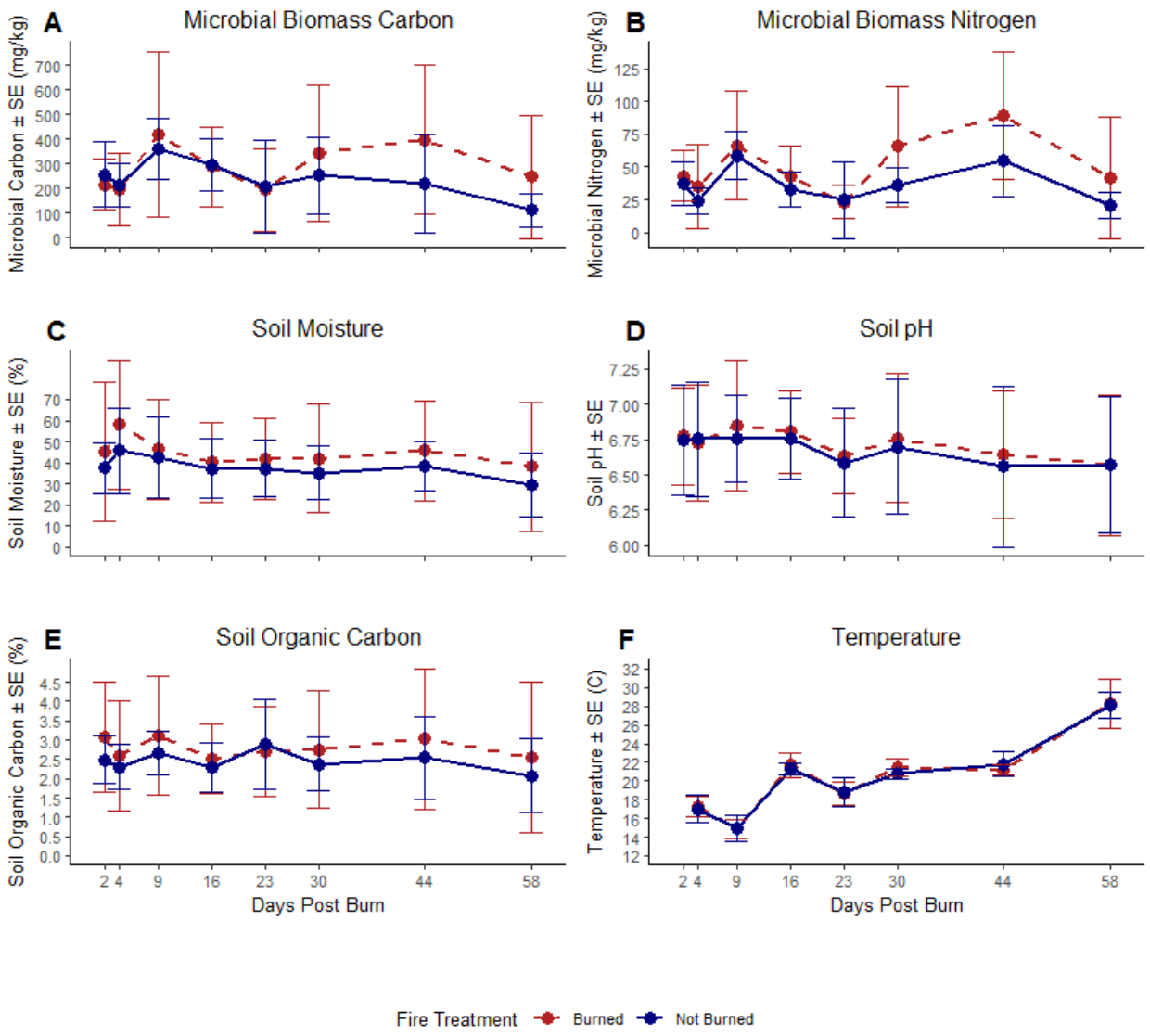

Figure 4. Shows response of A) soil microbial biomass carbon (mg/kg), B) soil microbial biomass nitrogen (mg/kg), C) soil moisture (%), D) soil pH, E) soil organic carbon (%), and F) temperature (°C) taken in air or water to the fire treatment over time, starting with 2 days after the burn. Day 2 is the first day that allows comparison between fire treatments; temperature response not available until day 4. Estimates are means with error bars depicting ± standard error. No significant differences between treatments or time as detected using a repeated measures ANOVA. The burn treatment is represented by red dashed lines and the not burned treatment by green solid lines.

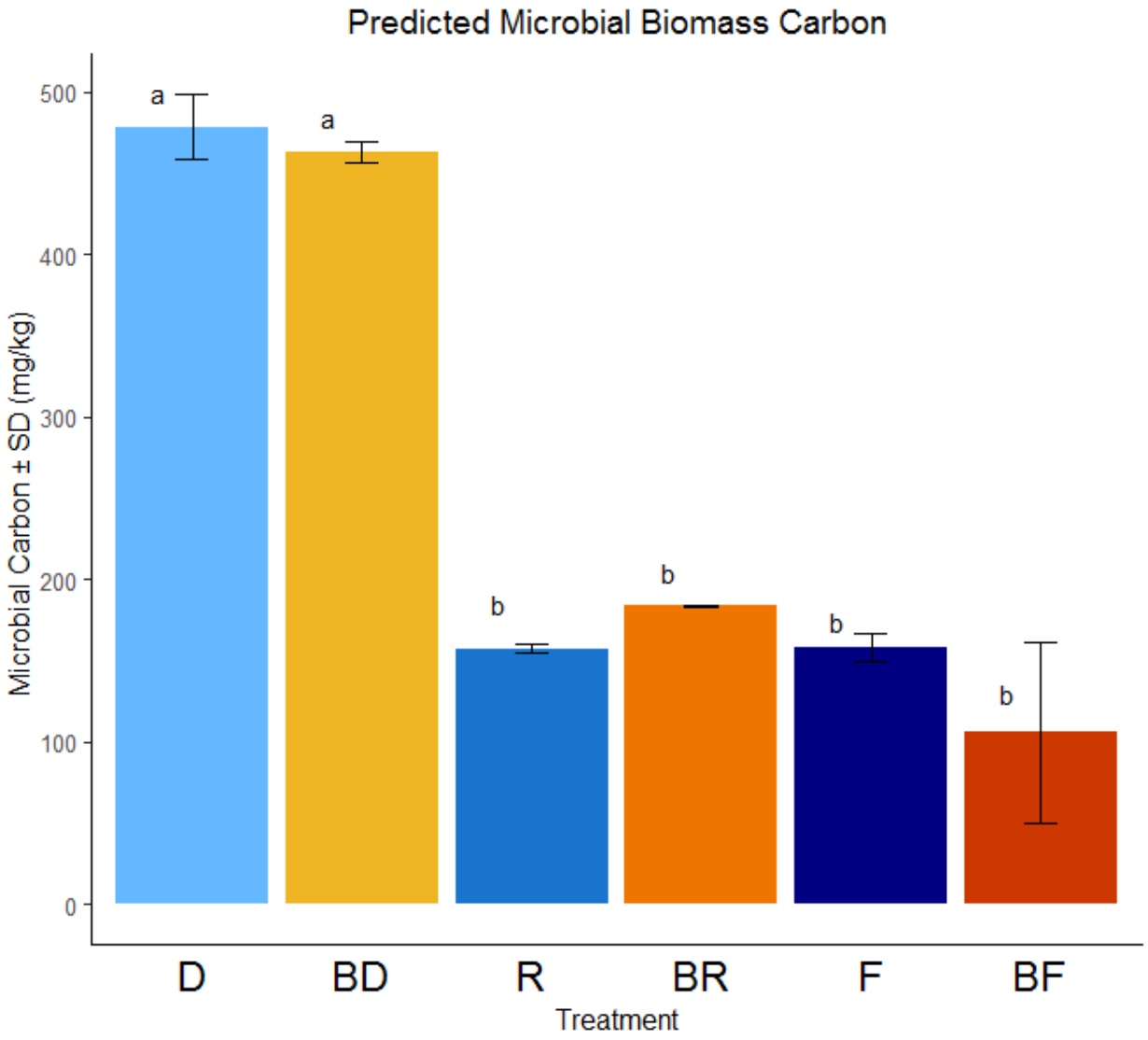

Figure 5. Predicted mean response of soil microbial biomass carbon (mg/kg) to treatment. Means predicted from the median value of all components of the best model (Soil moisture, pH, temperature, and soil organic carbon). Different letters represent significant differences between treatments, as shown in Table 4. Burn treatment codes are as follows: wetlands with a **B** were burnt prior to flooding treatment while those without were not burned. Flooding treatments are as follows: **D** dry treatment, **R** wetland flooded and allowed to recede, **F** wetland continuously flooded.

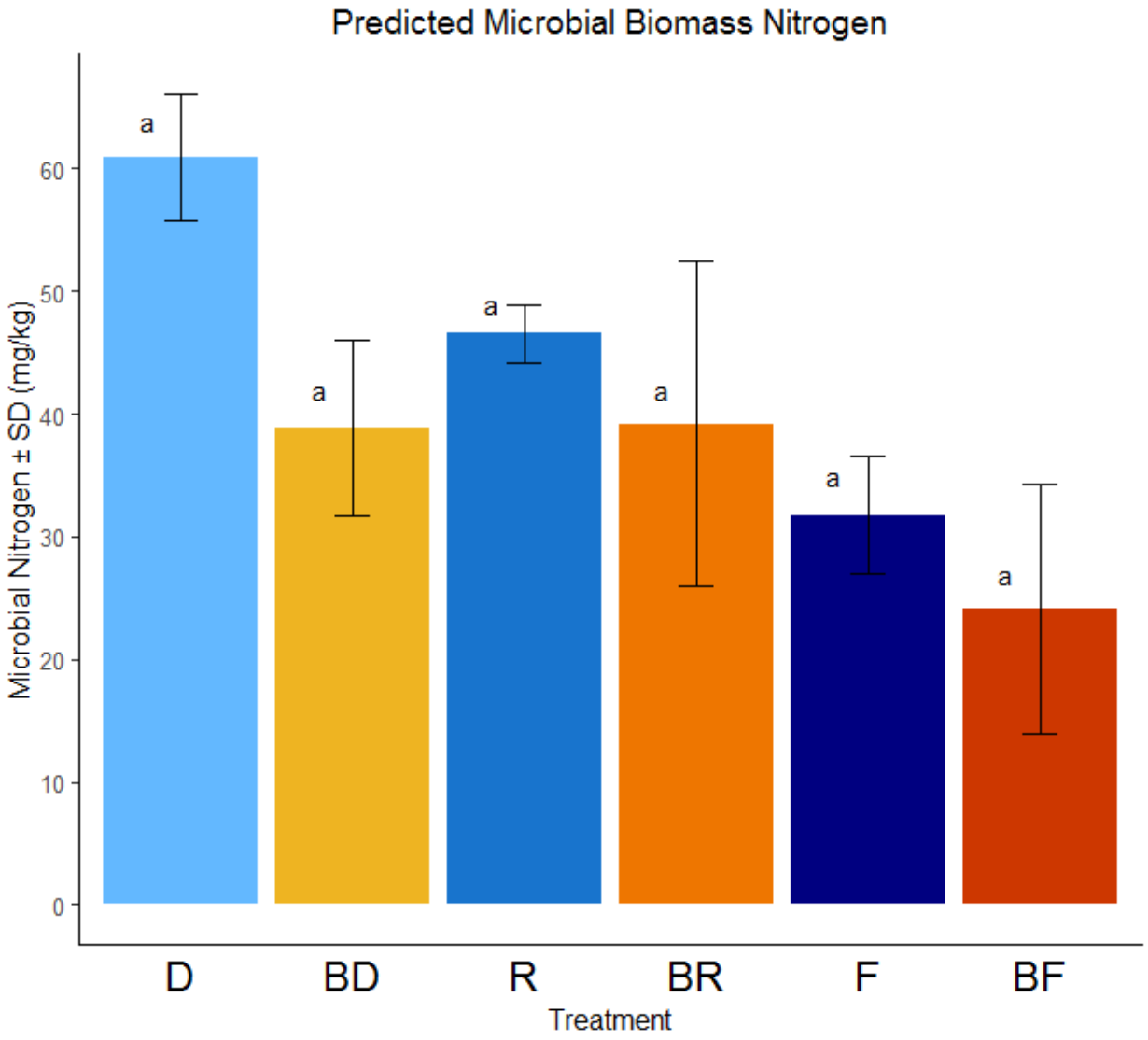

Figure 6. Predicted mean response of soil microbial biomass nitrogen (mg/kg) to treatment. Means predicted from the median value of all components of the best model (soil organic carbon and pH). Different letters represent significant differences between treatments, as shown in Table 4. Burn treatment codes are as follows: wetlands with a **B** were burnt prior to flooding treatment while those without were not burned. Flooding treatments are as follows: **D** dry treatment, **R** wetland flooded and allowed to recede, **F** wetland continuously flooded.

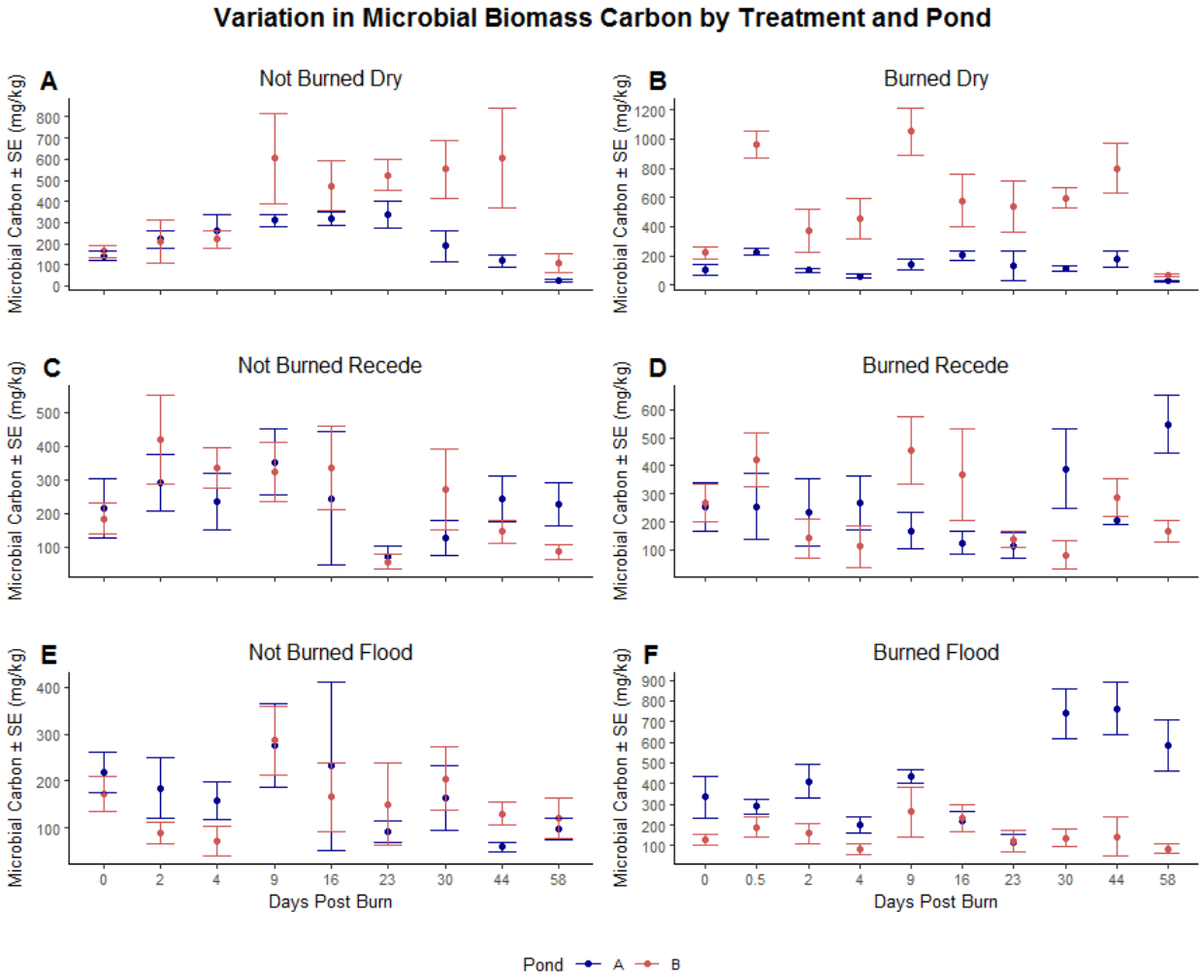

Figure 7. Soil microbial biomass carbon variation by treatment in mg/kg ± SE. Each of the six treatments was comprised of two ponds and four subsamples within those ponds. This depicts the variation of the mean within each pond in the study. Note, none have the same two ponds, labels are for ease of reading and merely denote that two ponds make up each treatment.

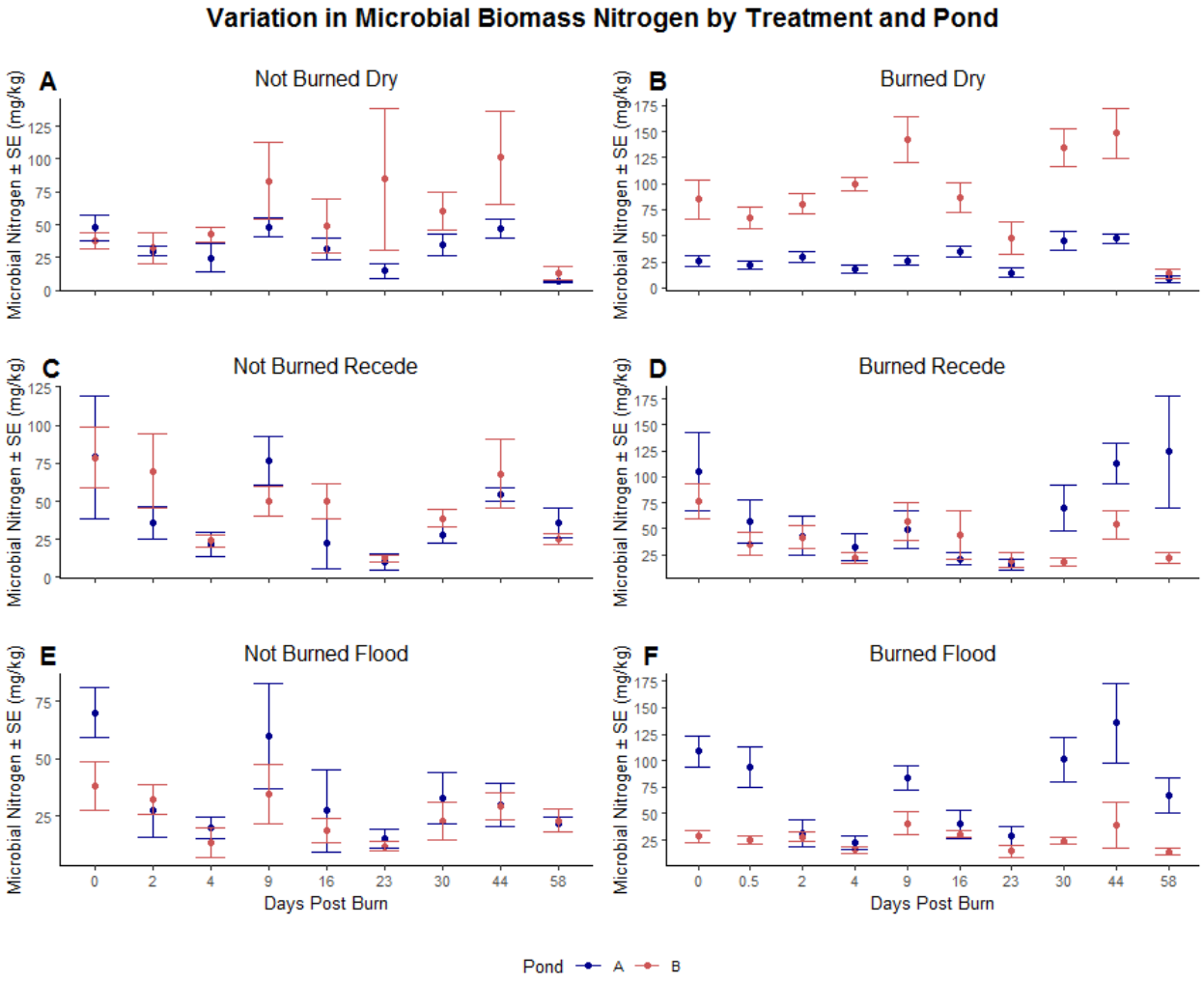

Figure 8. Soil microbial biomass nitrogen variation by treatment in mg/kg ± SE. Each of the six treatments was comprised of two ponds and four subsamples within those ponds. This depicts the variation of the mean within each pond in the study. Note, none have the same two ponds, labels are for ease of reading and merely denote that two ponds make up each treatment.

CHAPTER 4

DISCUSSION

At the onset of the study, it was speculated that the continuously flooded and not burned sites would serve as the reference condition, since these most closely reflect natural wetlands in the area. However, this did not take into account the disturbance effect that the initial flooding would have on the soil. Therefore, not burned and dry treatment sites were the true reference conditions and have been treated as such in my analysis. Holden and Treseder (2013) found that disturbances on average lead to ~30% decreased in soil microbial biomass, regardless if the disturbance was biotic or abiotic and regardless of climate. More intense and longer-term disturbances see decreased soil microbial biomass due to time needed to recolonize or experience a microbial community shift when the previous microbes are no longer suited for the new conditions (Holden and Treseder, 2013; Nahlik; 2016). Since most wetland studies are starting with or using a fully inundated wetland as their reference state, the main disturbance that these wetlands experience is drought. Consequently, as these wetlands dry out, there is generally a decreased in microbial biomass as expected. Even though I set out to measure the same types of effects, my flooded treatment still experienced a large disturbance event that reference sites in other studies would not have experienced, making my true reference site the dry and undisturbed experimental units. Taking this into account, having a different starting reference point has likely led to the difference in many of my results compared to other wetland studies examining degraded and disturbed wetland conditions.

Soil and Microbial Response to Disturbances

Wildfires in wetlands have been shown to result in decreased microbial biomass, while many prescribed burns have been found to have no effect or a short positive effect (Zhao et al., 2012; Holden and Treseder, 2013; Medvedeff et al., 2013). I observed a decrease in microbial biomass nitrogen, but not microbial biomass carbon, immediately following the prescribed burn but at no other time during the study (Table 6). Similarly, other studies have also found a lack of microbial response to burning due to low intensity burns, high local humidity, and the additional nutrients from the burn dispelling any

negative burn effects (Kara and Bolat, 2009; Medvedeff et al., 2013). Although no significant responses were detected from the fire treatment over the length of my study, some studies have found it can take up to a year for some soil effects to appear following a burn (Medvedeff et al., 2013). When I used predicted values, microbial biomass nitrogen had a negative trend in the fire treatment, suggesting that burning may have impacted availability of soil nitrogen and limited microbial uptake and cycling (Salvia et al., 2012). Overall, it is likely that the fire treatment was not intense enough to cause widespread microbial die-off and therefore, there was no interaction effect between the fire and hydrological treatments.

Microbial biomass has been found to increase with increasing availability of soil oxygen and moisture (Mettrop et al., 2014). However, I found results contrary to this idea with predicted microbial biomass carbon reduced in the recede and flood treatments compared to the dry treatment and predicted microbial biomass nitrogen having a similar, but non-significant, trend. Although the soils in the receding treatment likely had higher soil moisture conducive to microbial growth, the microbes also experienced a large disturbance event (i.e., flooding followed by drying) which has been found to negatively impact soil microbes (Holden and Treseder, 2013). Urbanová and Bárta (2016) found decreased microbial biomass in drained fens and bogs compared to pristine ones even though the aerobic conditions would have theoretically been better suited for microbial growth, indicating that aerobic conditions are not necessarily the controlling factor for microbial biomass fluctuation. I did not find a significant difference between my receding and flooded treatments, in contrast to observed natural wetlands that mimic our treatments (Weaver et al., 2012). This may, in part, may be due to the short duration of my study and differences may become apparent as the treatments continue, as some suggest that constructed wetlands can take several years to mature and see the disappearance of disturbance effects (Weaver et al., 2012; Holden and Treseder, 2013).

Decreased microbial biomass in drained and disturbed wetlands has been attributed to related shifts in other soil characteristics like organic matter quality and quantity, bulk density, and pH (Jiang et al., 2013; Urbanová et al., 2018). I found pH to be slightly higher and microbial biomass to be lower in

both the receding and flooded treatments compared the dry treatments, suggesting a negative pH to microbial biomass relationship that has been found in other studies (Fisk et al. 2003; Urbanová and Bárta, 2016). Similar correlations were also found between measured soil moisture and pH. Other studies that have found a positive correlation between the two suggest this is caused by reduced redox potential upon soil wetting (Kozlowski and Pallardy, 1997; Misra and Tyler, 1999; DeLaune, et al., 2013). The steady increase in mean daily temperature found as the study progressed may have played an important role in the sharp decline of microbial biomass observed near the end of the study in the dry treatment due to higher moisture and temperature stress that could not be mediated by flooded conditions (Schimel et al., 2007).

Soil Microbial Biomass Variability

Although not tested, the temporal fluctuation in both microbial biomass carbon and microbial biomass nitrogen suggests that other environmental factors with the ability to change rapidly are likely responsible for the observed fluctuations (i.e., soil temperature, root exudates, etc.). The best model for microbial biomass carbon found that soil moisture, soil organic carbon, pH, and temperature were responsible for much more of the observed variation in microbial biomass carbon than natural variation found between the experimental ponds. Studies have suggested that soil type, organic content, and moisture are the most important factors in determining these fluctuations over seasons, but I saw large fluctuations in the span of days with minor changes in soil moisture and soil organic carbon (Devi and Yadava, 2006; Moche et al., 2015). My samples were collected 11 times over the span of 3 months and found large variability even as close as 5 days apart. Only one other study has taken microbial biomass samples *in situ* at frequencies less than a month, and also found large fluctuations in biomass that could not be fully explained by changes in temperature or soil moisture (Brooks et al., 1998). These large daily microbial fluctuations suggest that single time point assessments of microbial biomass may not be an appropriate way to evaluate soil response to changes and overall health.

Table 6. Observed Soil microbial biomass responses matched to predicted responses and suporting literature from table 1.

Parameter	Microbial Biomass Response Observed	Predicted Microbial Biomass Response	Citation
Fire	Initial slight decrease in microbial biomass nitrogen, but no long-term effects of fire.	**No Effect:** If the fire doesn't burn hot enough or passes over soil quickly, it will have little to no effect on microbial biomass, however any amendments to the soil, like ash, may have an impact.	Kara and Bolat, 2009; Medvedeff et al., 2013
Receding Water	Decreased in comparison to the dry and undisturbed treatment. Not significantly different from continuously flooded treatment.	**Decreased Microbial Biomass:** As water recedes, microbes may face competition for nutrients from plants. Wetland drainage may also lead to accelerated litter decomposition which limits available organic matter available to microbes.	Ladd et al., 1995; Baldwin and Mitchell, 2000; Schimel et al., 2007; Moche et al., 2015; Urbanová and Bárta, 2016; Urbanová et al., 2018
Dry	Highest microbial biomass. Likely due to no disturbance event.	**High Microbial Biomass:** This is only in the specific case of our treatments since the dry treatment will be the least disturbed.	Urbanová et al., 2018
Flood	Decreased in comparison to the dry and undisturbed treatment. Not significantly different from continuously recede treatment.	**Decreased Microbial Biomass:** As floods persist, soil conditions become anaerobic leading to community shift or microbial die-off.	Baldwin and Mitchell, 2000; Gonzalez-Quinones et al., 2011; Nahlik, 2016; Sutfin et al., 2016
pH	Slight negative correlation.	**Negatively Correlated:** Several studies have found a large proportion of microbes do not function well above a pH of 7.5 and many have adapted to the acidity of bogs and swamps.	Dalal, 1998; Ma et al., 2017; Weaver et al., 2012; Urbanová et al., 2018
Disturbance	More disturbance lead to decreased soil microbial biomass.	**Negatively Correlated:** More disturbed ecosystems have lower microbial biomass fluctuation but also have much lower biomass values.	Schimel et al.; 2007; Holden and Treseder, 2013; Jiang et al.; 2013; Nahlik; 2016; Urbanová and Bárta, 2016; Urbanová et al., 2018
Temperature	It is thought that increase of temperature at end of study lead to decreased microbial biomass.	**Mesophilic Range:** Very hot temperatures lead to microbial stress and death while very low temperatures can cause stupor and death.	Gonzalez-Quinones et al., 2011; Jiang et al., 2013
Variability	Mean values tend to fluctuate together but variability between samples is very high.	**Fluctuate Together:** Regardless of treatment, microbial biomass fluctuates in similar trends though not necessarily in the same proportion.	Brooks et al., 1998; Tscherko and Kandeler, 1999; Ruan, 2004; Jiang et al., 2013
Soil Moisture	Soil moisture did not have a significant effect by itself but was likely the driving factor behind the disturbance effect.	**Mesophilic Range:** Low and very high soil moisture cause microbial stress.	Gonzalez-Quinones et al., 2011; Jiang et al., 2013; Ren et al., 2017

CHAPTER 5

CONCLUSIONS

Though I had originally predicted to find the highest amount of microbial biomass in the receding and burned treatments, I found the opposite to be true for my study. This is mostly attributed to the potentially large disturbance event created by the flooding that both the recede and flood treatments experienced. It is likely that the present microbial community is more closely representative of a terrestrial community, but I expect that over the course of the next year or two, the continuously flooded wetlands will have a community shift that will leave them more similar to a true wetland microbial community. If I were to continue this study, I would add in components to measure changes in microbial community and microbial efficiency. Not only do I think that the flooded microbial community will shift towards an obligate anaerobic community, but I also would expect a shift in the receding treatment community towards microbes with thick cell walls that help to prevent desiccation upon drying and lysing when re-wetted. Recording soil gas exchange is a good way to discern microbial efficiency, which would help to assess how the microbes handle the hydrological stressors that the treatments enacted. Finally, the last addition I would make to this study would be to extend it for several years until the continuously flooded wetlands had become stable and could serve as a baseline for the study.

This study demonstrated that the starting state of the wetland is an important factor in the overall outcomes. If the experimental sites had started out fully flooded, observed outcomes may have been more consistent with those found in the literature. However, it can still be recommended that wetland managers attempt to preserve wetlands in their current hydrological state. These large hydrological disturbances in either direction have been found to reduce microbial biomass, so it can be assumed that there is an associated and comparable change in wetland carbon storage. Since burning the experimental wetlands did not appear to have a significant effect on soil microbial biomass or the measured soil variables, this

could a suitable method for wetland managers to use as vegetation control, without resulting in immediate impact microbial-mediated wetland functions.

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APPENDIX A

Paired t-test comparing pre-burned soil conditions to post-burned soil conditions on the same day of the study. Cmic = microbial biomass carbon and Nmic = microbial biomass nitrogen. Significance was considered at $p > 0.05$ and denoted by *.

Parameter	**t-value**	**DF**	**p-value**
Cmic (mg/kg)	-1.462	5	0.204
Nmic (mg/kg)	2.827	5	0.037*
Soil Moisture (%)	-1.664	5	0.157
pH	3.782	5	0.013*
Soil organic carbon (%)	-0.508	5	0.633
C:N	-2.813	5	0.037*
Total Carbon (%)	-2.103	5	0.089
Total Nitrogen (%)	1.563	5	0.179
Cmic:Soil organic carbon	-2.160	5	0.083
Nmic:Total Nitrogen	-0.027	5	0.979
Cmic:Nmic	-3.903	5	0.011*

APPENDIX B

Results from repeated measures ANOVA for microbial biomass and predictor values. Trt = treatment, Cmic = microbial biomass carbon, and Nmic = microbial biomass nitrogen. No treatment effects were significant apart from the intercepts being significantly different from 0.

	Trt DF	Error DF	F-value	p-value
Cmic (mg/kg)				
(Intercept)	1	48	28.070	<.0001
Fire Treatments	1	6	0.652	0.450
Hydrological Treatments	2	6	0.539	0.609
Fire*Hydrological Trts	2	6	0.278	0.766
Nmic (mg/kg)				
(Intercept)	1	48	32.177	<.0001
Fire Treatments	1	6	1.490	0.268
Hydrological Treatments	2	6	0.321	0.737
Fire*Hydrological Trts	2	6	0.066	0.937
Soil Moisture (%)				
(Intercept)	1	48	87.358	<.0001
Fire Treatments	1	6	0.550	0.486
Hydrological Treatments	2	6	4.985	0.053
Fire*Hydrological Trts	2	6	0.138	0.874
Soil organic carbon (%)				
(Intercept)	1	48	48.306	<.0001
Fire Treatments	1	6	0.141	0.720
Hydrological Treatments	2	6	0.108	0.899
Fire*Hydrological Trts	2	6	0.228	0.803
pH				
(Intercept)	1	48	2891.870	<.0001
Fire Treatments	1	6	0.037	0.854
Hydrological Treatments	2	6	1.268	0.347
Fire*Hydrological Trts	2	6	0.083	0.921
Temperature (˚C)				
(Intercept)	1	48	2342.342	<.0001
Fire Treatments	1	6	0.012	0.917
Hydrological Treatments	2	6	0.068	0.935
Fire*Hydrological Trts	2	6	0.305	0.748

	Trt DF	Error DF	F-value	p-value
Total Carbon (%)				
(Intercept)	1	48	50.417	<.0001
Fire Treatments	1	6	0.287	0.612
Hydrological Treatments	2	6	0.161	0.855
Fire*Hydrological Trts	2	6	0.202	0.823
Total Nitrogen (%)				
(Intercept)	1	36	22.536	<.0001
Fire Treatments	1	6	0.390	0.555
Hydrological Treatments	2	6	0.169	0.848
Fire*Hydrological Trts	2	6	0.052	0.950
Total Carbon:Nitrogen (%)				
(Intercept)	1	36	38.401	<.0001
Fire Treatments	1	6	0.466	0.520
Hydrological Treatments	2	6	0.342	0.723
Fire*Hydrological Trts	2	6	0.409	0.681
Cmic:Nmic				
(Intercept)	1	48	129.610	<.0001
Fire Treatments	1	6	0.259	0.629
Hydrological Treatments	2	6	1.342	0.330
Fire*Hydrological Trts	2	6	0.876	0.464
Nmic:Total N				
(Intercept)	1	36	27.681	<.0001
Fire Treatments	1	6	1.600	0.253
Hydrological Treatments	2	6	1.033	0.412
Fire*Hydrological Trts	2	6	0.775	0.502
Cmic:Soil organic carbon				
(Intercept)	1	48	238.214	<.0001
Fire Treatments	1	6	1.880	0.219
Hydrological Treatments	2	6	3.357	0.105
Fire*Hydrological Trts	2	6	0.617	0.571

APPENDIX C

Means and standard error for all soil characteristics and ratios separated by parameter, then days post burn, then treatment. Cmic = microbial biomass carbon and Nmic = microbial biomass nitrogen. Days post burn start with day 0 (before the burn) and day 0.5 (same day as the burn but directly following the burn). All other days are the number of days since the burn.

Soil Parameter Measured	Days Post Burn	N	Mean	SE
Cmic (mg/kg)				
Not Burned Dry	0	12	200.46	18.81
Burned Dry	0.5	6	390.96	119.30
Burned Dry	2	2	234.09	133.87
Burned Flood	2	4	206.15	34.91
Not Burned Dry	2	2	215.12	6.33
Not Burned Flood	2	4	276.46	83.02
Burned Dry	4	2	256.77	196.98
Burned Flood	4	4	165.20	42.00
Not Burned Dry	4	2	240.61	19.86
Not Burned Flood	4	4	199.62	55.72
Burned Dry	9	2	596.70	455.47
Burned Flood	9	4	330.39	68.63
Not Burned Dry	9	2	456.66	147.15
Not Burned Flood	9	4	309.84	17.52
Burned Dry	16	2	389.79	188.54
Burned Flood	16	2	225.39	8.85
Burned Recede	16	2	246.88	121.60
Not Burned Dry	16	2	396.27	75.43
Not Burned Flood	16	2	198.63	33.20
Not Burned Recede	16	2	289.66	45.15
Burned Dry	23	2	334.11	201.35
Burned Flood	23	2	117.69	5.57
Burned Recede	23	2	127.35	11.14
Not Burned Dry	23	2	430.81	93.90
Not Burned Flood	23	2	121.96	28.69
Not Burned Recede	23	2	62.59	6.82
Burned Dry	30	2	355.23	239.21
Burned Flood	30	2	437.59	300.36
Burned Recede	30	2	235.56	153.03
Not Burned Dry	30	2	370.94	182.22
Not Burned Flood	30	2	184.63	20.18
Not Burned Recede	30	2	198.20	72.21
Burned Dry	44	2	489.12	311.03
Burned Flood	44	2	452.96	309.64
Burned Recede	44	2	246.71	41.26
Not Burned Dry	44	2	362.60	245.08
Not Burned Flood	44	2	95.04	35.23

Soil Parameter Measured	Days Post Burn	N	Mean	SE
Not Burned Recede	44	2	193.86	48.92
Burned Dry	58	2	48.16	21.04
Burned Flood	58	2	333.91	247.94
Burned Recede	58	2	357.48	190.55
Not Burned Dry	58	2	65.70	41.61
Not Burned Flood	58	2	109.02	10.95
Not Burned Recede	58	2	154.96	70.73
Nmic (mg/kg)				
Not Burned Dry	0	12	65.07	8.27
Burned Dry	0.5	6	49.93	11.33
Not Burned Dry	2	2	30.99	1.42
Burned Dry	2	2	55.04	25.60
Not Burned Flood	2	4	40.22	9.95
Burned Flood	2	4	37.23	3.39
Not Burned Dry	4	2	33.58	8.94
Burned Dry	4	2	58.91	41.06
Not Burned Flood	4	4	19.74	2.29
Burned Flood	4	4	23.26	3.42
Not Burned Dry	9	2	65.53	17.93
Burned Dry	9	2	84.43	58.26
Not Burned Flood	9	4	55.40	8.76
Burned Flood	9	4	57.51	9.24
Not Burned Dry	16	2	40.20	8.69
Burned Dry	16	2	60.81	25.64
Not Burned Flood	16	2	23.01	4.38
Burned Flood	16	2	35.29	4.64
Not Burned Recede	16	2	35.98	13.83
Burned Recede	16	2	32.41	11.56
Not Burned Dry	23	2	49.61	35.10
Burned Dry	23	2	30.98	16.50
Not Burned Flood	23	2	13.44	1.63
Burned Flood	23	2	21.53	6.93
Not Burned Recede	23	2	11.32	1.26
Burned Recede	23	2	17.44	2.26
Not Burned Dry	30	2	47.50	13.12
Burned Dry	30	2	89.95	45.04
Not Burned Flood	30	2	27.62	5.01
Burned Flood	30	2	62.67	38.44
Not Burned Recede	30	2	33.24	5.57
Burned Recede	30	2	43.79	26.44
Not Burned Dry	44	2	74.11	27.14
Burned Dry	44	2	98.10	50.64
Not Burned Flood	44	2	29.52	0.15
Burned Flood	44	2	87.23	48.45
Not Burned Recede	44	2	61.19	6.78
Burned Recede	44	2	83.38	29.51
Not Burned Dry	58	2	9.90	3.38
Burned Dry	58	2	10.91	2.60
Not Burned Flood	58	2	22.13	0.70
Burned Flood	58	2	40.96	26.72

Soil Parameter Measured	Days Post Burn	N	Mean	SE
Not Burned Recede	58	2	30.52	5.41
Burned Recede	58	2	73.05	51.26
Soil Moisture (%)				
Not Burned Dry	0	12	32.78	5.64
Burned Dry	0.5	6	36.40	10.71
Not Burned Dry	2	2	25.58	6.05
Not Burned Flood	2	4	43.39	4.46
Burned Dry	4	2	36.35	29.87
Burned Flood	4	4	68.87	11.17
Not Burned Dry	4	2	22.09	6.39
Not Burned Flood	4	4	57.75	4.78
Burned Dry	9	2	25.07	18.27
Burned Flood	9	4	57.17	8.12
Not Burned Dry	9	2	21.33	2.50
Not Burned Flood	9	4	53.01	6.52
Burned Dry	16	2	23.60	15.94
Burned Flood	16	2	50.63	14.58
Burned Recede	16	2	46.88	2.21
Not Burned Dry	16	2	20.61	5.28
Not Burned Flood	16	2	45.52	7.00
Not Burned Recede	16	2	45.49	0.16
Burned Dry	23	2	21.23	15.86
Burned Flood	23	2	49.37	3.86
Burned Recede	23	2	54.92	2.53
Not Burned Dry	23	2	22.78	8.35
Not Burned Flood	23	2	48.95	0.47
Not Burned Recede	23	2	40.39	0.79
Burned Dry	30	2	20.27	14.28
Burned Flood	30	2	55.76	24.10
Burned Recede	30	2	50.34	12.77
Not Burned Dry	30	2	20.00	5.75
Not Burned Flood	30	2	42.74	0.16
Not Burned Recede	30	2	42.82	3.72
Burned Dry	44	2	27.96	14.81
Burned Flood	44	2	59.45	23.22
Burned Recede	44	2	50.21	11.07
Not Burned Dry	44	2	25.53	5.54
Not Burned Flood	44	2	43.38	3.29
Not Burned Recede	44	2	46.20	7.69
Burned Dry	58	2	8.95	2.92
Burned Flood	58	2	60.15	30.28
Burned Recede	58	2	45.29	7.91
Not Burned Dry	58	2	12.59	4.47
Not Burned Flood	58	2	44.25	1.25
Not Burned Recede	58	2	31.83	5.24
pH				
Not Burned Dry	0	12	6.91	0.11
Burned Dry	0.5	6	6.69	0.13
Not Burned Dry	2	2	6.45	0.49
Not Burned Flood	2	4	6.90	0.04

Soil Parameter Measured	Days Post Burn	N	Mean	SE
Burned Dry	4	2	6.49	0.54
Burned Flood	4	4	6.84	0.09
Not Burned Dry	4	2	6.47	0.52
Not Burned Flood	4	4	6.90	0.06
Burned Dry	9	2	6.50	0.51
Burned Flood	9	4	7.03	0.12
Not Burned Dry	9	2	6.40	0.20
Not Burned Flood	9	4	6.93	0.04
Burned Dry	16	2	6.71	0.43
Burned Flood	16	2	6.81	0.10
Burned Recede	16	2	6.90	0.02
Not Burned Dry	16	2	6.57	0.32
Not Burned Flood	16	2	6.80	0.01
Not Burned Recede	16	2	6.90	0.20
Burned Dry	23	2	6.42	0.33
Burned Flood	23	2	6.76	0.06
Burned Recede	23	2	6.73	0.02
Not Burned Dry	23	2	6.31	0.50
Not Burned Flood	23	2	6.75	0.06
Not Burned Recede	23	2	6.70	0.04
Burned Dry	30	2	6.47	0.52
Burned Flood	30	2	6.72	0.17
Burned Recede	30	2	7.10	0.17
Not Burned Dry	30	2	6.32	0.55
Not Burned Flood	30	2	6.95	0.19
Not Burned Recede	30	2	6.84	0.04
Burned Dry	44	2	6.34	0.56
Burned Flood	44	2	6.70	0.11
Burned Recede	44	2	6.89	0.18
Not Burned Dry	44	2	6.26	0.63
Not Burned Flood	44	2	6.85	0.22
Not Burned Recede	44	2	6.58	0.45
Burned Dry	58	2	6.28	0.64
Burned Flood	58	2	6.61	0.22
Burned Recede	58	2	6.82	0.07
Not Burned Dry	58	2	6.16	0.57
Not Burned Flood	58	2	6.74	0.01
Not Burned Recede	58	2	6.82	0.04
Soil organic carbon (%)				
Not Burned Dry	0	12	2.97	0.04
Burned Dry	0.5	6	2.92	0.26
Not Burned Dry	2	2	2.09	0.76
Not Burned Flood	2	4	2.70	0.51
Burned Dry	4	2	2.77	0.28
Burned Flood	4	4	2.52	1.69
Not Burned Dry	4	2	2.57	0.61
Not Burned Flood	4	4	2.17	0.74
Burned Dry	9	2	2.74	0.17
Burned Flood	9	4	3.31	1.61

Soil Parameter Measured	Days Post Burn	N	Mean	SE
Not Burned Dry	9	2	2.73	0.71
Not Burned Flood	9	4	2.65	0.83
Burned Dry	16	2	3.19	0.14
Burned Flood	16	2	2.33	0.19
Burned Recede	16	2	2.03	0.83
Not Burned Dry	16	2	2.63	0.78
Not Burned Flood	16	2	2.37	0.75
Not Burned Recede	16	2	1.90	0.12
Burned Dry	23	2	2.23	0.40
Burned Flood	23	2	3.26	1.13
Burned Recede	23	2	2.66	1.24
Not Burned Dry	23	2	3.51	0.23
Not Burned Flood	23	2	3.07	1.51
Not Burned Recede	23	2	2.08	0.22
Burned Dry	30	2	2.58	0.18
Burned Flood	30	2	3.31	0.93
Burned Recede	30	2	2.37	2.09
Not Burned Dry	30	2	2.73	0.35
Not Burned Flood	30	2	2.28	0.86
Not Burned Recede	30	2	2.11	0.34
Burned Dry	44	2	2.74	0.39
Burned Flood	44	2	3.68	1.24
Burned Recede	44	2	2.66	2.48
Not Burned Dry	44	2	3.16	0.08
Not Burned Flood	44	2	1.87	1.38
Not Burned Recede	44	2	2.58	0.17
Burned Dry	58	2	0.91	0.15
Burned Flood	58	2	3.58	0.34
Burned Recede	58	2	3.19	2.23
Not Burned Dry	58	2	1.47	0.52
Not Burned Flood	58	2	2.35	0.90
Not Burned Recede	58	2	2.43	0.70
Temperature (°C)				
Not Burned Dry	0	12	N/A	N/A
Burned Dry	0.5	6	N/A	N/A
Burned Dry	2	2	N/A	N/A
Burned Flood	2	4	N/A	N/A
Not Burned Dry	2	2	N/A	N/A
Not Burned Flood	2	4	N/A	N/A
Burned Dry	4	2	15.97	0.14
Burned Flood	4	4	17.85	0.33
Not Burned Dry	4	2	15.34	0.06
Not Burned Flood	4	4	17.88	0.42
Burned Dry	9	2	13.78	0.00
Burned Flood	9	4	15.49	0.32
Not Burned Dry	9	2	13.11	0.00
Not Burned Flood	9	4	15.76	0.11
Burned Dry	16	2	23.22	0.89
Burned Flood	16	2	20.97	0.36
Burned Recede	16	2	21.00	0.28

Soil Parameter Measured	Days Post Burn	N	Mean	SE
Not Burned Dry	16	2	22.14	0.03
Not Burned Flood	16	2	20.97	0.25
Not Burned Recede	16	2	20.94	0.00
Burned Dry	23	2	17.25	0.25
Burned Flood	23	2	18.72	0.39
Burned Recede	23	2	20.06	0.27
Not Burned Dry	23	2	16.89	0.22
Not Burned Flood	23	2	20.11	0.11
Not Burned Recede	23	2	19.36	0.64
Burned Dry	30	2	22.28	0.78
Burned Flood	30	2	20.73	0.17
Burned Recede	30	2	21.28	0.39
Not Burned Dry	30	2	20.81	0.09
Not Burned Flood	30	2	20.97	0.14
Not Burned Recede	30	2	20.53	0.81
Burned Dry	44	2	21.36	0.47
Burned Flood	44	2	21.56	0.16
Burned Recede	44	2	20.70	0.37
Not Burned Dry	44	2	20.53	0.20
Not Burned Flood	44	2	23.28	0.33
Not Burned Recede	44	2	21.61	0.67
Burned Dry	58	2	29.70	1.31
Burned Flood	58	2	25.84	2.28
Burned Recede	58	2	29.22	1.28
Not Burned Dry	58	2	28.81	0.02
Not Burned Flood	58	2	26.58	1.14
Not Burned Recede	58	2	28.86	0.14
Total Nitrogen (%)				
Not Burned Dry	0	12	0.20	0.04
Burned Dry	0.5	6	0.19	0.07
Not Burned Dry	2	2	0.14	0.08
Not Burned Flood	2	4	0.21	0.09
Burned Dry	4	2	0.18	0.12
Burned Flood	4	4	0.17	0.06
Not Burned Dry	4	2	0.09	0.02
Not Burned Flood	4	4	0.12	0.01
Burned Dry	9	2	0.14	0.08
Burned Flood	9	4	0.25	0.07
Not Burned Dry	9	2	0.13	0.05
Not Burned Flood	9	4	0.15	0.02
Burned Dry	16	2	0.10	0.05
Burned Flood	16	2	0.11	0.09
Burned Recede	16	2	0.14	0.06
Not Burned Dry	16	2	0.10	0.06
Not Burned Flood	16	2	0.17	0.06
Not Burned Recede	16	2	0.11	0.02
Burned Dry	23	2	0.11	0.09
Burned Flood	23	2	0.23	0.13
Burned Recede	23	2	0.19	0.03
Not Burned Dry	23	2	0.19	0.10

Soil Parameter Measured	Days Post Burn	N	Mean	SE
Not Burned Flood	23	2	0.17	0.02
Not Burned Recede	23	2	0.13	0.03
Burned Dry	30	2	0.12	0.10
Burned Flood	30	2	0.24	0.23
Burned Recede	30	2	0.17	0.03
Not Burned Dry	30	2	0.12	0.02
Not Burned Flood	30	2	0.16	0.01
Not Burned Recede	30	2	0.10	0.00
Burned Dry	44	2	0.26	0.14
Burned Flood	44	2	0.28	0.19
Burned Recede	44	2	0.16	0.02
Not Burned Dry	44	2	0.14	0.08
Not Burned Flood	44	2	0.11	0.00
Not Burned Recede	44	2	0.14	0.05
Burned Dry	58	2	N/A	N/A
Burned Flood	58	2	0.28	0.25
Burned Recede	58	2	0.22	0.07
Not Burned Dry	58	2	0.06	0.04
Not Burned Flood	58	2	0.12	0.06
Not Burned Recede	58	2	0.15	0.06
Total Carbon (%)				
Not Burned Dry	0	12	3.37	0.40
Burned Dry	0.5	6	4.98	1.20
Not Burned Dry	2	2	2.72	1.23
Not Burned Flood	2	4	3.59	1.18
Burned Dry	4	2	2.95	1.71
Burned Flood	4	4	2.76	0.58
Not Burned Dry	4	2	1.84	0.16
Not Burned Flood	4	4	2.27	0.25
Burned Dry	9	2	2.50	1.21
Burned Flood	9	4	3.44	0.68
Not Burned Dry	9	2	2.45	0.55
Not Burned Flood	9	4	2.80	0.31
Burned Dry	16	2	2.35	0.77
Burned Flood	16	2	2.27	0.82
Burned Recede	16	2	2.60	0.78
Not Burned Dry	16	2	2.10	0.38
Not Burned Flood	16	2	2.58	0.10
Not Burned Recede	16	2	2.06	0.22
Burned Dry	23	2	2.20	1.15
Burned Flood	23	2	3.50	1.16
Burned Recede	23	2	3.02	0.48
Not Burned Dry	23	2	3.42	1.28
Not Burned Flood	23	2	3.13	0.25
Not Burned Recede	23	2	2.12	0.10
Burned Dry	30	2	2.41	1.14
Burned Flood	30	2	3.49	2.36
Burned Recede	30	2	2.43	0.83
Not Burned Dry	30	2	2.47	0.36
Not Burned Flood	30	2	2.56	0.52

Soil Parameter Measured	Days Post Burn	N	Mean	SE
Not Burned Recede	30	2	1.87	0.48
Burned Dry	44	2	3.26	1.24
Burned Flood	44	2	3.58	2.04
Burned Recede	44	2	3.00	0.46
Not Burned Dry	44	2	2.64	0.75
Not Burned Flood	44	2	1.76	0.12
Not Burned Recede	44	2	2.63	0.49
Burned Dry	58	2	0.75	0.32
Burned Flood	58	2	3.32	2.56
Burned Recede	58	2	3.31	0.94
Not Burned Dry	58	2	1.60	1.04
Not Burned Flood	58	2	2.05	0.60
Not Burned Recede	58	2	2.67	1.00
Carbon:Nitrogen (%)				
Not Burned Dry	0	12	18.84	1.60
Burned Dry	0.5	6	33.68	5.81
Not Burned Dry	2	2	21.83	3.00
Not Burned Flood	2	4	18.97	1.50
Burned Dry	4	2	18.08	2.68
Burned Flood	4	4	19.67	3.53
Not Burned Dry	4	2	20.69	2.55
Not Burned Flood	4	4	19.38	0.97
Burned Dry	9	2	20.09	3.24
Burned Flood	9	4	14.78	1.44
Not Burned Dry	9	2	21.01	4.32
Not Burned Flood	9	4	19.23	1.51
Burned Dry	16	2	25.87	5.74
Burned Flood	16	2	39.66	24.57
Burned Recede	16	2	19.25	2.57
Not Burned Dry	16	2	27.10	11.13
Not Burned Flood	16	2	17.76	5.85
Not Burned Recede	16	2	20.28	4.95
Burned Dry	23	2	29.38	12.42
Burned Flood	23	2	18.33	5.02
Burned Recede	23	2	15.88	0.02
Not Burned Dry	23	2	20.54	3.89
Not Burned Flood	23	2	18.72	0.21
Not Burned Recede	23	2	17.90	4.90
Burned Dry	30	2	36.68	20.00
Burned Flood	30	2	48.66	36.09
Burned Recede	30	2	13.96	2.09
Not Burned Dry	30	2	21.14	0.09
Not Burned Flood	30	2	15.89	2.74
Not Burned Recede	30	2	18.66	4.83
Burned Dry	44	2	14.29	3.05
Burned Flood	44	2	15.12	3.05
Burned Recede	44	2	18.35	1.13
Not Burned Dry	44	2	22.94	7.27
Not Burned Flood	44	2	15.91	1.65
Not Burned Recede	44	2	19.96	3.23

Soil Parameter Measured	Days Post Burn	N	Mean	SE
Burned Dry	58	2	N/A	N/A
Burned Flood	58	2	16.92	5.73
Burned Recede	58	2	15.22	0.31
Not Burned Dry	58	2	27.09	0.66
Not Burned Flood	58	2	19.17	4.00
Not Burned Recede	58	2	18.30	0.81