SOIL MICROBIAL COMMUNITY RESPONSES FOLLOWING SEVERE WILDFIRES IN THE LINVILLE GORGE, BURKE COUNTY, NC

A Thesis
by
MICHAEL SCOTT HUFFMAN

Submitted to the Graduate School
at Appalachian State University
in partial fulfillment of the requirements for the degree of
MASTER OF SCIENCE

August 2015
Department of Biology

SOIL MICROBIAL COMMUNITY RESPONSES FOLLOWING SEVERE WILDFIRES IN THE LINVILLE GORGE, BURKE COUNTY, NC

A Thesis
by
MICHAEL SCOTT HUFFMAN
August 2015

APPROVED BY:

_____
Dr. Michael D. Madritch
Chairperson, Thesis Committee

_____
Dr. Howard S. Neufeld
Member, Thesis Committee

_____
Dr. Ray S. Williams
Member, Thesis Committee

_____
Dr. Susan L. Edwards
Chairperson, Department of Biology

_____
Max C. Poole, Ph.D.
Dean, Cratis D. Williams School of Graduate Studies

Abstract

SOIL MICROBIAL COMMUNITY RESPONSES FOLLOWING SEVERE WILDFIRES IN THE LINVILLE GORGE, BURKE COUNTY, NC

Michael Huffman
B.S., Appalachian State University
M.S., Appalachian State University

Thesis Chairperson: Michael D. Madritch

Wildfires represent a significant disturbance to forested ecosystems of the southern Appalachian Mountains. The belowground community response to a wildfire is often linked to the chemical, physical, and biological properties of soils. Fundamental processes such as soil respiration are strongly influenced by changes in soil biogeochemical parameters caused by wildfire, and a number of studies have examined the belowground response to wildfire. However, due to the complex interactions between soil properties and processes, the microbial response is often unpredictable. While the impacts of wildfire on coarse soil microbial community metrics is well documented, and it is generally accepted that fire reduces microbial biomass, there remains a lack of knowledge with regards to the fine-scale composition of microbial communities impacted by fire. Forest soils are hyper-diverse, and these complex communities mediate critical environmental processes such as soil respiration and nutrient cycling. This study investigates the composition of soil bacterial communities in burned and unburned soils in an attempt to understand how these diverse communities respond to wildfire.

Since 2000 five severe wildfires have occurred in the Linville Gorge, providing a unique opportunity to examine the response of soil microbial communities and soil respiration rates following wildfires. This study had three main objectives: (1) to measure basic belowground biogeochemical parameters at paired burned and unburned sites in three burn areas (0, 7, and 14 years post-burn), (2) to describe and compare soil microbial community structure between burned and unburned soils, and (3) to analyze the relationship between functionally important microbial groups and soil biogeochemistry. Soil respiration, soil C, and soil N were all influenced by wildfires. Among all six sites, I observed 52,564 bacterial operational taxonomic units (BOTUs) across 21 distinct phyla and identified a core bacterial community comprised of 12,289 BOTUs that were shared among all sites. Fires decreased microbial diversity (inverse Simpson diversity, $P<0.001$) and reduced the abundance of nitrifying bacteria in the most recently burned soils. The results of this study indicate that wildfire resulted in changes to soil chemical parameters and caused a significant shift in bacterial community composition and reductions in overall diversity. However, a large portion of ubiquitous taxa persists in burned and unburned soils. I suggest that it is the small community of rare and unclassified taxa that are responsible for observed differences in soil biogeochemical metrics. The inability to classify a majority of the taxa that are unique to burned soils highlights the need for more studies that address the functional roles of soil communities.

Acknowledgments

I would first like to thank my advisor Dr. Michael D. Madritch for agreeing to chair this project, his interest and support in the development and completion of this project, and his valued comments, suggestions, and advice throughout this research. I thank my other committee members, Dr. Howard Neufeld and Dr. Ray Williams for their advice, comments and suggestions. I extend a great thanks to Dr. Matt Estep and Dr. Patrick Schloss as well as to Sarah Westcott for the helpful assistance provided during sequence analysis and processing.

This thesis was supported by the Cratis D. Williams Graduate School, the Appalachian State University Biology Department, and the Grandfather Ranger district of the U.S.D.A Forest Service.

Dedication

I would like to dedicate this thesis to my family who have always supported and believed in me. I cannot express my appreciation enough for all of their words of encouragement and support in all I have accomplished. And finally to my Pop, for instilling in me the importance of education and always expressing interest in discussing these “funny little soil creatures.”

Table of Contents

List of Tables

List of Figures

Foreword

This thesis will be submitted to the journal of *Soil Biology and Biogeochemistry,* an international peer-reviewed journal owned by Elsevier and published by Elsevier Ltd.; it has been formatted according to the style guide for that journal.

1. Introduction

1.1. Community interactions

Soil microbes play an integral role in critical ecosystem processes including nutrient cycling, energy flow, and decomposition of organic matter. Microbial communities are particularly sensitive to minor changes in the physical, chemical, and biological properties of soils (Hernandez et al., 1997; Neary, 1999; Ginzburg et al., 2012**,** DeBano, 1991**;** Van Der Heijden et al., 2008). By mediating decomposition and nutrient cycling, soil microbial communities directly influence the rate of CO_2 efflux from soils to the atmosphere, hereafter referred to as soil respiration (Buchmann, 2000**;** Cisneros-Dozal et al., 2006; Hanson et al., 2000). Soil respiration constitutes the second largest terrestrial flux of carbon to the atmosphere after net primary production (Raich and Schlesinger, 1992**;** Houghton and Woodwell, 1989). Carbon released via respiration in temperate deciduous forests alone is estimated at 1060 g $C/m^{-2}/yr^{-1}$ and this flux accounts for 20% of the global CO_2 transfer (98 Gt C/yr) to the atmosphere, while fossil fuel burning contributes about 32 Gt C/yr (Luyssaert et al., 2007; IPCC, 2007). Consequently, small changes in soil respiration rates could have significant impacts on atmospheric CO_2 concentrations (Houghton and Woodwell, 1989; Raich Schlesinger, 1992; Bond-Lamberty et al., 2010).

Wildfires are an important mechanism of disturbance and have the potential to alter microbial communities and soil properties, including soil respiration (Neary, 1999**;** Gonzalez-Perez, 2004**;** Certini, 2005**;** Knoepp et al., 2008**;** Wang et al., 2012). The impacts of wildfires on belowground systems are primarily the result of the direct heat transfer to soils, combustion of living plant material, reduction of soil organic matter, as well as increased erosion of topsoil and loss of fine root biomass (DeBano, 1991**;** Gonzales-Perez et al., 2004**;** Certini, 2005). The response of an ecosystem to wildfire is influenced by fire

severity, which is highly variable and heterogeneous across a burn area due to variations in topography, microclimate, fuel moisture levels, and a suite of other environmental factors (DeBano, 1991). This unpredictability makes it challenging to generate any broad conclusions about the effects of wildfire, and there is need for an integration of empirical field data on ecosystem dynamics from small-scale studies into landscape and regional scale models to utilize information at larger temporal and spatial scales. Future studies should aim to examine short and long-term ecosystem response to wildfires across a range of fire severities and spatial scales both large and small (Knoepp et al., 2008**;** Neary, 1999; Gonzalez-Perez et al., 2004; Certini, 2005**;** Restaino and Peterson, 2013). With the frequency and intensity of wildfires projected to increase due to climate change (Dale et al., 2001), it is imperative to understand how these fire disturbances will impact belowground ecological communities that are responsible for driving essential ecosystem services.

1.2. Wildfire

Wildfires were once a frequent disturbance in the Southern Appalachian Mountains and the common occurrence of wildfires shaped the vegetation structure, soil environment, and ecological dynamics of these forests, resulting in many fire-adapted ecosystems (Hart et al., 2005, Newell and Peet, 1998). Historical records indicate that Native Americans and early European settlers commonly implemented low-intensity controlled ground fires to increase productivity on agricultural lands, maintain landscape structure, and sustain historic fire regimes (Brose et al., 2001). The fire suppression movement began in the late 1890's under the direction of Gifford Pinchot, the founder of the Forest Service. Management strategies centered on complete suppression of all natural and human-caused wildfires resulting in longer fire return intervals (Fowler and Konopik, 2007). The

implementation of strict fire suppression strategies led to significant changes in wildfire regimes across much of the southeast U.S., initiating major changes in fire-adapted forest ecosystems (Brose et al., 2001). Decades of fire suppression led to large increases in fuel loads and shifts in vegetation structure for millions of hectares of North American forests. These changes have spurred increases in the frequency of large, high intensity wildfires. From 1960 to 1999 the area burned by wildfires averaged 1,642,000 ha annually, while between 1999 and 2003 that number increased 38% to an average of 2,271,000 ha, with over 1.5 billion dollars spent on fire suppression tactics (Stephens and Ruth 2005). It was not until the late 1960's that new research into fire ecology identified fire suppression as a policy that was adversely affecting forest habitats (Stephens and Ruth 2005, Fowler and Konopik 2007).

Wildfires constitute an important disturbance in forest ecosystems, providing a number of ecological services that aid in maintaining forest structure. However the effect of a wildfire is dependent on the severity of the fire (Certini, 2005**;** Knoepp et al., 2008). Prescribed, or controlled fires, with low to moderate severity burning, can be beneficial to the ecosystem without causing permanent changes to the vegetation or soil properties. These fires often act to reduce competition in the understory, remove invasive species, and recycle nutrients to the soil. Typically, low to moderate severity fires do not transfer large amounts of heat energy below the surface of the soil, leaving much of the rhizosphere intact, promoting re-sprouting of vegetation and recolonization of the soil by microflora that survived the burn (Neary, 2008). In contrast, large intense fires can be detrimental to an ecosystem, leading to long-lived and sometimes permanent alterations (Neary et al., 2008). Extreme temperatures reached during high severity wildfires can transfer substantial amounts of heat energy to the soil while also eliminating all living and dead organic matter

at the surface. Aboveground mortality coupled with heat-induced mortality in soils often leads to significant reductions in root and microbial biomass, changes in water repellency and infiltration rates, elevated soil temperatures, and chemical alteration to soils through deposition, combustion, and volatilization of organic compounds (Neary, 1999).

1.3. Below-ground microbial processes

Soils are the basis for all terrestrial life, providing essential nutrients, water, and substrate required for vegetation to thrive (Knoepp et al., 2008). Soils also offer a vital ecosystem service as carbon reservoirs, representing the largest terrestrial pool of organic carbon globally with total storage estimated to be 2011 Gt of carbon and forest soils alone containing >750 Gt of C (Batjes, 1996**;** Raich and Schlesinger, 1992; IPCC, 2007). Soil processes are linked to the physical, chemical, mineralogical, and biological composition of the soil, all of which are susceptible to alterations when exposed to wildfires. Modifications of soil properties caused by wildfire are driven by the combustion of organic matter at the soil surface and the direct transfer of heat to the soil (Knoepp et al., 2008; Gonzalez-Perez et al. 2004). Respiration rates vary based on several factors including land-use history, soil physical parameters, vegetation type, disturbance, and regional climate. However, the influence of soil moisture and temperature often overshadows all other factors (Schlesinger, 1977; Bond-Lamberty, 2004**;** Cleveland et al, 2007). Total soil respiration is a product of autotrophic root respiration, associated rhizosphere organisms, and respiration from heterotrophic bacteria and fungi present in the organic and mineral soil horizons. A number of studies have partitioned respiration into heterotrophic and autotrophic sources, and the majority of research indicates that microbial respiration dominates total soil CO_2 efflux in forest soils (Buchmann, 2000**;** Hanson et al., 2000**;** Cisneros-Dozal et al., 2006).

Soil bacterial and fungal microbes directly regulate the transfer of carbon from terrestrial ecosystems via soil respiration, decomposition of organic matter, and nutrient cycling (Dooley and Treseder, 2012). Additionally, these microbes are sensitive to minor alterations to soil properties that are typically associated with wildfires. First and foremost, fires impact the soil micro-fauna through direct heating during the burn event and directly following a wildfire. The primary effect of heating on soil organisms is a reduction in microbial biomass (DeBano, 1999). Peak temperatures reached during a wildfire are directly influenced by fire severity and typically exceed 200°C in the upper soil horizons (Weber et al., 2014). In the most extreme cases, temperatures have been recorded at > 500°C on the forest floor, well above the lethal temperature threshold of bacterial or fungal microbes (Neary et al., 2008). Soil heating experiments in both the laboratory and field indicate fatal temperatures for most microbes are less than 100°C (Dunn et al., 1985). However, the inherently heterogeneous nature of wildfire severity can result in variable responses of microbial biomass and abundance to soil heating caused by fires.

Several studies have demonstrated a pronounced negative effect of fire on belowground microbial biomass (Banning, 2008**;** Dooley and Treseder, 2012; Makita-Barbato et al., 2015). For instance, Prieto-Fernandez et al. (1998) reported an almost complete loss of the microbial community directly following a wildfire, with an average reduction of 65% in microbial C and N two years after the fire. However, Hamman et al. (2007) quantified the effects of low- and high-severity fires on environmental variables commonly associated with limiting microbial activity and observed no significant changes in microbial biomass following a fire but rather a shift in microbial community structure that was correlated with changes in soil moisture, pH, and soil carbon exhibited in high-severity burn sites.

Soil respiration response to forest fires is mixed. In some cases soil respiration increases following wildfires, possibly from increased soil temperature, shifts in microbial community structure, and/or priming of microbial activity through nutrient inputs (Cleveland et al. 2007). However, some researchers have reported no significant changes or decreased soil respiration as a result of altered soil chemistry, reduced soil moisture capacity, and diminished root and microbial activity (Hernandez 1997**,** Wang et al. 2012). Banning et al. (2008) heated soils to between 90 and 120°C and observed significant decreases in microbial biomass and respiration when compared with unheated controls but found no effect of heating on total microbial community structure as measured via microbial metabolite profiles. The results of these studies indicate that wildfires reduce microbial biomass and abundance, and the extent of the reduction is largely dependent on the severity of the fire.

Direct heat-induced mortality of soil microbes can result in significant negative impacts to the microbial community; however, heating of soils is usually short-lived following a fire. Equally important to the overall recovery of the microbial community is the effect that fire has on soil nutrients such as carbon and nitrogen availability. High temperatures reached during severe fires can alter C and N pools and reduce soil organic matter (SOM) (Hart et al., 2005). The clearest impacts of wildfires on soils is the loss of SOM and reduction of the litter layer through volatilization of minor constituents or complete oxidation. Combustion of the organic layer can have a fertilizing effect directly following a wildfire, releasing large quantities of stored carbon and nitrogen into the uppermost soil layers, thereby stimulating microbial activity (Choromanksa et al., 2002; Gonzalez-Perez et al., 2004; Kara et al. 2009). Prieto- Fernandez et al. (1998) found that shortly following a wildfire, the organic C and organic N both increased several-fold and

remained higher than the levels in unburned soils; however, microbial C and N were both significantly lower in burned soils compared to unburned. Consequently, while fires appear to increase C and N in recently burned soils, these increases are not always sufficient to stimulate microbial activity, which suggests that nutrient availability is not the only factor influencing microbial recovery and that direct effects of fire on microbes may be too significant to overcome in burned soils.

Changes in fire severity result in significantly altered environmental conditions, potentially influencing the survival and growth of certain fractions of the soil's microbial community (Hamman et al., 2007). Wildfires can increase primary production and maintain aboveground biodiversity by promoting seedling regeneration and reducing overcrowding in the understory (Wade, 2000). It appears that while fires positively affect the aboveground communities, the impacts to belowground microbial communities are negative, leading to immediate reductions in microbial activity, biomass, and abundance following a severe fire by reducing microbial biomass while concurrently altering soil physical and chemical characteristics. However, the specific effects of fire on fine-scale belowground microbial communities remain largely unknown.

The objective of this study was to examine the response of soil microbial communities and their influence on soil respiration following wildfires in the Linville Gorge of the southern Appalachian Mountains. My goal was to increase the understanding of how these complex communities respond to wildfires and how this response may be linked to observable changes in belowground biogeochemical processes by evaluating soil respiration, extracellular enzymatic activity, and microbial community composition. My main hypothesis is that recently burned soils will support a significantly different microbial community and that these differences would be correlated with biogeochemical indices.

2. Methods

2.1. Sampling site

Linville Gorge is located in Burke County, North Carolina, USA, within the Grandfather Ranger district of the Pisgah National Forest (Figure 1). Elevations range from 400 m at the riverbed to 1,250m on the upper ridges. Precipitation is highest in the summer months and averages 1,250 to 1,625 mm annually. Peak temperatures occur during June to August (14-17^{o}C average minimum, 21-27^{o}C average maximum) with the coolest temperatures occurring in February (-2 to 0^{o}C average minimum, 8-12^{o}C average maximum) based on data from nearby weather stations (Morganton, Boone, Blowing Rock, and Grandfather Mountain, NC, weather stations) (Newell and Peet, 1998).

Upper ridgelines and summits have Typic or Lithic Dystrochrepts soils in the Ashe, Buladean, and Chestnut soil series. These soils are characterized as being deep, excessively to well drained, with a loamy surface and a loamy subsoil. They were formed from weathering of gneiss in the Blue Ridge geological province and are found on moderately sloping to very steep mountain uplands. All three soil series are strongly to moderately acidic, have very high to medium water infiltration rates, and low cation exchange capacities (Knight, 2006).

Due to its rugged topography, only about 5% of Linville Gorge has experienced logging, with the remainder relatively untouched. Thermic oak-pine communities dominate the upper ridges and slopes where this study was conducted. Historically, these communities experienced frequent, uncontrolled wildfires, with 7-12 yr average fire intervals (Newell and Peet, 1998). However, fire suppression practices led to the exclusion of large, uncontrolled wildfires prior to 2000. Since 2000, five large wildfires have occurred in the Linville Gorge. The Brushy Ridge Fire in 2000 burned approximately

10,000 ha; Pinnacle Fire in 2007 burned approximately 3,500 ha; Shortoff Fire also in 2007 burned approximately 3,200 ha; Sunrise Fire in 2008 burned approximately 3,200 ha; and most recently, the Table Rock Fire in 2013 burned approximately 2,600 ha. It is important to note that variations in topography and pre- vegetation structure in the Linville Gorge strongly influence the heterogeneity of fire severity across the landscape. Wimberley and Reilly (2007) reported that fire severity during the 2000 fire was highest on upper hillslopes and ridges, and this is where the sampling sites for this study were located to try to account for variations in fire severity. Nonetheless, the occurrence of these wildfires provides a unique opportunity to examine the belowground response of microbial communities to forest fires.

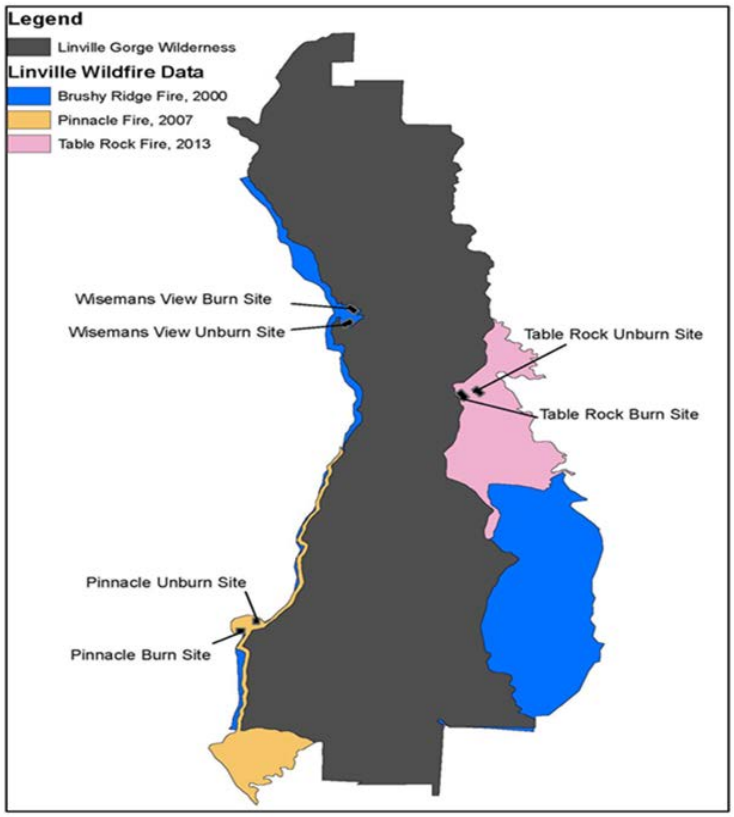

Figure 1: Site Map of sampling locations within the Linville Gorge, Burke County, NC.

2.2 Sampling Methods

The study region consisted of three areas that had been exposed to moderate to severe wildfires. Each burned sampling area was paired with an adjacent unburned control area that had not experienced a wildfire (N=6 plots). I identified sample areas using burn perimeter maps provided by the US Forest Service supported by visual indicators of a wildfire occurrence. Visual indicators included standing and fallen trees that had visible burn scars, blackened soils, ash deposition, and high productivity in the herbaceous layer. Each sampling plot was approximately 50x50 meters, with ten 2 m diameter circular subplots located randomly within the perimeter of each of the six plots. Burned and control plots were located within the perimeters of the Brushy Ridge (Wisemans's View area; WV), Pinnacle (PNCL), and Table Rock (TR) fire (Figure 1). Control plots were located <100m from burned plots to control for above ground community variation and had not experienced severe fires based on visual indicators. Permitting restrictions did not allow for sampling within the boundaries of the Linville Gorge Wilderness area, therefore all sampling plots were located outside of the Linville Gorge Wilderness Area boundary.

2.3. Soil Respiration, Soil Carbon, and Soil Nitrogen

All sampling took place during the summer of 2014 from June to August. This period is considered to be the peak stage of soil respiration and microbial activity (Raich and Schlesinger, 1992). For each subplot, soil respiration measurements were recorded at the center using a Li-COR 8100, yielding 60 measurements of soil respiration. Directly following respiration measurements, five soil samples from the top 15 cm of the soil horizon were collected using a soil corer. Between each sample, the corer was brushed with a wire brush and sterilized with ethanol to reduce the possibility of microbial contamination. Cores were taken at the center of each subplot and at four equally spaced

locations on the diameter of the subplot. The five cores from each subplot were pooled into a single sample, yielding 60 individual soil samples. Soils were stored in sterile Whirl-pac® bags in a cooler until returned to the lab. Once in the lab, soils were stored at -20ºC until further analysis. Soils were not sieved to reduce the possibility of cross contamination. Soil samples were subsampled for biogeochemical analyses. A portion of which was freeze dried for total C and N. Total C and N content of soils was determined via combustion analyses on a FlashEA 1112 NC Analyzer (Thermo Fisher Scientific, Waltham, MA)

2.4. Enzyme Activity

To characterize the functional microbial response to wildfires, the activity of six extracellular enzymes were measured the activity of in burned and unburned soils: cellobiohydrolase and β-glucosidase (involved with the degradation of cellulose), leucine aminopeptidase (involved with the degradation of proteins), phenol oxidase and peroxidase (involved with the degradation of aromatic compounds), and urease (degrades urea). Enzyme assays were based on protocols by Carriero et al.,(2000) and Saya-Cork et al. (2002) and are described in detail by Madritch et al. (2007). Commercially available substrates were used to ensure that enzyme assays were standardized and could be compared to other published studies.

2.5. DNA Extraction and Amplification

Composite DNA was extracted from approximately 0.5 g of pooled soil samples using MPBIO FastDNA™ SPIN Kit for Soils (MP Biomedical, Solon, OH) according to the manufacturer's instructions. Partial bacterial 16S rRNA genes were amplified from composite DNA samples by polymerase chain reaction (PCR) using primers 515 forward

and 806 reverse (Caporaso, 2012). To yield 25 µL reaction mixtures, 1 µL of approximately 20 ng/µL DNA template, 1 µL of each 5 µM primer, 12 µL of Nuclease-Free water (Quiagen, Hilden, Germany), and 10 µL Q5 High-Fidelity 2X Master Mix (New England BioLabs, Ipswich, MA) were added to each reaction mixture. The PCR cycling parameters for the bacterial partial 16S rRNA gene were denature at 94 ºC for 180 s, followed by 35 cycles of 94 ºC for 45 s, 55 ºC for 60 s, and 72 ºC for 90 s, followed by 600 s extension at 72 ºC. Positive and negative controls were used for each PCR reaction set. Following electrophoresis at 80 V for 20 min on 1%, 1X TAE agarose gel amplified products were separated and quantified using the Molecular Imager Gel Doc XR system (Bio-Rad Laboratories, Hercules, CA). Bands of interest were gel purified. The products were sent to the Genomics Core Facility in Manchester, WV, for sequence analysis. Sequences were analyzed with the Illumina MiSeq platform (Illumina Inc., San Diego, CA)

2.6. Sequence processing

Illumina MiSeq pair-end reads were processed using MOTHUR version 1.35.1 using the MOTHUR MiSeq standard operating procedure (Schloss et al. 2009). The Make.contigs command was used to trim primer sequences, indexing barcodes, illumina adapters, and finally to join forward and reverse reads into contigs. Screen.seqs was then used to screen sequences and to remove any sequence that was longer than 275bp, contained at least one ambiguous base, or had >8 nt homopolymers. Sequences that occurred more than once in the dataset were combined to leave only unique sequences. A customized SILVA database (Pruesse et al., 2007) was created using the pcr.seqs command with the start and stop positions of 13,862 and 23,444 respectively. These start and stop values correspond to the v4 region of hypervariable 16S rRNA amplified by 515f and 806r primers used in this study. All unique sequences were aligned to this customized

database. Aligned sequences were then screened to ensure that all reads were the same length. Clustering and OTU classification of unique sequences were done using cluster.split and the Ribosomal Database Project (RDP) taxonomic database release 9 (Cole et al. 2009). To control for differences in the number of sequence reads per sample, while still capturing the greatest amount of genetic diversity, the number of sequence reads in each sample was normalized by randomly subsampling of the group with the lowest number of reads (1242).

2.7. Statistical Analyses

Based on our sampling design, I lacked the statistical power to determine the effect of time since burn on belowground response. As year of burn is confounded with site, we concentrated our analyses on determining the belowground effects of fires and not time since burn. We employed nested analysis of variance (ANOVA) with fire treatment nested within area to determine the effect of fire and site on soil physical and chemical characteristics, with a significance threshold $p < 0.05$. For microbial community analysis, bacterial operational taxonomic units (BOTUs), were grouped by treatment and site to test for differences in community structure. MOTHUR was used to calculate abundance-based coverage estimates (ACE) of community richness, inverse Simpson indices, and test analysis of molecular variation (AMOVA) between burned and unburned groups. ACE estimates were used to correct for the occurrence of highly abundant BOTUs in our data set. Inverse Simpsons index of diversity is calculated by taking the reciprocal of Simpson's index (D). A higher value for the inverse Simpson index of diversity represents higher diversity in a community. Inverse Simpson is preferred over Shannon's index because it accounts for differences in sampling effort (Pielou, 1975) and places emphasis on abundant species (Pielou, 1975). A random forest algorithm was implemented to

identify the BOTUs that were responsible for driving the observed differences in soil communities (Schloss et al., 2009). Venn diagrams were created in MOTHUR to compare community variation among sites and between burned and unburned soils. MOTHUR also identified a core group of ubiquitous BOTUs present among all sites that I term the "core microbiome." Finally, I examined the correlation between functionally important groups of soil bacteria and soil chemical parameters between burned and unburned sites.

3. Results

3.1. Soil Respiration

Both site and burn history influenced soil respiration (Figure 2A). Soil respiration was lower in burned (B) than in unburned (UB) soils (Figure 2A). Wisemans View (WV) burn plot was burned 14 years ago and showed the highest respiration rates when compared with Table Rock (TR) and Pinnacle (PNCL) burn sites, and WV sites also exhibited the least amount of variation between treatments. The lowest levels of respiration were observed at the most recently burned, TR sites (Figure 2A).

3.2. Soil C, N, and enzyme activity

Both site and burn history influenced soil C content, with burning consistently increasing soil C (Figure 2B). The highest levels of soil C were observed at the PNCL burned site (20.2%). PNCL sites also exhibited the greatest variation between treatments, with PNCL unburned soil C being 10.2%. TR burn and unburned treatments showed the least amount of variation in soil C (8.9% and 9.4% respectively).

Site and burn history also influenced soil N content (Figure 2C). Similar to soil C, the highest soil N levels were found at the PNCL burned site (0.62%) with the next highest levels occurring in the TR unburned site (0.49%). WV and PNCL followed a similar trend with the burned sites containing higher levels of soil N than the unburned sites. As with soil C, both WV burned and unburned had the lowest soil N at 0.16% and 0.25%, respectively. While burning increased soil N at PNCL and WV, the TR burn sites had lower N than did TR unburned sites (Figure 2C).

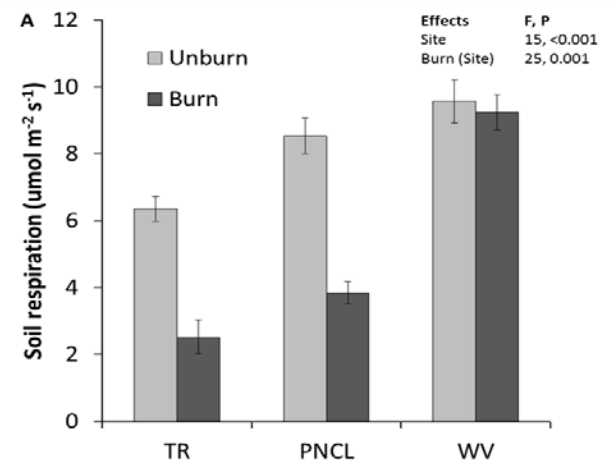

Figure 2A: Response of mean flux of soil respiration to burn history in Linville Gorge.

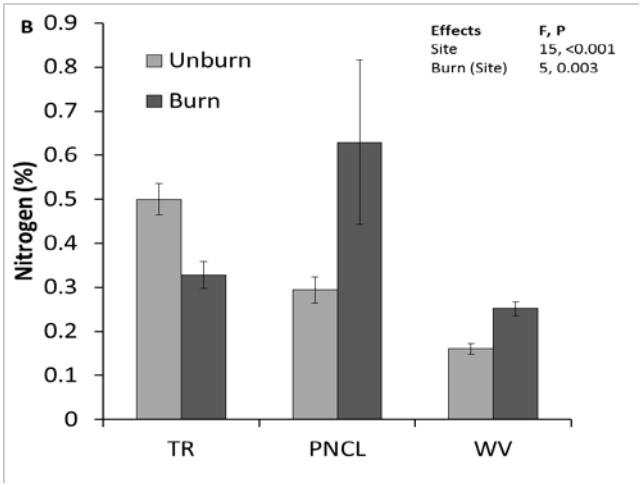

Figure 2B: Response of soil nitrogen to burning in Linville Gorge.

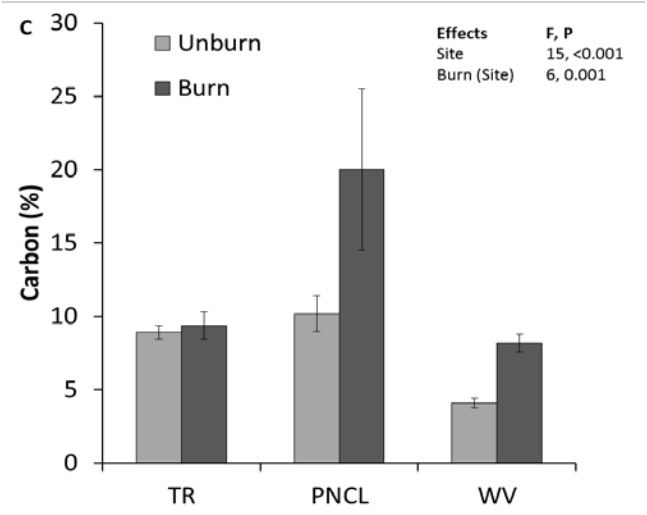

Figure 2C: Response of soil nitrogen to burn history in Linville Gorge.

Of the six enzymes analyzed, only urease and phenol oxidase activity showed a significant response to either site or burn history. Urease activity decreased in response to burning. WV exhibited the highest urease activity levels, followed by TR, and then PNCL (Figure 3A). Variation in phenol oxidase activity across sites was similar to that of variation in urease. However, phenol oxidase activity increased, rather than decreased, in response to burn history (Figure 3B).

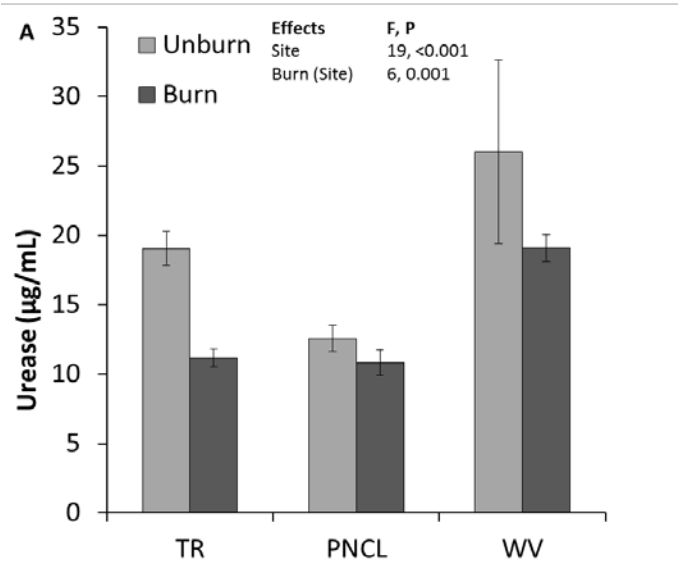

Figure 3A: Response of urease activity to burn history in Linville Gorge.

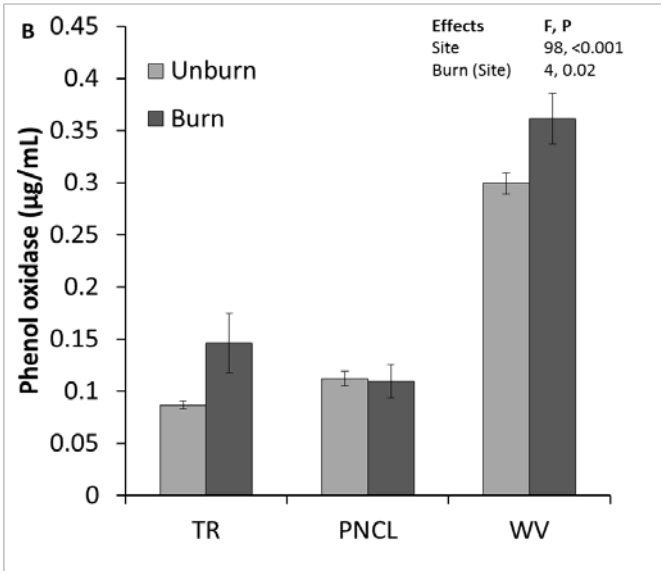

Figure 3B: Response of phenol oxidase activity to burn history in Linville Gorge.

3.3. Bacterial community analysis

For the 60 samples, 10.2 x 10^5 raw Illumina sequence reads were obtained at length of 275 nt before trimming. Of these, approximately 2,000,000 sequences passed quality filtering and were used for analysis in MOTHUR. Sample coverage was estimated at approximately 95% for each site. A total of 52,564 bacterial operational taxonomic units (BOTUs) were observed among all samples with approximately 4,500 OTUs observed per site; 14% of all OTUs could not be classified beyond kingdom.

There was a significant effect of burn treatment on inverse Simpson's index of diversity (*P<0.001*) (Figure 4A), but burn treatment and site only showed a marginal effect on ACE estimates of community richness (*P=0.08*) with PNCL being the only group having a higher estimate of richness in the burned treatment compared to the unburned treatment (Figure 4B). The average number of OTUs observed at each site was not influenced by burn history (Figure 4C).

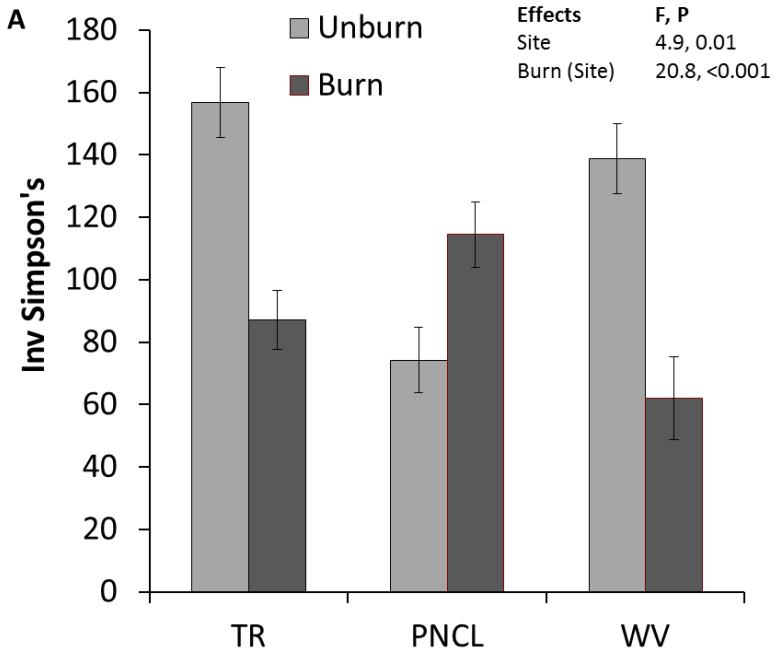

Figure 4A: Response of community diversity, as measured by Inverse simpson's index, to burn history in the Linville Gorge.

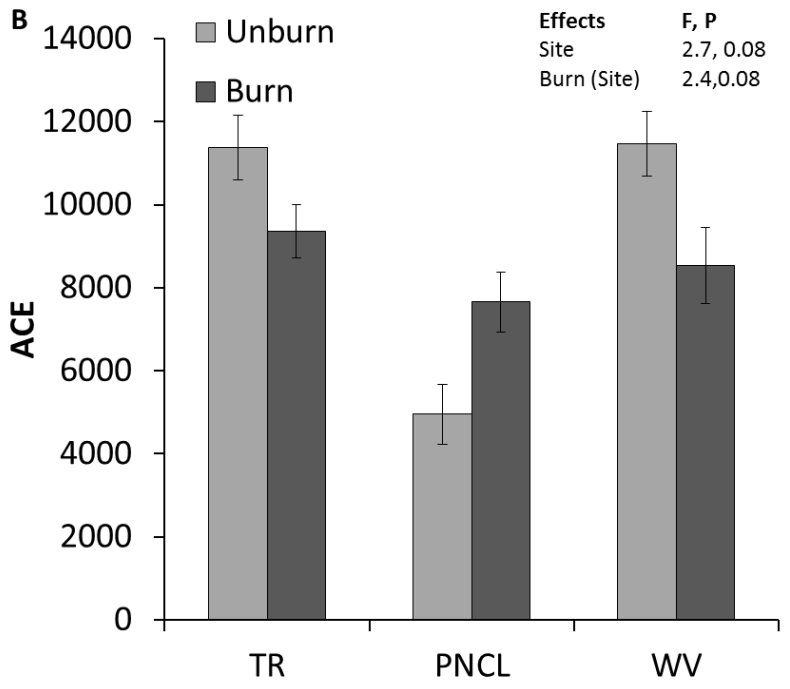

Figure 4B: Response of community abundance to burn history in the Linville Gorge.

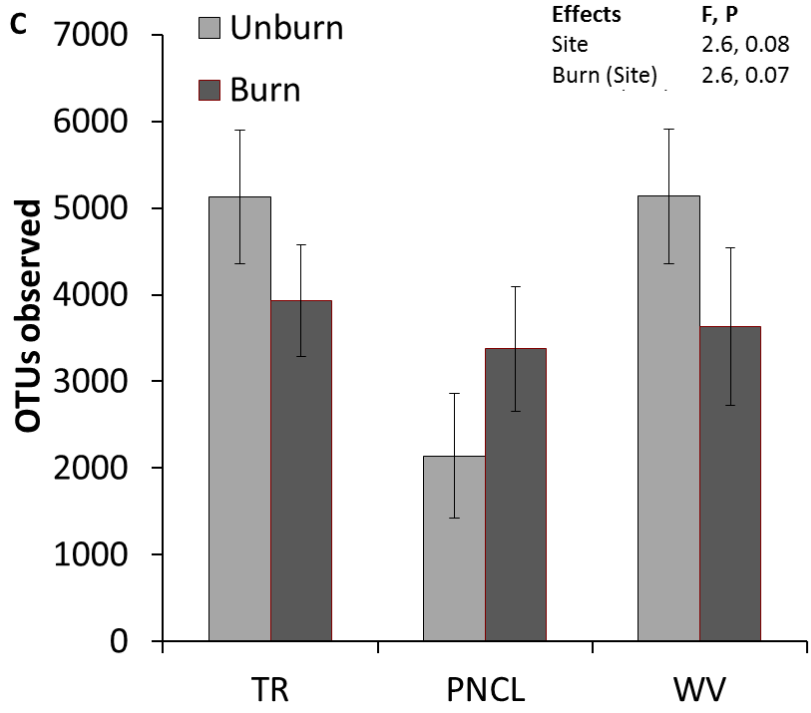

Figure 4C: Average number of BOTU's observed at each sampling location in Linville Gorge.

To test the hypothesis that wildfire alters community composition, sites were grouped into burned and unburned treatments. Community composition was significantly different between burned and unburned sites (AMOVA, *P=0.011*). In addition, nonmetric multidimensional scaling (NMDS) was performed on total community composition to determine if there was a difference in observed communities. Clustering of all six sites was observed, showing varying degrees of community separation based on both site and burn history (Figure 5).

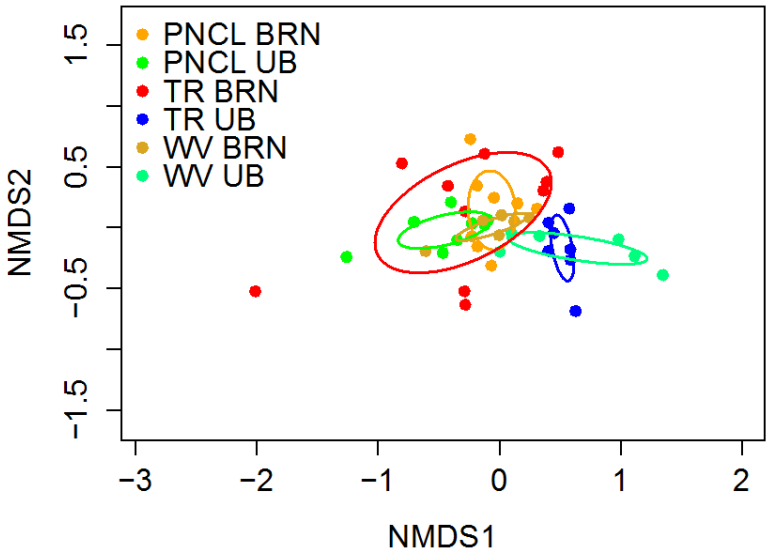

Figure 5: Non-metric multi-dimensional scaling of BOTU's, grouping of BOTU's varied among sites and between treatments.

Classified BOTUs belonged to 21 phyla among all sites and several dominant phyla were observed that included *Acidobacteria, Proteobacteria, Actinobacteria,* and *Bacteroidetes.* Three phyla, *Planctomycetes*, *Verrucomicrobia,* and *Chlamydiae* were grouped into a superphyla referred to as PVC (Kamneva et al., 2012), and comprised 11% of the total observed community. These 7 most abundant phyla accounted for 94.4% of sequences among all sites (Figure 6).

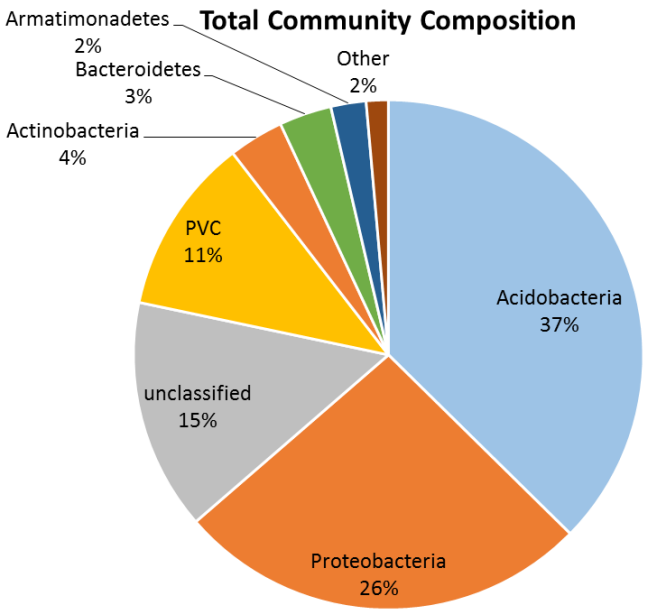

Figure 6: Total community composition of sampled soils in Linville Gorge.

Roughly a quarter, 23% (12,289) of all BOTUs were present at each site, and shared BOTUs accounted for 96% of sequence reads (a surrogate for abundance metric) among all sites (Figure 7A). Roughly three-quarters, 77% of total observed BOTUs were not shared between all sites (Figure 7B) but accounted for only 4% of sequence reads among all sites. Therefore, a small portion of sequence reads from all sites (<5%) accounted for nearly 80% of the variation among sites for each site (Figure 8A-8B)

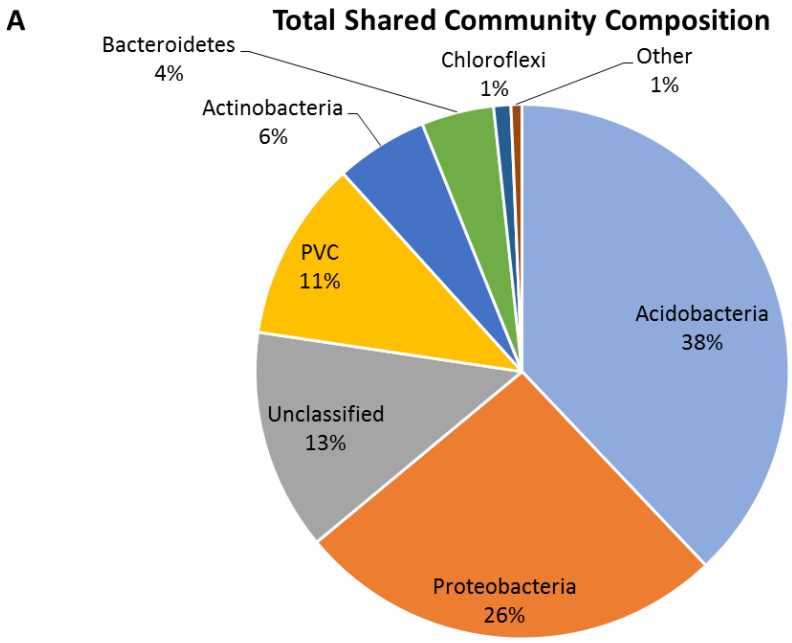

Figure 7A: Community composition of shared BOTU's among all sampling locations in Linville Gorge.

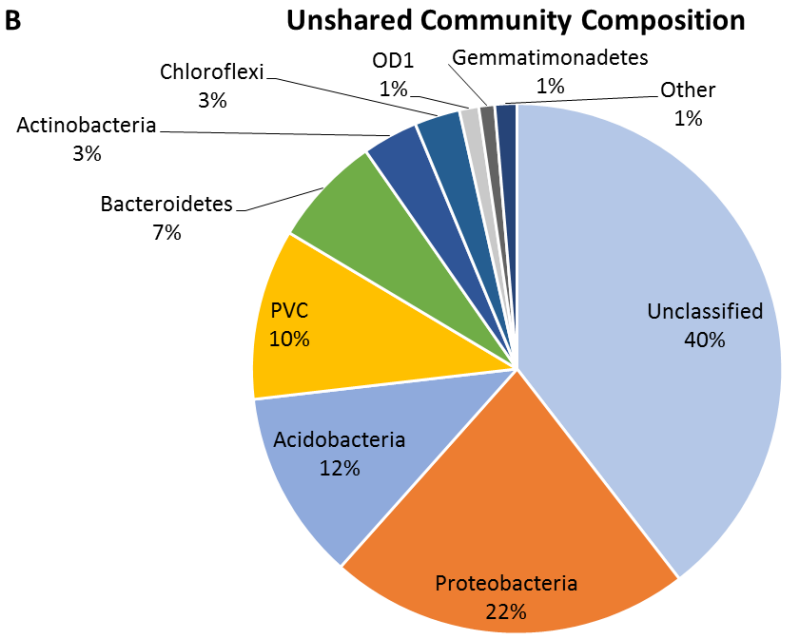

Figure 7B: Community composition of unshared BOTU's among all sampling locations in Linville Gorge.

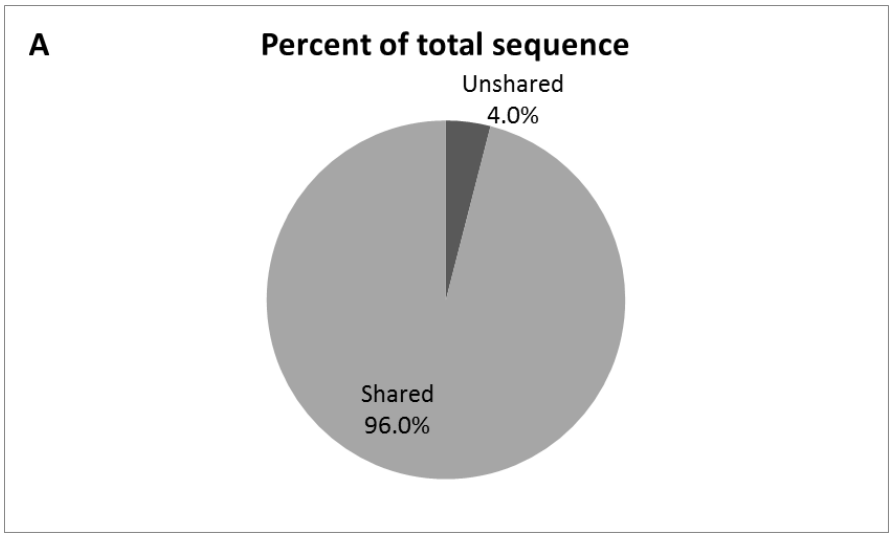

Figure 8A: Percentage of total sequence counts in shared and unshared communities.

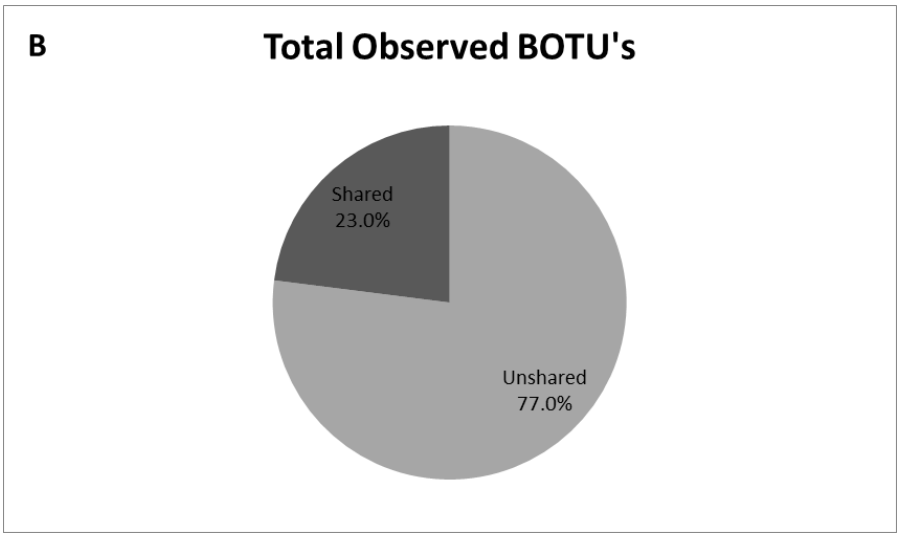

Figure 8B: Percentage of observed BOTU's present in shared and unshared communities.

I identified and summarized the microbial communities unique to unburned (Figure 9) and burned (Figure 11) treatments. Burned and unburned sites had a similar composition of unique BOTUs, and the unique communities of unshared taxa were dominated by Unclassified BOTUs (40%) (Figures 9A-9C, Figures 10A-10C). The WV unburned site had a large percentage of Bacteroidetes (15%) (Figure 9C), but this community shift was not observed at any other site. At the TR site there was a noticeable reduction in the functionally important nitrifying bacterial phyla *Nitrospira* in the burned site community when compared to the community of the unburned site (Figure 11). A random forest

algorithm identified unclassifed BOTUs as the most responsible for driving the observed differences between unburned and burned communities (Figure 12). The taxonomic classification of the eight most important OTUs that describe the difference between unburned and a burned treatment are provided in Table 1, and is again dominated by unclassified bacteria.

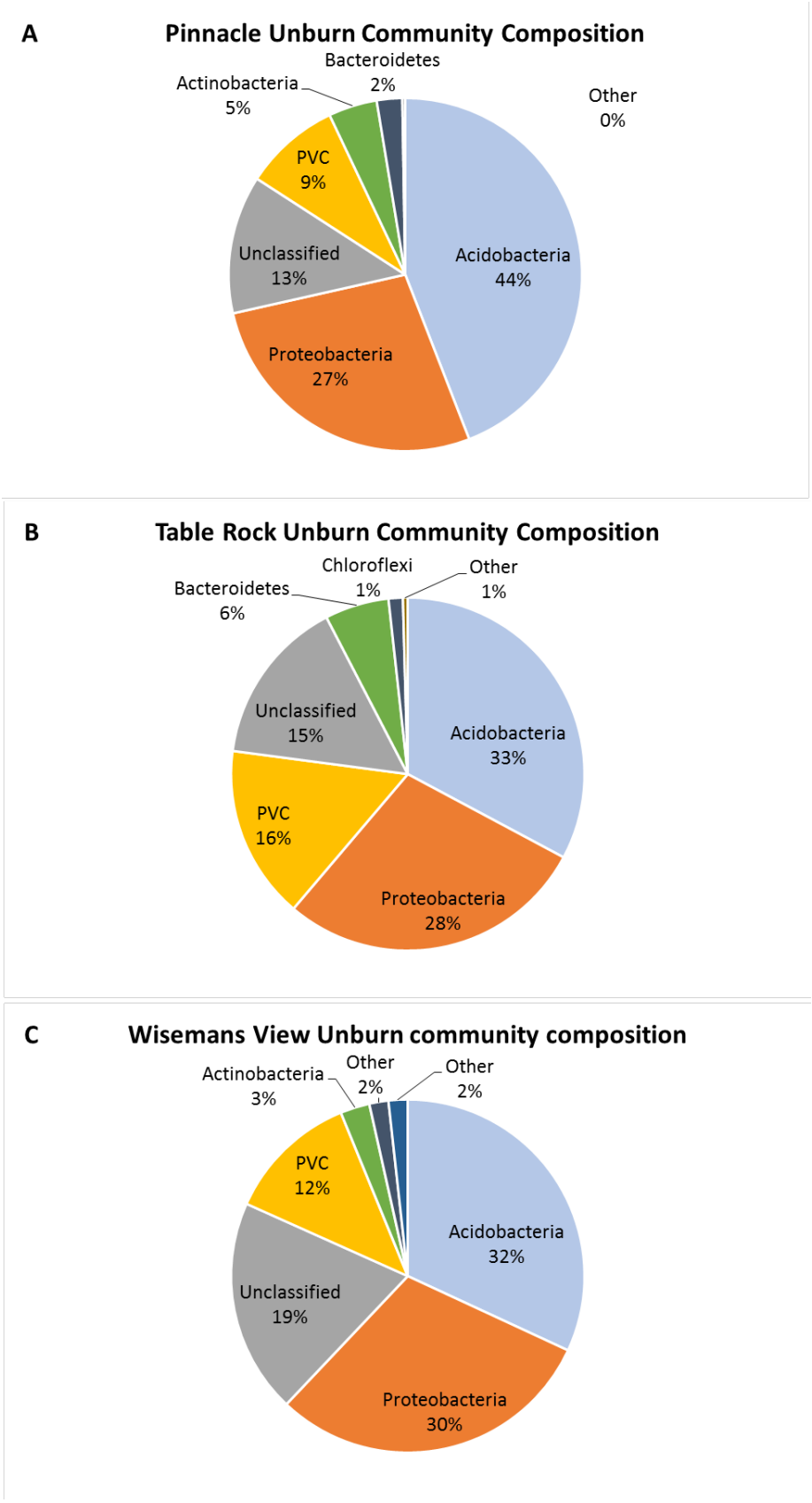

Figure 9A-9C: Taxonomic composition of unique BOTU's at unburned sampling locations in Linville Gorge.

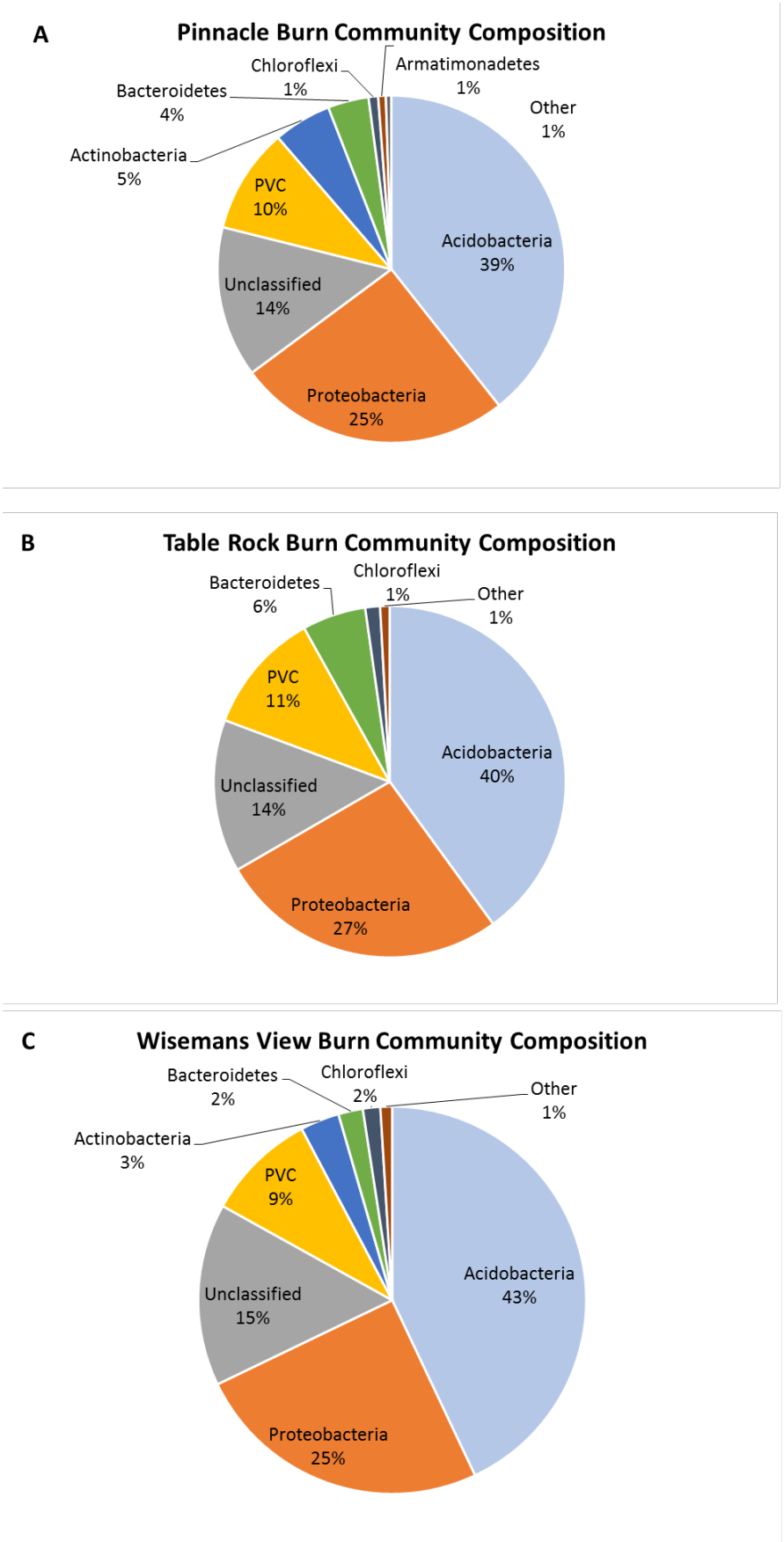

Figure 10A-10C: Taxonomic composition of unique BOTU's at burned sampling locations in Linville Gorge

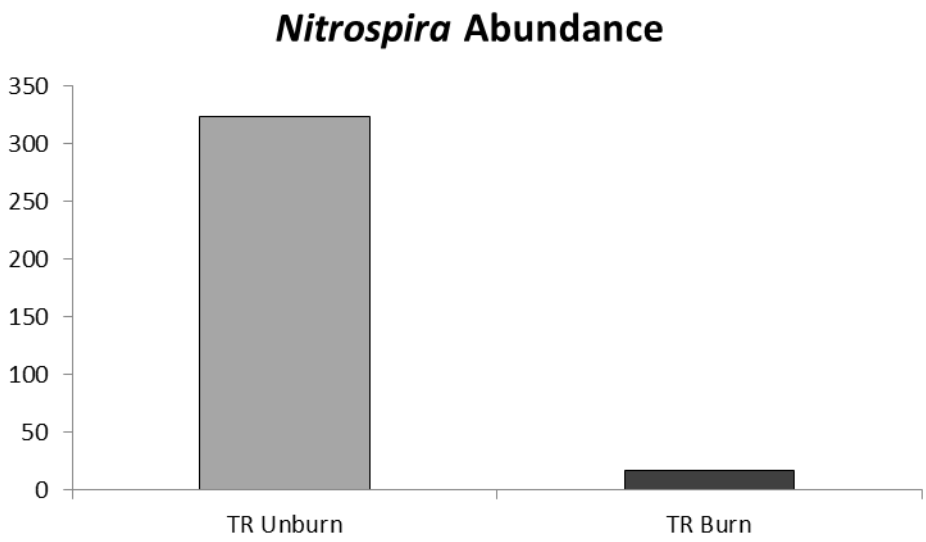

Figure 11: Shift in abundance of nitrifying bacteria between Table Rock burn and unburn sites

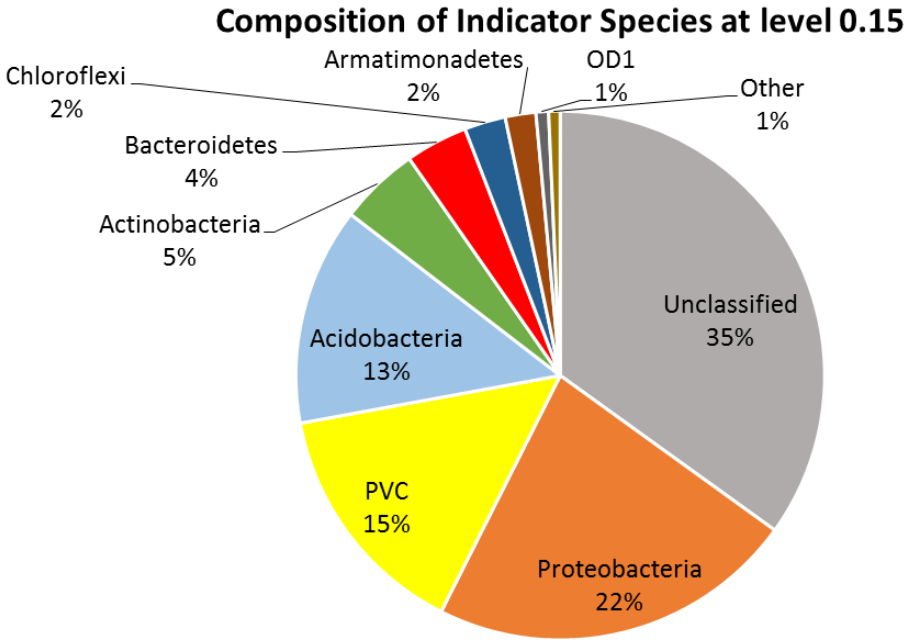

Figure 12: Taxonomic composition of indicator species driving differences in burned and unburned communities in Linville Gorge.

Table 1.

Taxonomy of 8 BOTU's responsible for the observed differences in burned and unburned communities in Linville Gorge.

BOTU	MDA	Phylum	Class	Order	Family	Genus
Otu006900	0.21	unclassified	unclassified	unclassified	unclassified	unclassified
Otu004865	0.20	Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiacaea	Chitinimonas
Otu015524	0.20	Actinobacteria	Actinobacteria	Solirubrobacterales	unclassified	unclassified
Otu004036	0.19	Bacteroidetes	Sphingobacteria	Sphingobacteriales	unclassified	unclassified
Otu002936	0.18	Actinobacteria	Actinobacteria	Acidimicrobiales	unclassified	unclassified
Otu003037	0.18	Chlamydiae	Chlamydiae	Chlamydiales	unclassified	unclassified
Otu006388	0.18	unclassified	unclassified	unclassified	unclassified	unclassified
Otu017207	0.18	unclassified	unclassified	unclassified	unclassified	unclassified

4. Discussion

4.1. Impacts of wildfire

It is well established that intense wildfires have significant effects on aboveground communities and processes (Reilly et. al., 2006**;** Hart et. al., 2005**;** Neary, 2000) and that fire can also have direct impacts on soil physical, chemical, and biological characteristics (Neary, 1999**;** Certini, 2005**;** DeBano, 1991; Dooley and Treseder, 2012). I have demonstrated that in addition to commonly measured coarse biogeochemical metrics, wildfire also has an important impact on soil microbial communities. The results of my study indicate that wildfire results both in significant changes to soil biogeochemistry and belowground bacterial communities.

4.2. Biogeochemical responses to fires

My study found that wildfire significantly reduced soil respiration, somewhat in agreement with previous investigations. In a meta-analysis of the response of soil respiration to fire in forest ecosystems Wang et al. (2012) reported that fire decreased soil respiration by 13.5%. Similarly, Hamman et al. (2007) observed significantly lower soil respiration rates in both low- and high-severity burn sites than in unburned areas, and Campany et al. (2006) also reported decreased soil respiration in burned plots within the Linville Gorge. Reductions in soil respiration following wildfire are likely a result of several interacting factors. First, heterotrophic microbes rely on the aboveground community to provide essential nutrients in the form of litter fall and root exudates into the rhizosphere. Severe wildfires directly impact the aboveground community via overstory mortality and reduction in organic matter, leading to altered soil processes (Ginzburg et al., 2012; Whitaker et al., 2014). The loss of over story species often leads to reduced root respiration and decreased C inputs through litter fall, likely causing surviving microbes to

become C limited. Reduced microbial activity, increased topsoil erosion from loss of fine roots, decreased autotrophic root respiration, and combustion of SOM leading to reduced substrate availability for surviving microbes likely decreased soil respiration in response to recent wildfires in the Linville Gorge.

My results indicate that fire increased soil C. While previous studies have also reported that fire increased soil organic C (Boerner et al., 2005), the effects of fire on soil C are not consistent. Several studies have noted decreased levels of C in burned soils (Neff et al., 2005; Pourreza et al., 2014; Holden et al., 2013), and other studies indicate little to no effect of fire (Hamman et al., 2007; Makita-Barbato et al., 2015). Inconsistencies in soil C responses to fire could be attributed to differences in fire severity, soil type, vegetation, and sampling methods among studies. The increased soil C observed here could be a result of C inputs in the form of charcoal following fires. However, increased soil C in my study was most pronounced in older burn sites, suggesting that soil C may be accumulating and remaining in the soil for several years after a fire. Soil C accumulation could be the result of decreased decomposition driven by reduced microbial activity. In a study of low-severity fire in Oak-Pine forests, Dumas et al. (2000) reported lower total respiration and decomposition rates in burned plots compared to unburned plots. Fire generally increased soil N in this study with elevated levels of N occurring in the older burned sites of PNCL and WV compared to unburned sites at PNCL and WV. In contrast, the most recently burned TR site had lower soil N when compared with the TR unburned site and this was correlated with a noticeable reduction in nitrifying bacteria at the TR burned sites. These results are consistent with the findings of Dunn et al. (1985), who reported high temperatures reached during wildfires directly impact nitrifying bacteria which are particularly sensitive to soil heating in comparison to other soil microbes. Certini (2005),

in a review of effects of fire on properties of forest soils, also reported that severe wildfire often reduces N directly following fire as a result of conversion of organic N to N_2 during combustion of organic matter and increased leaching in burned soils. Choromanska et al. (2002) reported that fire history has the potential to impact N levels with higher levels of N observed in soils that had previously been exposed to fire when compared with soils that had not experience burning. Similarly Hart et al. (2005) notes that in the years following a fire, N pools can surpass pre-fire levels if the successional community includes N-fixing species, but this response is dependent on the prompt plant recolonization in the burned area. My findings indicate that wildfires negatively impact functionally important nitrifying bacteria at one site following a burn and that this reduction is correlated with reduced soil N levels. There were no observable differences in the abundance of nitrifying bacteria among the PNCL and WV burned and unburned site, suggesting that the recovery period for this functionally important group is less than 7 years, the age of the intermediate burn site of PNCL.

Soil microbes produce extracellular enzymes in order to mineralize organic matter and release nutrients that would otherwise be inaccessible. Consequently, enzymatic activity can be used to assess the functional response of soil communities (Allison et al., 2005). Fire decreased urease activity at all sites and was lowest in the PNCL site, which also exhibited the highest levels of soil N. Reductions of nitrifying bacteria as a result of soil heating could be responsible for the observed decreases in urease, as this enzyme is associated with the hydrolysis of urea into carbon dioxide and ammonia (Xue et al., 2014). Hernandez et al. (1997) reported wildfire reduced urease activity, likely due to fact that wildfires decrease labile forms of N and increase recalcitrant forms. In contrast, Allison et al. (2005) suggest that an abundance of labile soil N could lead to decreased urease activity,

as a consequence of microbial economics whereby enzyme production is dependent on microbial demand and availability of N for enzyme synthesis.

Fire had the opposite effect on phenol oxidase activity and increased activity levels. Phenol oxidase production is associated with the degradation of lignin and other recalcitrant forms of soil organic matter and is an indicator of fungal activity (Baldrian, 2008). Boerner et al. (2005), also noted elevated phenol oxidase activity in burned soils, possibly resulting from changes in substrate type and availability following a wildfire.

While biogeochemical assays are a valuable tool for evaluating fire effects on the soil environment, they only provide a snapshot of current conditions at the time of sampling; and it is important to note that these metrics can respond to seasonal and temporal variation (Boerner et al., 2005; Hamman et al., 2008; Ginzburg et al., 2012). Nonetheless, by pairing biogeochemical parameters with the composition of bacterial soil communities, it is possible to understand the lasting effects of fire on soil communities.

4.3. Microbial community response to fire

Analysis of the bacterial community supported the hypothesis that the microbial community composition varies with burn treatment. Results of the AMOVA between burned and unburned groups indicated a difference in soil communities between the two treatments. The high BOTU count (mean 5,000 BOTUs per site across 21 distinct phyla) supports the concept that forest soils harbor hyper-diverse microbial communities. Marginal differences in ACE community richness estimates between burn and unburned sites indicate that burning decreases overall community richness. The phylum level composition among all sites was similar to those reported in other studies of soil bacterial communities (Janssen, 2006; Cleveland et al., 2007; Weber et al., 2014), with *Acidobacteria* and *Proteobacteria* representing the dominant phyla across all groups. The dominance of *Acidobacteria* is

expected as the soils in Linville Gorge are moderately to highly acidic (Knight, 2006), and the low pH in Linville soils is probably a major factor shaping the overall composition of these communities.

A core community of BOTUs was observed at all sites and likely represents the core microbiome for these soils. This core group that was shared among all site plots exhibited a similar composition to the overall microbial community with the dominant taxa being *Acidobacteria* and *Proteobacteria*. While these abundant taxa constitute a large portion of soil communities, relatively little is known about their functional roles in the environment (Fierer et al., 2007). The dominance of these abundant taxa in this core group suggests that these phyla are ubiquitous in soil environments and possibly resistant to disturbances, persisting even after the occurrence of wildfires. More importantly, of the over 50,000 observed BOTUs, 23% were shared among all sites. However, this relatively small number of BOTUs accounted for approximately 95% of the total sequences (a surrogate for abundance) observed at all sites. Therefore the unique communities that represent 76% of the total observed BOTUs accounted for only approximately 4% of the total observed sequences, 80% of the phylogenetic variation between site. The majority of these BOTUs are represented by rare taxa that only occur once in the dataset. When examining the composition of BOTUs that were unique to each site, there was a noticeable shift in community composition with *Acidobacteria* being replaced by unclassified bacteria as the dominant taxa. While the taxonomy and function of these microbes are largely unknown, these rare and unclassified microbes drive the observed differences in community composition between burned and unburned sites.

Analyzing communities by site revealed several distinct trends; first, the TR burn site contained the largest number of observed BOTUs; then, the WV burn; and finally,

PNCL burn had the lowest observed number of BOTUs. However, ACE and inverse Simpson diversity metrics were both highest in the PNCL burned site compared to the PNCL unburned site, while the opposite trend was observed at TR and WV sites. A possible explanation for this trend could be that in recently burned soils there is a greater reduction in microbial community richness, partially in response to the large reductions in soil microbial biomass that immediately follows a burn event (Dooley and Treseder, 2012). Wildfires may further reduce rare taxa that are sensitive to changes in the soil chemical and physical parameters, while abundant taxa that are adapted to a wider range of soil environments remain common. Furthermore, decreased C levels in recently burned soils may result in increased competition for limited nutrients favoring the dominance of abundant taxa. In contrast, the PNCL burn site exhibited elevated levels of soil C and N, and increased community diversity when compared to TR and WV burn sites. The longer recovery time at PNCL sites could be responsible for elevated soil nutrient levels and increases in soil nutrients and may be a factor influencing the observed increases in microbial diversity at the PNCL burn site. Other studies have shown correlations between nutrient availability, microbial activity, and microbial biomass (Barcenas-Moreno et al., 2011; Frey et al., 2008). Elevated soil C and N is a possible explanation for the higher inverse Simpson diversity at PNCLB sites compared to WV and TR. Similarly, low levels of both soil C and N at the WVB site could be limiting diversity in the microbial community.

Sequence data identified which BOTUs were driving the differences between burned and unburned sites and these taxa are described in Table 1. Of the eight most abundant BOTUs that differed most between treatments, three were unable to be classified and occurred 25 times. The second most important taxa was in the subgroup *beta-*

proteobacteria, which have been noted for their abundance in the early stages of root development (Weber et al. 2014). Root development was not examined in this study, but Miller (2000) reports that roots are susceptible to death directly following fires from direct heat-transfer to the soil and that root recovery may occur immediately following burning or could be delayed until the following spring depending on burn severity and specific plant physiology.

I report one of the few studies to date that compares bacterial communities from burned and unburned soils of similar vegetation types using the Illumina MiSeq platform. Weber et al. (2014) analyzed soil communities via high throughput amplicon sequencing of bacterial 16S rRNA gene fragments in burned and unburned soils but focused on differences related to variations in vegetation type. The overall community composition described here agrees with data reported in other studies of general soil communities (Janssen, 2006). However, a large portion of our sequences (13%) could not be classified beyond Kingdom, and these unclassified sequences were responsible for most of the observed differences in sampling sites. In a similar study of fungal communities of soils affected by wildfire, Oliver et al. (2015) noted that 15% of their sequence data could not be classified beyond the kingdom level and reported that recurring burns had no observable effect on richness or diversity of the fungal community but burned sites maintained a fungal community; that was distinct from unburned soils. While the relatively large proportion of unidentified BOTUs limits my ability to describe the function and physiology of the microbes driving the difference in communities, it does not detract from the results presented here.

4.4. Limitations

As the frequency and intensity of wildfires increases with climate change, there is a critical need to understand how fire disturbances will effect soil microbial communities and respiration. Severe wildfires are often more intense than are prescribed burns (Certini, 2005) and could have different impacts on soil ecosystems than those reported from controlled burns. Valuable information can be gained from studying the effects of natural wildfires. However, field studies of the effects of wildfires present several significant challenges. First, wildfires are unpredictable in location and timing, so the collection of pre-fire samples from paired burned and unburned sites is not possible. Second, because these fires are uncontrolled, it is not easily possible to generate replicated, randomized treatment plots. Due to these limitations, the approach used in this study was to sample soils from severely burned sites and adjacent non-burned sites within the Linville Gorge at several time points in locations where severe wildfires had been reported. In order to capture the natural variation at each sampling location, a number of soil samples (10) were collected from each location. The burned and unburned sites for each sampling location were located within 100m of each other, so they experienced the same climate conditions pre- and post- fire, and were nearly identical in topography, soil type, and above ground community composition. Therefore, we have used the differences in soil physical, chemical, and biological observed between sites to infer effects of wildfire.

The importance of fungal communities to ecosystem recovery following wildfires is well documented, and several studies have shown that fire has significant effects on fungal community composition (Van Der Heijden et al., 2008; Neary et al., 2005; Barcenas-Moreno et al., 2011; Holden et al., 2013).

This study, however, did not address the effect of fire on fungal communities. Nonetheless, bacterial communities are critical components in ecosystem recovery following wildfires and therefore were the primary focus of this study.

When comparing the recovery of above- and below-ground communities to wildfire, it is important to note the complex linkages between the two systems (Hart et al., 2005). The connections that drive plant-species linkages with soil communities center on the quantity and quality of resources produced and competition for nutrients (Wardle, 2002). Plants act as a C pump, providing heterotrophic soil microbes with organic matter via litter fall, root exudation and turnover, and growth substrates. In response, C stimulates microbial activity leading to increased availability of limiting nutrients to plants. However, the recovery of soil communities may lag behind that of plant communities. For example, Holden et al. (2013) reported that fungal hyphal length recovery lagged behind plant community recovery and was more closely related to levels of C in SOM, which can take up to 15 years to return to pre-fire levels (Treseder et al., 2004).

Soil microbial communities are hyper-diverse, and to date, relatively little is known about the ecological role of the majority of these organisms due to their resistance to standard laboratory culturing techniques (Tringe et al., 2005; Fierer et al., 2007). Based on this enormous belowground diversity, it is not surprising that I was unable to classify large numbers of the BOTUs responsible for the observed differences in sites beyond kingdom. However, it is also important to note that while burning resulted in changes to the bacteria community composition, these shifts may not necessarily affect ecosystem process rates. Because of the hyper-diverse nature of soil communities, there is likely a large amount of functional redundancy among microbial communities (Allison et al., 2008); and many of

the unclassified bacteria observed in burned sites likely operate in a similar functional capacity to the abundant taxa that were shared between all sites. Nonetheless, wildfire has a significant impact on soil community composition and respiration.

5. Conclusion

There is increasing recognition that microbes are important in ecosystem processes, and great progress has been made in characterizing the response of these organisms to wildfires. Despite these advances, microbial ecology still lacks a comprehensive understanding of the functional consequences of changes in microbial community composition. My findings indicate that wildfire in the Linville Gorge Wilderness Area of Burke County, NC, resulted in changes in the composition of bacterial soil communities; and a coarse examination of soil biogeochemical parameters revealed that wildfire also had significant effects on soil respiration, C, and N. I was able to correlate reductions in the abundance of nitrifying bacteria with decreased levels of soil N in recently burned soils. However, a large majority of the taxa observed in this study remained unchanged between burned and unburned plots (95% of total sequences); it is the small portion of rare taxa (4%) that are primarily impacted by fire disturbances. My inability to classify the majority of the community that was unique to each site highlights the need for more studies whose focus is to define the functional roles of these diverse soil communities and their response to disturbance. Increased efforts toward classification and assessment of the physiological response of microbial communities under controlled conditions would contribute greatly to our understanding of these complex communities.

References

Allison, S.D. Lu. Y., Weihe, C., Gulden, M.L., Martiny, J.B.H, Treseder, K.H., 2013. Microbial abundance and composition influence litter decomposition response to environmental change. Ecology 94.3, 714-725.

Allison, S.D., Martiny, J.B.H, 2008. Resistance, resilience, and redundancy in microbial communities. Proceedings of the National Academy of Science U.S.A 105, 11512-11519

Baldrian, P., Valášková, V., 2008. Degradation of cellulose by basidiomycetous fungi. FEMS microbiology reviews 32.3 501-521.

Banning N.C., Murphy D.V., 2008. Effect of heat-induced disturbance on microbial biomass and activity in forest soil and the relationship between disturbance effects and microbial community structure. Applied Soil Ecology. 40, 109–119.

Batjes, N.H., 1996. Total carbon and nitrogen in the soils of the world." European Journal of Soil Science 47.2, 151-163.

Barcenas-Moreno, G., Garcia-Orenes, F., Mataix-Solera, J., Mataix-Beneyto, J., Baath, E., 2011. Soil microbial recolonisation after a fire in a Mediterranean forest. Biology of Fertile Soils 47, 261-272.

Boerner R.E.J., Brinkman J.A., Smith A., 2005. Seasonal variations in enzyme activity and organic carbon in soil of a burned and unburned hardwood forest. Soil Biology and Biochemistry37, 1419–1426.

Bond-Lamberty, B., Wang, C., Gower, S.T., 2004. A global relationship between the heterotrophic and autotrophic components of soil respiration? Global Change Biology 10.10, 1756-1766.

Brose, P., Schuler, T., Van Lear, D., Berst, J., 2001. Bringing fire back: the changing regimes of the Appalachian mixed-oak forests. Journal of Forestry 99.11, 30-35.

Buchmann, N., 2000. Biotic and abiotic factors controlling soil respiration rates in Picea abies stands. Soil Biology and Biochemistry 32.11, 1625-1635.

Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Lozupone, C.A.,Turnbaugh, P.J., Fierer, N., Knight, R., 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proceedings of the National Academy of Science U.S.A. 108 (Suppl. 1), 4516-4522.

Carreiro, M.M., Sinsabaugh, R.L., Repert, D.A, Parkhurst D.F., 2000. Microbial enzyme shifts explain litter decay responses to simulated nitrogen deposition. Ecology 81.9, 2359-2365.

Certini, G., 2005. Effects of fire on properties of forest soils: a review. Oecologia 143,1-10.

Choromanska, U., DeLuca, T.H., 2001. Prescribed fire alters the impact of wildfire on biochemical properties in a ponderosa pine forest. Soil Science Society Of America Journal 65, 232-238.

Choromanska U., DeLuca, T.H., 2002. Microbial activity and nitrogen mineralization in forest mineral soils following heating: evaluation of post-fire effects. Soil Biology and Biochemistry 34, 263–271.

Cisneros-dozal, L.M., Trumbore, S., Hanson, P., 2006. Partitioning sources of soil- respired CO2 and their seasonal variation using a unique radiocarbon tracer. Global Change Biology 12.2, 194-204.

Cleveland, C.C., Nemergut, D.R., Schmidt, S.K., Townsend, A.R., 2007. Increases in soil respiration following labile carbon additions linked to rapid shifts in soil microbial community composition. Biogeochemistry 82.3, 229-240.

Dale, V.H., Joyce, L.A., McNulty, S., Neilson, R.P., Ayres, M.P., Flannigan, M.D., Hanson, P.J., Irland, L.C., Lugo, A.E., Peterson, C.J., Simberloff, D., Swanson, F.J., Stocks, B.J., Wotton, B.M., 2001. Climate change and forest disturbances: climate change can affect forests by altering the frequency, intensity, duration, and timing of fire, drought, introduced species, insect and pathogen outbreaks, hurricanes, windstorms, ice storms, or landslides. BioScience 51.9, 723-734.

DeBano, L.F., 1991. The effect of fire on soil properties. Proceedings management and productivity of western-Montane. Forest Soils.

DeBano L.F., Neary D.G., Elliott P.F., 1998. Fire's effects on ecosystems. New York: Wiley.

Dixon, R.K., Brown, S., Houghton, R.A., Solomon, A.M., Trexler, M.C., Wisniewski, J., 1994. Carbon pools and flux of global forest ecosystems. Science 263.5144, 185-190.

Dooley SR, Treseder KK. 2012. The effect of fire on microbial biomass: a meta-analysis of field studies. Biogeochemistry 109, 49–61.

Dunn, P.H., Barro, S.C., Poth, M., 1985. Soil moisture affects survival of microorganisms in heated chaparral soil. Soil Biology and Biochemistry 17, 143- 148.

Dumas, S., Neufeld H.S., Melany C.F. 2007. Fire in a thermic oak-pine forest in Linville Gorge Wilderness Area, North Carolina: importance of the shrub layer to ecosystem response. Castanea 72.2, 92-104.

Fierer, N., Bradford, M.A., Jackson, R.B., 2007. Toward an ecological classification of soil bacteria. Ecology 88, 1354-1364.

Fowler, C., Konopik, E., 2007. The history of fire in the southern United States. Human Ecology Review 14.2, 165-176.

Frey, S.D., Drijber, R., Smith, H., Melillo, J., 2008. Microbial biomass, functional capacity, and community structure after 12 years of soil warming. Soil Biololgy and Biochemistry 40.11, 2904-2907.

Ginzburg, O., Steinberger, Y., 2012. Effects of forest wildfire on soil microbial community activity and chemical components on a Temporal-seasonal scale. Plant Soil 360, 243-257.

González-Pérez, J.A., Gonzalez-Vila, G.A., Gonzalo, A., Knicker, H., 2004. The effect of fire on soil organic matter—a review. Environment International 30.6, 855-870.

Hamman, S.T., Burke, I.C., Stromberger, M.E., 2007. Relationships between microbial community structure and soil environmental conditions in a recently burned system. Soil Biology and Biochemistry 39, 1703-1711.

Hanson, P. J., et al. 2000. Separating root and soil microbial contributions to soil respiration: a review of methods and observations. Biogeochemistry 48.1, 115- 146.

Hart, S.C., DeLuca, T.H., Newman, S.G., MacKenzie, M.D., Boyle, S.I., 2005. Post-fire vegetative dynamics as drivers of microbial community structure and function in forest soils. Forest Ecology and Management 220,166–84.

Hernandez, T., Garcia, C., Reinhardt, I., 1997. Short-term effect of wildfire on the chemical, biochemical and microbiological Wildfire Effects on Boreal Soil Fungi 45 properties of Mediterranean pine forest soils. Biology of Fertile Soils 25,109 16.

Houghton, R. A., Woodwell, G.M., 1989. Global climatic change. Science America (United States) n. page. 260.4.

IPCC, 2007: Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change [Solomon, S., D. Qin, M. Manning, Z. Chen, M. Marquis, K.B. Averyt, M.Tignor and H.L. Miller (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Janssen, P.H., 2006. Identifying the dominant soil bacterial taxa in libraries of 16S rRNA and 16S rRNA genes. Applied Environmental Microbiology 72, 1719-1728.

Kara, O., Bolat, I., 2009. Short-term effects of wildfire on microbial biomass and abundance in black pine plantation soils in Turkey. Ecological Indicators 9, 1151– 1155

Knoepp, J. D., DeBano, L. F., Neary, D. G. 2008. Effects of fire on soil, soil chemical characteristics. Wildland Fire in Ecosystems: Effects of Fire on Soils and Water 4, 53-71.

Knoepp, J.D., Vose, J.M., Swank, W.T., 2004. Long-term soil responses to site preparation burning in the southern Appalachians. Forest Science 50, 540-550.

Mabuhay, J.A., Isagi, Y., Nakagoshi, N., 2006. Wildfire effects on microbial biomass and diversity in pine forests at three topographic positions. Ecological Restoration 21, 54-63.

Madritch, M.D., Lindroth, R.L., 2007. Soil microbial communities adapt to genetic variation in leaf litter inputs. Oikos 120.11, 1696-1704.

Mikita-Barbato, R.A., Kelly, J.J., Tate, R.L., 2015. Wildfire effects on the properties and microbial community structure of organic horizon soils in the New Jersey Pinelands. Soil Biology and Biochemistry 86, 67-76.

Neary, D.G., Klopatek, C.C., DeBano, L.F., Elliott, P.F., 1999. Fire effects on belowground sustainability: a review and synthesis. Forest Ecology and Management 122, 51-71.

Neff, J. C., Harden, J. W., Gleixner, G., 2005. Fire effects on soil organic matter content, composition, and nutrients in boreal interior Alaska. Canadian Journal of Forest Research 35.9, 2178-2187.

Newell, C.L., Peet, R.K., 1998. Vegetation of Linville Gorge Wilderness, North Carolina. Castanea 63, 275-322.

Pielou, E. C. 1975. Ecological Diversity, 8.

Pietikainen J, Fritze H. 1993. Microbial biomass and activity in the humus layer following burning: Short-term effects of two different fires. Canadian Journal of Forest Research 23,1275–85.

Prieto-Fernandez, A., Acea, M.J., Carballas, T., 1998. Soil microbial and extractable C and N after wildfire. Biology of Fertile Soils 27, 132-142.

Pourreza, M., Hosseini, S.M., Sinegani, A.A.S., Matinizadeh, M., Dick, W., 2014. Soil microbial activity in response to fire severity in Zagros oak (Quercus brantii Lindl.) forests, Iran, after one year. Geoderma 213, 95-102.

Raich, J.W., Schlesinger, W.H., 1992. The global carbon dioxide flux in soil respiration and its relationship to vegetation and climate. Tellus 44B, 81-99.

Restaino, J.C., Peterson, D.L., 2013. Wildfire and fuel treatment effects on forest carbon dynamics in the western United States. Forest Ecology and Management 303, 46-60.

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

Schlesinger, W.H., 1977. Carbon balance in terrestrial detritus. Annual Review of Ecology and Systematics 8, 51-81.

Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B.,

Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., Sahl, J.W., Stres, B.,Thallinger, G.G., Van Horn, D.J., Weber, C.F., 2009. Introducing mothur: opensource, platform-independent, community-supported software for describing and comparing microbial communities. Applied Environmental Microbiology 75, 7537-7541.

Stephens, S.L., Ruth, L.W., 2005. Federal forest-fire policy in the United States. Ecological Applications 15.2, 532-542.

Van Der Heijden, M.G.A., Bardgett, R.D., Van Straalen, N.M., 2008. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecology Letters 11.3, 296-310.

Wade, D.D., 2000. Chapter4: Fire in Eastern Ecosystems. Wildland Fire in Ecosystems: effects of fire on flora. 53-97.

Wang, Q., Zhong, M., Wang, S., 2012. A meta-analysis on the response of microbial biomass, dissolved organic matter, respiration, and N mineralization in mineral soil to fire in forest ecosystems. Forest Ecology and Management 271, 91-97.

Weber, C.F., Lockhart, J., Charaska, E., Aho, K., Lohse, K.A., 2014. Bacterial composition of soils in ponderosa pine and mixed conifer forests exposed to different wildfire burn severity. Soil Biology and Biochemistry 69, 242-250.

Wimberly, M.C., Reilly, J.M., 2007. Assessment of fire severity and species diversity in the southern Appalachians using Landsat TM and ETM+ imagery. Remote Sensing of Environment 108.2, 189-197.

Figure Legend

Figure 1: Site Map of sampling locations within the Linville Gorge, Burke County, NC.

Figure 2: Carbon and nitrogen responses to burn history in Linville Gorge. Mean flux of soil respiration (2A), mean percent soil carbon (2B), and mean percent soil N (2C) varied among sites and between burn treatments. Bars represent the mean of 10 subplots and error bars represent standard error.

Figure 3: Extracellular enzyme activity response to burn history in Linville Gorge. Mean urease activity (3A) and mean phenol oxidase activity (3B) varied among sites and between burn treatments. Bars represent the mean of 10 subplots and error bars represent standard error.

Figure 4: Community richness and diversity metrics in response to burn history in Linville. Inverse Simpson diversity index values (4A) Abundance-Corrected Estimates (ACE) (4B), and Mean observed BOTUs (4C) varied among sites and between burn treatments. Bars represent the mean of 10 subplots and error bars represent standard error.

Figure 5: Nonmetric multidimensional scaling of BOTUs, grouping of BOTUs varied among sites and between burn treatments.

Figure 6: Total community composition of sampled soils in the Linville Gorge. Both sequence counts (6A) and taxonomic composition (6B) of observed BOTU's. Acidobacteria and Proteobacteria dominated sequences.

Figure 7: Community composition of shared BOTUs and unshared BOTUs among all sampling locations in Linville Gorge. Taxonomic composition of BOTUs shared at all sampling locations (7A) and those BOTUs not observed among all sites (7B) were dominated by acidobacteria, proteobacteria, and unclassified bacteria.

Vita

Michael Scott Huffman was born in Garner, North Carolina, to Karen Edwards and Scott Huffman. He graduated from Asheville High School in June of 2008. That August, he entered Appalachian State University; and in December of 2012, he was awarded the Bachelor of Science degree. In the fall of 2013, he accepted a teaching assistantship in the Department of Biology at Appalachian State University and began study toward a Master of Science degree. The M.S. was awarded in August 2015.