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Nitrogen Cycling and Spatial Heterogeneity Following Fire and
Restoration Treatments in the Ponderosa Pine/Douglas-fir Ecosystem

By:

Michael J. Gundale

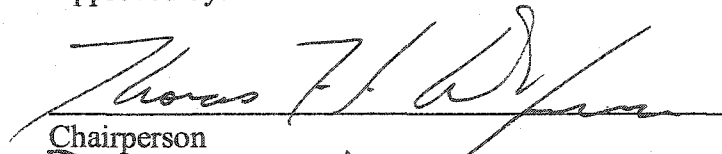B.S. University of Montana, Missoula, MT, 1996

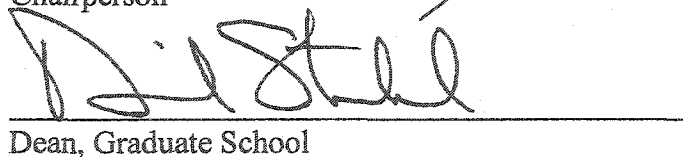M.S. Michigan Technological University, Houghton, MI, 2001

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Department of Ecosystem and Conservation Sciences
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Michael J. Gundale, Ph.D., April 2005   College of Forestry and Conservation

Nitrogen Cycling and Spatial Heterogeneity Following Fire and Restoration Treatments in the Ponderosa Pine/Douglas-fir Ecosystem

Chairperson: Thomas H. DeLuca *THD*

Lower elevation ponderosa pine ecosystems of the Rocky Mountain West (U.S.) historically experienced a frequent, low-intensity fire regime that promoted dominance of large diameter ponderosa pine (*Pinus ponderosa*). An abrupt change in this historical disturbance regime occurred upon Euro-American settlement of the West in the late 1800s and early 1900s. A century of fire exclusion likely allowed less fire-tolerant species to become more dominant and C rich organic matter to accumulate. Some investigators hypothesize that these changes in forest structure and composition have resulted in reduced nutrient turnover relative to historical conditions. Land managers throughout the West are introducing surrogates of natural disturbance into the ponderosa pine community in an effort to reduce the risk of stand replacing wildfire and to restore historical stand structure and function. Within this dissertation I present an introduction and four manuscripts of original research that focus on how fire and restoration treatments influence various aspects of ecosystem function, with emphasis on the internal N cycle. In the first manuscript, I report numerous soil physical, chemical and biological parameters measured following four restoration treatments, and find that N, more than any other soil parameter, was influenced by restoration treatments. In the second manuscript I determine that spatial heterogeneity of available N following restoration treatments has a positive relationship with understory diversity, which was driven by divergence in species composition on high and low N patches. In the third manuscript, I investigate the potential role of charcoal on soil solution chemistry and growth of a native species that thrives following wildfire, *Koeleria macrantha*. Data in this manuscript suggests that charcoal can have a large effect on soil solution chemistry, including increased N cycling, and altered growth of *K. macrantha*. In the last manuscript I present data that leads to a better understanding of how several charcoal properties vary as a function of temperature and substrate.

DEDICATION

   I dedicate this dissertation to my wife Kelley, for her endless support and confidence in my abilities. This dissertation could not have been completed without her. I also dedicate this dissertation to my daughter Brule, who continually helps me put life into perspective. I also wish to dedicate this dissertation to my brother David, with whom I shared many childhood adventures in the Northwoods of Minnesota. My interest in science is an extension of these adventures. Lastly, I would like to dedicate this dissertation to my parents, John and Marlene, for exposing me to the natural world at a young age, and encouraging me to be curious in life.

ACKNOWLEDGMENTS

This dissertation would not be possible without the contributions of numerous people. First and foremost, I wish to acknowledge my advisor, Tom DeLuca. Tom has been both a mentor and a friend during the last four years. I believe the relationship between a student and advisor is the single most important element that shapes the graduate school experience. Tom has made this a great experience for me.

I thank my committee members, Carl Fiedler, Hans Zuuring, Anna Sala, and Ray Callaway. I have had numerous valuable discussions and courses with them which have helped me become a better scientist. I am grateful for their contribution to my education.

I thank several co-authors who provided input on the manuscripts included in this dissertation. These include, Tom DeLuca, Carl Fiedler, Kerry Metlen, Jim Gannon, Philip Ramsay, and Mick Harrington. I would additionally like to thank John Graham, Matt Jolly, and Heiko Langer for their valuable input in various aspects of laboratory and statistical analysis.

I would also like to thank my fellow graduate students within the DeLuca lab group for valuable discussions and for contributing to a positive learning environment. These people include Derek MacKenzie, Valerie Kurth, Rachel Brimmer, Tricia Burgoyne, Joss McKinnon, and Vince Archer. I would like to thank Tarn Ream, Francine Farrell, Tracey Brown, Peter Grum, Casey Ruggiero, Jared Kinear, Heather Roberts, Josh Kamensky, and Clarice Pina for their help with laboratory and field work.

Lastly, I thank the USDA Joint Fire Sciences Program, NSF, the Bertha Morton Scholarship, and the College of Forestry for funding during the last four years.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

DISSERTATION OVERVIEW

This dissertation was written in partial fulfillment of the Ph.D. requirements at the University of Montana and is comprised of an introductory review chapter, followed by four research manuscripts in various stages of the publication process. The title and brief description of each chapter is provided below.

Chapter 1: N Cycling, Succession and Feedbacks in Ponderosa Pine/Douglas-fir Ecosystems: An Overview

In this chapter, I establish the context for the following research manuscripts, by reviewing pertinent literature. I first describe why N is limiting in ponderosa pine/Douglas-fir ecosystems and discuss the numerous components of N cycling that are influenced by fire. Additionally, I propose a mechanism by which N greatly influences successional processes, with emphasis on physiological trade-offs, nitrogen-use-efficiency, and positive feedbacks. Lastly, I describe the need to consider these processes in the context of restoration management.

Chapter 2: Restoration Treatments in a Montana Ponderosa Pine Forest: Effects on Soil Physical, Chemical, and Biological Properties

In this manuscript, I investigate the response of numerous soil physical, chemical and biological properties following four restoration treatments in the ponderosa pine/Douglas-fir ecosystem, including prescribed fire, thinning, prescribed fire and thinning, and an untreated control. These approaches reflect a range of treatments currently available to forest managers that likely have greatly different effects on ecosystem function. This study is part of the national Fire and Fire Surrogates Study and

1

is one of 13 similar studies throughout the United States. This manuscript has been accepted by the journal of *Forest Ecology and Management*.

Chapter 3: Nitrogen Spatial Heterogeneity Influences Diversity Following Restoration Treatments in a Ponderosa Pine/Douglas-fir Forest, Montana

In this manuscript, I investigate the resource heterogeneity hypothesis, which states that an area with high spatial resource heterogeneity will support more species than an equal area with homogeneous resource availability. I investigate this hypothesis within the experimental framework of the previous manuscript, with the goal of understanding mechanisms responsible for diversity responses following restoration treatments. This manuscript is accepted to the journal *Ecological Applications*.

Chapter 4: Charcoal and Litter Extracts Alter Soil Solution Chemistry and Growth of *Koeleria macrantha* in the Ponderosa Pine/Douglas-fir Ecosystem

In this manuscript, I propose that charcoal can have numerous effects on soil that may effectively delay the successional transition from early- to late-succesional communities. I investigate this hypothesis by measuring numerous parameters important in N cycling on forest soils amended with a factorial combination of charcoal and litter extract from a late-successional species, *Arctostaphylos uva-ursi*. I present a second experiment where I compare growth of an early-successional species, *Koeleria macrantha,* grown in a control, and in treatments where soil was amended with charcoal made from Douglas-fir and ponderosa pine bark. Finally, I present a third experiment where *K. macrantha* is grown with soil amended with varying concentrations of charcoal generated at different temperatures.

2

Chapter 5: Temperature and Substrate Influence the Chemical Properties of Charcoal in the Ponderosa Pine/Douglas-fir Ecosystem

In this chapter, I attempt to reconcile some of the unexpected results in the previous chapter by evaluating numerous properties of charcoal when made from different substrates and at different temperatures. Specifically, I compare several properties of charcoal potentially important in N cycling, generated from wood and bark of both Douglas-fir and ponderosa pine at 350 and 800 °C. Additionally, I discuss the implications that substrate and temperature effects may have on the activity of charcoal in ponderosa pine/Douglas-fir forests.

3

Chapter 1

N Cycling, Succession and Feedbacks in Ponderosa
Pine/Douglas-fir Ecosystems: An Overview

Introduction

Nitrogen (N) is one of the most limiting nutrients in northern terrestrial

ecosystems (Vitousek and Howarth 1991). All other soil nutrients can be derived from

geologic materials and thus become available to organisms through chemical weathering

of parent materials. In contrast, N is derived entirely from the atmosphere and requires

fixation from N_2 gas to a reduced amine form (Figure 1). Nitrogen fixation is

accomplished almost entirely by soil microbes, with only a very small additional fraction

fixed abiotically in nature (Alexander 1991). Nitrogen fixation is energetically costly,

requires either a host plant, high C conditions, or anaerobic microsites, and can be

completed by only a small group of organisms. Thus, it is believed that N fixation

contributes, on an annual basis, only a small portion to the total N capital of forest

ecosystems (Stevenson and Cole 1999, Hattenschwiler and Vitousek 2000).

Because of this relatively small annual N input, a majority of N that is assimilated

by plants does not come directly from fixation, but rather, is recycled N derived from the

decomposition of plant material. Plant assimilated N returns to the soil as organic matter

and becomes available for further uptake as heterotrophic microbes cleave the amine

bond in organic N molecules (Figure 1). The rate of N mineralization from organic

inputs depends largely on the chemistry of litter (Attiwill and Adams 1993).

Like many other northern terrestrial ecosystems, the ponderosa pine/Douglas-fir

(*Pinus ponderosa/Pseudotsuga menziesii*) ecosystem of the inland western United States

4

has been described as N limited (Mandzak and Moore 1994). This limitation is likely the result of low mean annual temperatures and a dry climate, both factors that limit microbial activity (Alexander 1991, Attiwill and Adams 1993). Further, the coniferous vegetation that dominates forests in this climate produces litter with high C:N ratios, and a large carbon allocation to secondary chemistry, both factors that diminish N mineralization from litter (Northrup et al. 1995). Thus, despite a substantial long-term accumulation of N in ponderosa pine/Douglas-fir forests, N exists in a form that is largely unavailable to the plant community.

Many factors influence the rate of N cycling in ponderosa pine/Douglas-fir ecosystems; however, fire has been identified as one of the most influential factors (Neary et al. 1999). Forests in the northern portion of this ecosystem historically experienced fire return intervals between 10 and 50 years, but have not experienced any fire since Euro-American colonization of the west (Arno 1980, Barrett and Arno 1982, Arno et al. 1995a, Fule et al. 1997, Mast et al. 1999, Moore et al. 1999). This change in fire frequency has likely had a large influence on N cycling in this system.

The objective of this introduction is to first review the numerous ways that N cycling is influenced by fire at different time scales, including immediately following fire, months to years following fire, and decades following fire. Second, I will review the limited literature on N cycling in ponderosa pine/Douglas-fir forests in the extended absence of fire. I will then explore the role that N cycling has on forest successional dynamics in this system, with an emphasis on a positive feedback mechanism that leads to a quick transition to dominance of late-successional communities. Lastly, I will

5

discuss restoration management in this system, and discuss the role that N cycling likely plays in successful restoration of diversity and ecosystem function in this system.

The Influence of Fire on Nitrogen Cycling

Immediate Responses

Fire directly influences the N cycle by combusting organic matter, where reduced-N molecules are denatured and oxidized. Nitrogen begins to volatilize at about 200 °C (Prichett and Fisher 1987), leading to a significant loss of N to the atmosphere. At a temperature of 500 °C, nearly half of the N in organic matter may be volatilized (Neary et al. 1999). DeBano et al. (1979) studied the influence of fire intensity on N cycling in a California chaparral forest, and found that a dry intense fire (812 °C) resulted in a 67% loss of N through combustion. Due to the low heat transfer in soils, the heat generated from fire often does not penetrate very deeply; therefore, loss of N occurs primarily in the surface organic layer.

Despite a significant loss of N during combustion, an ash residue is left behind containing high concentrations of both organic and inorganic N. Consequently, the enriched ash deposit leads to an initial increase of inorganic N into the mineral soil. Christensen (1973) reported that fire in California chaparral left an ash residue that was extremely high in NH_4^+ and organic N. Similarly, DeBano et al. (1979) measured a high concentration of NH_4^+ in the ash residue following laboratory burning of chaparral soil. They further noted that this large NH_4^+ pulse was quickly acted upon by nitrifying bacteria, which resulted in a delayed NO_3^- pulse.

6

Numerous other studies have identified an immediate NH_4^+ pulse above and within the mineral soil following fire. Covington and Sackett (1990, 1992a) found a 20-fold increase in inorganic N immediately after fire in a southwestern ponderosa pine forest. Inorganic N was almost entirely in the form of NH_4^+. DeLuca and Zouhar (2000) found an immediate 10-fold increase in NH_4^+ directly after prescribed fire in a ponderosa pine ecosystem, in western Montana. Similarly, Monleon et al. (1997) found that burned ponderosa pine plots showed a significant 2-fold increase of inorganic N following fire. Prieto-Fernandez et al. (1993) found that a prescribed burn in *Pinus pinaster* forest in Spain resulted in a 13-fold increase of inorganic N following fire, which was almost entirely due to a 19-fold increase in NH_4^+. Preito-Fernandez et al. (1993) presented several hypotheses that explained why fire results in increased inorganic N primarily in the form of NH_4^+, including: NO_3^- volatilizes at a lower temperature than NH_4^+; heat likely diminishes nitrifying bacteria, slowing nitrification; and heating results in NH_4^+ release from protein-like components of organo-mineral complexes. The release of NH_4^+ is followed by autotrophic oxidation of NH_4^+ to NO_3^-, resulting in a lag in NO_3^- accumulation two months to one year after fire (Kaye and Hart 1998, DeLuca and Zouhar 2000).

While this dramatic increase of inorganic N availability following fire may be partly a result of a concentrated ash deposit, many other factors may simultaneously act to increase the soil inorganic N pool, including microbe mediated mineralization of organic matter, and root and microbe death resulting from soil heating. No study has clearly separated the relative contribution of these N-mineralization inputs to this post-fire inorganic N pulse.

Direct Effects of Fire on the Microbial Community

Soil microorganisms drive the conversion of organic N into mineral N (Table 1). The soil contains a diverse array of microbes, many of which are specialized to decompose specific substrates. Fire may influence mineralization rates by directly influencing the quantity or diversity of the microbial community, or the quality and quantity of organic substrates they decompose. These changes have consequences for both short- and long-term inorganic N availability to plants.

The heat generated from fire can quickly cause microbes to die. DeBano et al. (1979) measured temperatures at the mineral soil surface of 175 °C during a fire in the California chaparral. Following this heating, an immediate increase in N mineralization occurred, which was thought to be partly due to the death of microbes. The severity of microbe death is related to intensity and duration of the fire, and the water content of the soil (Neary et al. 1999). A low intensity, quickly moving grassland fire may not transfer enough heat into the soil to cause microbes to die, whereas, a slow-moving high-intensity forest fire may result in significant microbe death (Neary et al. 1999).

As soil moisture increases, the temperature at which microbes are affected decreases. Several studies have evaluated the interacting effect of soil moisture and heat on microbe death. Dunn et al. (1985) measured the impact of both water potential and heating on three microbial functional groups. As soil moisture and heat increased, fungi, bacteria and specifically nitrite oxidizer populations all decreased at a similar rate. Interestingly, Dunn et al. (1985) noted that heat stimulated some microbe spores to germinate. In a similar study, Choromanska and DeLuca (2002) heated replicate soils

8

with three different water potentials to three different temperatures. This experiment also showed that microbial biomass decreased as both temperature and moisture increased. Diaz-Ravina et al. (1996) also conducted a laboratory experiment that showed soil heating resulted in a dramatic drop in soil bacteria numbers and growth rates. In this 15-week experiment the bacterial population never fully recovered to that of the control. Heated soils initially showed higher levels of extractable C, which was thought to have been from dead bacteria. Furthermore, water extracts from heated soils were applied to control soils that resulted in an inhibition of bacteria in these soils. This suggests that in some soils, heating may result in the release or creation of inhibitory compounds. These compounds may be unstable or volatile, inhibiting microbes for only a short period of time. These studies suggest that there is a strong interacting effect of soil temperature and moisture on the microbial community. There are multiple hypotheses that attempt to explain why microbes are more affected by heat when the soil is moist including: greater latent heat transfer into the soil, greater production of lethal steam (Covington and DeBano 1990, Neary et al. 1999) and a predominance of microbes in their vulnerable, active stage rather than dormant spore stages (Dunn et al. 1985, Diaz-Ravina et al. 1996).

Despite the known influence of fire on soil microbes, this impact is often temporary. Dunn et al. (1979) found that the fire-induced temperature pulse resulted in high mortality of microorganisms, which was positively correlated with fire intensity. Despite the loss of microorganisms, complete soil sterilization never occurred. Incubation of these soils showed rapid growth of microbes, with least severe fire having the quickest response. DeLuca and Zouhar (2000) found that microbial biomass N

9

increased dramatically after a prescribed fire, suggesting that any direct negative effects of fire on microbes were extremely short lived.

Change in Organic Matter Quality

Because the direct effect of fire on microbes is only temporary, we must consider the role microbes play once they have become reestablished. The heat generated by fire may alter organic matter, making it a better or worse substrate for microorganisms. If a positive change in organic matter occurred, we would expect the microbial community to rapidly rebound shortly after fire. This increase should be highly correlated with an increase in respiration and N mineralization. Several studies support the idea that organic matter improves as a substrate for microorganisms following fire.

For example, White (1991, 1994) suggested that increased microbial activity following fire is at least partly the result of volatilization of organic compounds that are inhibitory to the microbial community. In another study, Pietikainen et al. (2000) subjected soil samples to a heat gradient. After heating, the humus of each soil sample was characterized based on seven molecular properties, using Fourier-transform infrared spectra analysis. Their results indicated that, indeed, heating of soils to mild, moderate and extreme temperatures resulted in a different molecular composition of humus. These samples were then inoculated with the microbial community present prior to the start of the experiment, and incubated for six months. Soils that were most severely heated in the study (200 °C) resulted in the highest microbial biomass C observed one month after heating but diminished to the lowest levels observed six months later. In contrast, microbial biomass in soil heated at the mildest temperature gradually increased over the

10

six months, never reaching a peak. This suggests that at the higher temperature, microbes quickly colonize labile organic matter generated by the death of microorganisms during heating. Slower microbial buildup in soils treated with a milder temperature could be the result of less high quality C available for decomposition. The ability of heat to alter humus, therefore, may partly explain the pulse of inorganic N observed after fire in many studies.

Fernandez et al. (1997) analyzed the quality of the remaining organic matter following burning in a Spanish *Pinus sylvestris* forest. They found that lignin, a molecule generally considered resistant to microbial decomposition, was also resistant to oxidation during fire, which led to its increase in the remaining organic matter. However, this increase was offset by an increase in lipids, which increased the overall percentage of labile C. This study also showed that the humus remaining after fire changed in quality, where humin greatly increased, and both fulvic and humic acids decreased. It is not known whether this change in humus quality carried any biological significance. These changes in organic C quality following fire were associated with higher C mineralization rates. Although not measured in this study, this would likely lead to an increase in N mineralization in these soils as well.

In support of these studies, many studies have shown that N mineralization increases after fire. DeLuca and Zouhar (2000) found that potentially mineralizable nitrogen (PMN), a relative index of N mineralization, increased 4-fold following a prescribed burn. In this same study biomass N increased immediately after fire. This suggests that a fire-induced change in organic matter quality led to an increase in the microbial population, which in turn led to an increase in N mineralization. Prieto-

11

Fernandez et al. (1993) found that prescribed fire greatly increased N mineralization potential in a conifer forest in Spain. White (1986) also found that fire significantly increased potentially mineralizable N immediately in the organic horizon and six months later in the mineral horizon. Monleon et al. (1997) measured net N mineralization after fire using an *in situ* incubation, and found no significant difference between burned and control plots for one year following fire. This suggests that the increased inorganic N pulse detected in this study was not a result of changes in the rate of organic matter decomposition, but rather ash residue or root and microbial death.

Long-Term Mineralization

As discussed, it is well documented that a pulse of increased N availability is created by fire. This pulse could be the combined effect of ash and char residue, root and microbe death, and increased N mineralization from organic matter due to alteration of organic matter quality. While all of these factors may contribute to the concentration of inorganic N in the soil, it appears that this increase is short-lived. Processes such as plant uptake, nitrification and leaching of NO_3^-, and denitrification act to reduce this pulse to pre-fire levels. Once the pulse of elevated inorganic N diminishes, decomposition processes again limit N availability. Fire, therefore, may influence this long-term mineralization potential by decreasing the quality and size of the remaining organic N pool (Monleon et al. 1997).

Monleon et al. (1997) found that burned plots had a significantly lower net N mineralization rate five years following fire. This is likely a result of net organic matter loss during combustion. This lower net mineralization rate was likely not detected at

12

year zero because of a temporary increase in easily degradable organic compounds that resulted from fire. Covington and Sackett (1986) found that within four years, burned plots no longer had elevated levels of inorganic N relative to the controls. Wright and Hart (1997) measured mineralization rates through an anaerobic incubation of soil receiving prescribed burns every two years for 20 years. Mineralization rates of these soils were 25% less than control plots, suggesting repeated fire may reduce N availability in the long run. DeLuca and Zouhar (2000) found lower PMN and microbial biomass N in plots treated with a prescribed burn than no-burn control plots 2, 3, 11 and 12 years after a fire. These studies demonstrate that fire, despite its dramatic influence on short-term increased N availability, appear to lower N mineralization on the scale of several years to decades following fire.

Spring vs. Fall Burning

The timing of wildfire and prescribed fire likely influences its effects on soil N cycling (Neary et al. 1999), however this remains poorly studied. In the northern Rocky Mountains, it is believed that a majority of historical wildfires occurred between the months of July and October, although intentional burning by Native Americans may have added a substantial spring fire component to the ponderosa pine system (Arno 1980, Barett & Arno 1982). Accurate data on the seasonality of historic fire frequencies do not exist.

Currently, most prescribed fires in the northern ponderosa pine system are conducted in the spring, which may have different effects on N cycling than fall prescribed fire. Soils and fuels are generally moister in the spring. The higher fuel

13

moisture generally lead to lower severity fires; however, as discussed, microbe and root mortality is generally higher when soils are moist because of the lethal steaming effect and reduced dormancy of organisms (Dunn et al. 1985, Choramasnska & DeLuca 2002, Diaz-Ravina 1996). Greater mortality of soil organisms in moist soil may translate to a more dramatic increase in soil inorganic N pools, and mineralization rates in spring compared to fall fires. Another factor that may potentially influence N cycling between spring and fall fires is the activity of vegetation during these seasons. Spring fires are followed by an active growing season, where burned patches may be quickly colonized by vegetation that may rapidly take up N from the soil (Platt et al. 1988, Brewer & Platt 1994). In contrast, the lower activity and uptake by most plant species in the fall may lead to a greater loss of N to leaching, particularly during the spring melt, when a majority of groundwater discharge occurs.

Summary of Nitrogen Dynamics Initiated by Fire

The impacts of fire on N dynamics occur at several control points along the N cycle. First, fire volatilizes N during combustion, leading to a net loss of N from the system. Second, during combustion, an ash and char residue is formed that is greatly enriched in N, primarily in the form of NH_4^+ and organic N. This ash contributes to a pulse of N that is readily available to plants. Other contribution to this immediate N pulse include root and microbial death caused by heating, and increased N mineralization as a result of a rebounding microbial community working on labile organic matter that was made available during heating and combustion. As time passes, N mineralization diminishes, leading to levels of N availability similar to pre-fire conditions. Often this N

14

availability drops below pre-fire levels, likely as a result of lost organic matter from combustion.

Nitrogen Cycling and Fire Exclusion

As described previously, N availability and turnover are greatly accelerated by fire, an effect than may last for decades. Numerous authors have speculated that in the prolonged absence of fire, a "tightening" of N cycling occurs (Covington and Sackett 1990, 1992, Kaye and Hart 1998, MacKenzie et al. 2004). In the most explicit evaluation of long-term N cycling following fire in the ponderosa pine ecosystem, MacKenzie et al. (2004) demonstrated that resin sorbed NH_4^+ and NO_3^- gradually decline for over a century following fire, with the steepest decline occurring in the first two decades. In another study, DeLuca et al. (2005) demonstrated that wilderness stands in the northern Rocky Mountains that experienced two or more fires within the last century have higher nitrifier activity, gross nitrification, and net nitrification than stands without fire within the last century. These studies strongly suggest that in the long-term absence of fire, nutrient cycling greatly diminishes.

Positive Feedbacks and Forest Succession

Successional models and trade-offs

Most successional models emphasize physiological trade-offs among species that correspond with dominance along temporal resource or stress gradients (Pickett 1976, Grime 1977, Tilman 1985). Tilman's (1985) Resource Ratio Hypothesis typifies this approach. According to this model, all species have evolved a unique position along a

15

trade-off spectrum between competition for soil resources and for light. In this model, each species has a minimum resource condition at which it can survive. When this minimum level is breached, it is replaced by a species that has evolved a more appropriate trade-off for these new conditions. Therefore, the plant community at any given time is in equilibrium with resource conditions, and composed of species with the most appropriate trade-off strategies. This equilibrium, however, is highly dynamic, with the rate of community change dependent on the rate of resource change.

As discussed earlier, a strong temporal N gradient exists following fire in the ponderosa pine/Douglas-fir ecosystem, where N is highly available shortly following fire, but gradually declines in the long-term absence of fire (MacKenzie et al. 2004, DeLuca et al. 2005). This change in N cycling is closely associated with a shift in species composition through time. In the ponderosa pine/Douglas-fir ecosystem, fast-growing forbs and grasses have been shown to dominate within decades following fire; whereas, late-successional understory communities become dominated by slower-growing perennials (Arno 1980, Arno et al. 1995a, Fule et al. 1997, Mast et al. 1999, Moore et al. 1999, MacKenzie et al. 2004).

Nitrogen-use-efficiency and positive feedbacks

The differences in the physiologies of early- and late successional understory communities in the ponderosa pine/Douglas-fir ecosystem appear to reflect a trade-off, central to which is a species nitrogen-use-efficiency. Nitrogen-use-efficiency (NUE) is a physiological term that expresses how much biomass is produced during the retention time of a given unit of N. Aerts and Chapin (2000) proposed that NUE is primarily a

16

function of two parameters, leaf N concentration and leaf lifespan, two attributes that are closely correlated with one another. They concluded that an evergreen morphology is the single most important plant characteristic that maximizes NUE.

Despite the high NUE that an evergreen morphology provides, this adaptation also presents numerous challenges that must be overcome. As a function of their longevity, each leaf has an increased probability of damage due to herbivory and other forms of disturbance. Thus, it appears that the evolution of long-lived leaves required an associated evolution of defense chemistry. The most abundant class of defense compounds found in leaves of NUE species are characterized as polyphenols (Hattenschwiler and Vitousek 2000). Polyphenols are constructed of two or more aromatic rings, and generally contain little or no N. Numerous polyphenol fractions have been shown to interfere with digestive enzymes of insects (Cooper and Owen-Smith 1985, Robbins et al. 1987, Bernays 1989), and thus provide effective defense against herbivory. This protection effectively increases the residence time of each unit of leaf N, and therefore greatly enhances NUE.

It has become recognized that this class of leaf compounds not only defends leaves against herbivory, but also can have strong negative effects on the soil microbial community and N cycling (Hattenschwiler and Vitousek 2000). Northrup et al. (1995) incubated litter samples from a variety of *Pinus muricata* phenotypes, which contained a range of polyphenol concentrations. They found that the ratio of dissolved organic N to inorganic N that was released from decomposing litter significantly increased as a function of polyphenol content. The authors suggested that polyphenols may allow species with high NUE to shortcut the N cycle, provided they could access organic N

17

through direct uptake or through mycorrhizael associations. Fierer et al. (2001) added polyphenol extracts from balsam poplar (*Populus balsamifera*) leaves to soils dominated by thinleaf alder (*Alnus tenuifolia*), an earlier successional species. They found that these extracts significantly diminished N availability, suggesting this species influence on soil chemistry may initiate successional change through its influence on N cycling.

There are two hypotheses that attempt to explain why secondary metabolites, such as polyphenols, influence N cycling. The first and more widely accepted hypothesis is that these compounds have a large organic C content and little or no N. Thus, it is hypothesized that these substrates provide a high C:N food source for microbes that requires substantial N immobilization in order to maintain microbial stoichiometry (Schimel et al. 1996). Because soil microbes collectively have an enormous surface area and are ubiquitously present throughout the soil, it is believed that plants cannot compete with microbes when high C:N environments exists. This is referred to as the immobilization hypothesis.

An alternative explanation is that polyphenols, and other secondary metabolites, can have a direct inhibitory effect on microorganisms responsible for N cycling. This inhibitory effect could be a function of toxicity to microbes, or through complexation and interference with microbial exo-enzymes (Baldwin et al. 1983, Field and Lettinga 1992). In support of this hypothesis, Lodhi and Killingbeck (1980) found that phenols and tannins extracted from ponderosa pine litter were toxic to *Nitrosomonas* spp. and *Nitrobacter* spp., both genera of autotrophic bacteria that mediate the conversion of NH_4^+ to NO_3^-. They paired this observation with very low numbers of these bacteria, and very little nitrification in late-successional ponderosa pine forests, and concluded that

18

accumulation of ponderosa pine litter inhibits nitrification in natural ponderosa pine forests. This interpretation is referred to as the inhibition hypothesis, and is generally less accepted than the immobilization hypothesis. Either hypothesis, however, may explain the negative effect that polyphenols appear to have on N cycling.

These studies suggest that polyphenols not only enhance plant NUE, but also reduce N availability in the soil. Aerts (1997, 1999) proposed that these two effects of polyphenols likely create a positive feedback within the plant community that leads to inevitable "tightening" of the N cycle, and dominance of late-successional species. Within this feedback, litter of highly NUE species decomposes slowly resulting in very little N mineralization or nitrification. This, in turn, leads to low N availability, which enhances the competitive advantage of highly NUE species over low NUE species. Early successional species, which have high N requirements, perform poorly in this late successional environment because the nutrient supply is too limited to sustain their high growth rates.

Summary of ecosystem function

It is clear that fire, and its influence on N cycling, has a major role in community composition, succession, and ecosystem function in the ponderosa pine/Douglas-fir ecosystem. Fire selectively removes the carbon rich materials that accumulate during succession, and simultaneously increases the inorganic N fraction in the soil. Further, it increases N mineralization and nitrification, which may last on the scale of years to decades following fire. These changes promote a fire maintained plant community, where N cycling is rapid, and low NUE species dominate (Figure 2). In the absence of

19

fire, N cycling gradually diminishes and higher NUE species become established. These species create a positive feedback, where slow decomposition of their own litter leads to low nutrient availability that further enhances their competitive advantage (Figure 2).

Restoration management

There is abundant evidence that fire has become less frequent in the ponderosa pine ecosystem within the last century. Studies of fire scars in numerous stands throughout the northern Rocky Mountains have shown that mean fire return intervals in the 1700s and 1800s ranged from 10-50 years (Arno 1980, Barrett and Arno 1982, Arno et al. 1995a, Arno et al. 1995b, Smith and Arno 1999); whereas, many low elevation pine forests in this region have not experienced any wildfire since the late 1800s.

It is commonly thought that historical fires were predominantly low-intensity ground fires that promoted open stand structures dominated by large diameter ponderosa pine, with early-successional understory communities. As discussed earlier, it is commonly thought that these fires maintained rapid N cycling that promoted dominance of native grasses and forbs. Accordingly, the reduction in fire frequency within the last century has allowed secondary succession to advance throughout the region, resulting in significantly higher tree densities, an increased dominance of species with a lower tolerance of fire, such as Douglas-fir, and more NUE understory communities. The absence of fire has also led to an increased presence of live and dead fuels resulting in an increased risk of stand replacing wildfire, thus jeopardizing the persistence of old-growth ponderosa pine communities.

In response to the fire hazard associated with high stand densities, land managers are increasingly using fuel reduction treatments to reduce the risk of stand-replacing crown fire associated with higher stand densities. An additional objective in many fuel reduction operations is to restore ecosystem function of historical fire-maintained ponderosa pine communities. Restoration treatments in the ponderosa pine/Douglas-fir ecosystem include prescribed fire, and thinning with and without prescribed fire. These restoration treatments likely effect ecosystem function in dramatically different ways (Moore et al. 1999).

Suding et al. (2004) proposed that successful restoration often requires consideration of positive feedbacks in degraded communities that lead to stability of the alternative degraded state. They proposed that simple re-introduction of historical disturbance may have two unexpected outcomes. First, a re-introduction of historical disturbance may not sufficiently overcome positive feedback present in degraded communities. Second, re-introduction of historical disturbance may lead to a new undesirable state that is dissimilar to the desired state (Suding et al. 2004). Further, they proposed that restoration of the desired community may require the establishment of positive feedback that maintain the desired community.

As described, early- and late-successional plant communities in the ponderosa pine ecosystem exhibit positive feedbacks. The early-successional plant community is dominated by fast-growing low NUE species that rely on high rates of N cycling. This is a relatively weak feedback that requires maintenance by periodic fire (Figure 2). Late-successional communities exhibit a strong positive feedback, where low N availability is driven by the litter chemistry associated with high NUE. Successful restoration of

historic fire maintained ponderosa pine communities likely requires consideration of these feedbacks.

In this dissertation, I present four chapters describing original research that lead to a better understanding of N cycling following fire and restoration treatments in the ponderosa pine/Douglas-fir ecosystem. First, I evaluate the response of numerous soil physical, chemical, and biological properties following four restoration treatments. Many of these soil variables are central components of the positive feedbacks in early- and late-successional communities, as previously discussed. In the second manuscript, I evaluate the relationship between N spatial heterogeneity and diversity following restoration treatments. In the third manuscript, I evaluate the influence of charcoal, a byproduct of fire, on soil solution chemistry, with a focus on N cycling. Recent studies have demonstrated that charcoal enhances N cycling. In this manuscript, I propose that charcoal may delay the onset of the late-successional positive feedback in ponderosa pine/Douglas fir forests, by sorbing polyphenols and enhancing N cycling. In the fourth manuscript I investigate how substrate and temperature influence numerous properties of charcoal, and discuss the potential importance of these factors in the context of restoration management.

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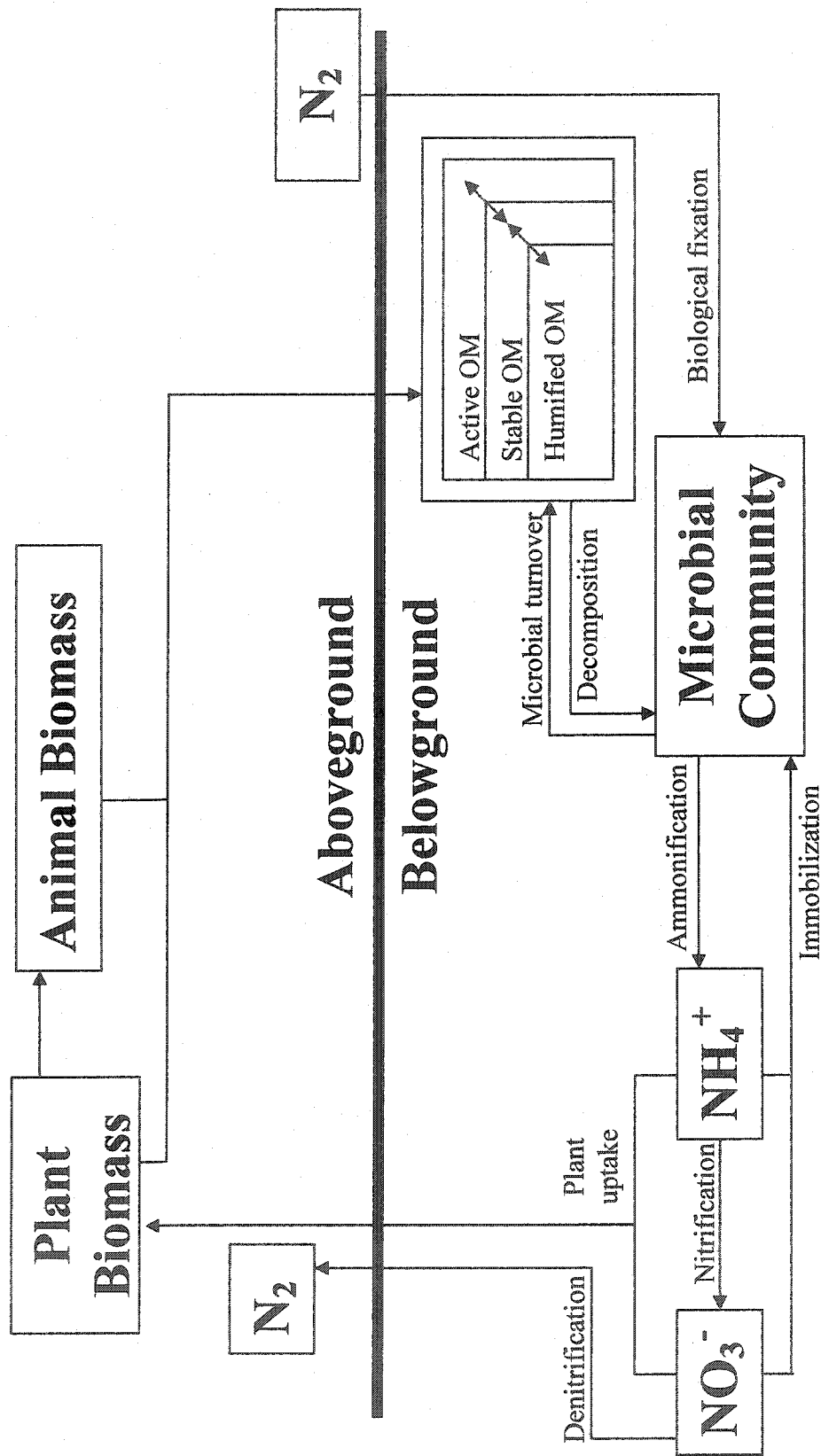
Figure 1: A simplified diagram of the nitrogen cycle.

30
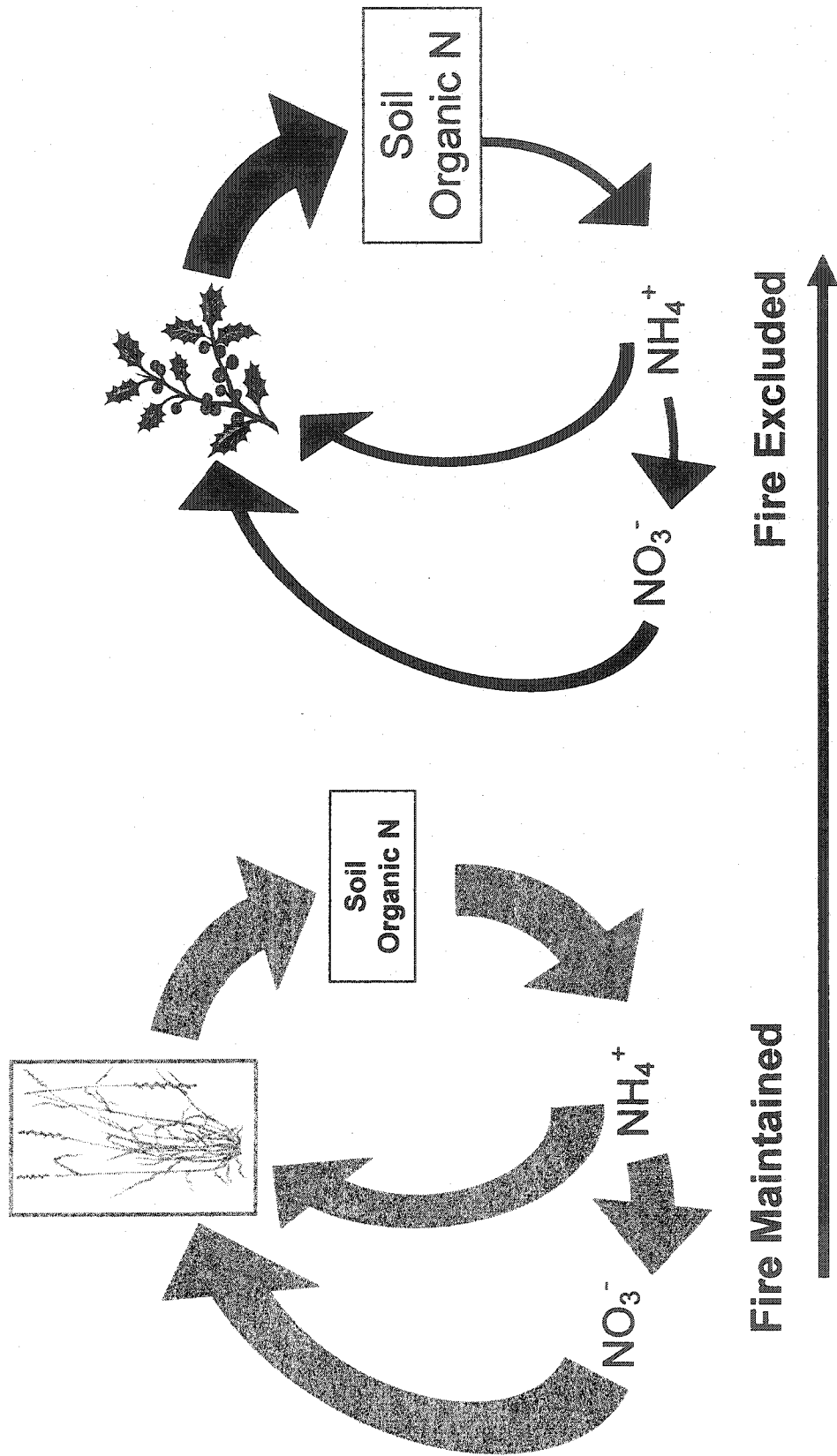
Figure 2: Diagram depicting the hypothesized change in N cycling in a ponderosa pine forests not experiencing fire for an extended period.

31

Chapter 2

Restoration Treatments in a Montana Ponderosa Pine Forest:
Effects on Soil Physical, Chemical, and Biological Properties

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Abstract

Low-elevation ponderosa pine ecosystems of the inland northwestern United States experienced frequent, low-severity fire that promoted open stands dominated by large diameter ponderosa pine (*Pinus ponderosa*). Fire exclusion has led to increased stand densities, often due to proliferation of less fire tolerant species, and an increased risk of stand-replacing wildfire. These fundamental changes have spurred interest in forest restoration treatments, including thinning, prescribed burning, and thinning combined with prescribed burning. We examined the response of numerous soil physical, chemical, and biological parameters to these treatments one and three years post-treatment, using a replicated field experiment. Individual restoration treatments were implemented in 9 ha units. We observed significantly lower C:N in the O horizon, and higher O horizon and mineral soil NH_4^+ concentrations in both BURN and THIN/BURN treatments during year 1. Soil NH_4^+ remained elevated through year 3 in the THIN/BURN treatment. Net N mineralization, nitrification, and NO_3^- concentration were significantly greater in the THIN/BURN than all other treatments during year 1, and

32

net nitrification rates remained elevated through year 3. A high C:N substrate decomposed more rapidly in both burn treatments relative to the unburned treatments. Treatments had no immediate effect on the soil microbial community; however, phospholipid fatty acid profiles differed 16-18 weeks following treatments due to higher actinomycetes in the THIN/BURN treatment. The large scale of our treatment units resulted in significant variation in fire severity among prescribed burns as a function of variation in fuel quantity and distribution, and weather conditions during burn days. Correlation analysis revealed that variation in fine fuel consumed was tightly correlated with net N mineralization and net nitrification. These differences in soil characteristics may influence stand productivity and understory species composition in the future.

Introduction

Lower elevation ponderosa pine ecosystems of the Rocky Mountain West (U.S.) historically experienced a frequent, low-intensity fire regime that promoted dominance of large diameter ponderosa pine (*Pinus ponderosa*) (Arno 1980, Barrett and Arno 1982, Arno et al. 1995a, Fule et al. 1997, Mast et al. 1999, Moore et al. 1999). The historical fire regime likely maintained more rapid nutrient cycling that corresponded with a diverse understory community of grasses and forbs (Hall 1977, Mutch et al. 1993, Kaye and Hart 1998, Moore et al. 1999, Smith and Arno 1999). An abrupt change in this historical disturbance regime occurred upon Euro-American settlement of the West in the late 1800's and early 1900's (Arno 1980, Barrett and Arno 1982, Arno et al. 1995a, Fule et al. 1997, Mast et al. 1999, Moore et al. 1999). A century of fire exclusion may have allowed less fire-tolerant species to become more dominant and C rich organic matter to

33

accumulate (MacKenzie et al. 2004). Some investigators hypothesize that these changes in forest structure and composition have resulted in reduced nutrient turnover relative to historical conditions (Covington and Sackett 1984, Kaye and Hart 1998, MacKenzie et al. 2004).

Land managers throughout the West are introducing surrogates of natural disturbance into the ponderosa pine community in an effort to reduce the risk of stand replacing wildfire. Management strategies to accomplish these goals often include silvicultural thinning or thinning followed by prescribed burning. A third option, less often employed, is the use of prescribed fire by itself. These restoration treatments likely affect ecosystem function in dramatically different ways (Moore et al. 1999). Central to ecosystem function are the numerous processes that occur within the soil that determine resource availability to the plant community. These belowground processes may ultimately influence site productivity, and initial composition and successional trajectory of the understory community. Thus, understanding these belowground responses to alternative restoration treatments may lead to more informed management decisions that may ultimately determine the success of restoration efforts.

Several studies focused on N cycling suggest that important belowground differences exist following these restoration treatments. Comparisons of prescribed fire with unburned controls (White 1986, Covington and Sackett 1992a, Monleon et al. 1997) consistently show that prescribed fire results in a substantial short-term increase in N mineralization and the availability of inorganic N. Additional studies (Kaye and Hart 1998, DeLuca and Zouhar 2000) have also included thinning treatments among these comparisons. Kay and Hart (1998) found that both thinning and prescribed burning

34

increased N mineralization and inorganic N availability relative to the control in the southwestern US; whereas, DeLuca and Zouhar (2000) found that only prescribed fire increased inorganic N pools in western Montana. Few studies have reported how other soil nutrient pools respond to restoration treatments.

The objective of our research was to determine how the initial application of restoration treatments, in a long-term restoration process, affect an array of soil physical, chemical, and biological properties. These treatments included silvicultural cutting, cutting followed by prescribed burning, prescribed burning alone, and an untreated control. To our knowledge, no published studies have simultaneously examined these restoration treatments under an experimental design where treatments are both replicated and implemented at a scale representative of operational restoration projects. Most studies evaluating fuel management and restoration in ponderosa pine have been conducted at the scale of 1 ha or smaller (Covington and Sackett 1984, White 1986, Covington and Sackett 1992a, Monleon et al. 1997, Kaye and Hart 1998). Larger treatment units allow a more natural spread of fire through stands and better reflect the heterogeneous effects of harvesting activities on fuel distributions compared to studies conducted at smaller scales. This experimental design allows us to examine differences among restoration treatments as well as examine within-treatment variation; variation that is likely to occur in operational-sized restoration projects. Finally, this study provides an analysis of ponderosa pine restoration in the northern half of its range, where far less research has been conducted.

Materials and Methods

Our study is part of the Fire and Fire Surrogates (FFS) national study network, which includes 13 research sites utilizing similar experimental designs and sampling protocols. The FFS study is a multiyear interdisciplinary study investigating the effectiveness of restoration treatments (cutting and burning) for reducing wildfire hazard. It is also examining treatment effects on vegetation, soils, insects, diseases, birds and small mammals, and wood utilization.

We implemented our study in an approximately 100-year-old second-growth ponderosa pine/Douglas-fir forest at the University of Montana's Lubrecht Experimental Forest in western Montana. Mean annual air temperature is 7 °C and mean annual precipitation is 50 cm, with 44% falling as snow (Nimlos 1986). We used a blocked experimental design consisting of three 36 ha blocks. Soil in block 1 is a clayey-skeletal, mixed Eutric Haplocryalfs. Soil within block 2 is a loamy-skeletal, mixed, frigid Typic Dystrocryepts. Soil in block 3 is a fine-silty, mixed Eutric Haplocryalfs. Experimental units ranged in elevation from 1230 m to 1388 m. Each block was quartered into square 9 ha units, and assigned one of four treatments (CONTROL, BURN, THIN, and THIN/BURN). We could not randomly assign one burn treatment in two of the blocks because positioning required consideration of preexisting firebreaks. All other treatments were randomly assigned. We measured most response variables prior to treatment implementation and determined that no preexisting differences existed among treatments (data not presented). Year one response data were collected in the summer of 2002, and year three response data were collected in the summer of 2004. Several variables (Table

36

3, 5) thought to be highly dynamic were sampled at three separate times during 2002. These are reported as week 1, week 4-5, and week 16-18.

Restoration Treatments

Our restoration treatments were designed to initiate the long-term process of moving stand density, structure, and species composition toward historical conditions, and to reduce hazard of stand-replacing wildfire. Restoration targets for these stand characteristics were based on old photographs, early stand descriptions (Anderson 1933), investigations of old-growth ponderosa pine in western Montana (Arno et al. 1995b, Fiedler 2000), and ongoing uneven-aged silvicultural research (Fiedler 1995, 1999). We employed a broad rather than discrete interpretation of historical reference conditions to guide our restoration treatment prescription development. This approach is consistent with Allen et al. (2002) and Brown et al. (2004) who suggest that reconstructed historical reference conditions are most useful when used as general guides in prescription development, rather than rigid restoration prescriptions, *per se.*

All stands were very similar prior to treatment implementation. Basal area density among the four treatments ranged from 20.6 to 23.8 m^2/ha (trees >10 cm diameter). Measured in basal area, pretreatment stands were approximately 60% seral species composition (primarily ponderosa pine) and 40% late-successional Douglas-fir. All treatment areas had nearly balanced uneven-aged diameter distributions prior to treatment, with individual tree diameters ranging from 10 to nearly 70 cm.

Our restoration prescription set a target reserve basal area of 11m^2/ha (trees >10 cm diameter) for each of the six units receiving cutting. Target stand structures were

37

uneven-aged, with a long-term goal of one-half to two thirds of the basal area in trees ≥ 50 cm diameter. Target species composition over the long-term is $\geq 90\%$ basal area composition of ponderosa pine. Units receiving cutting treatments were leave-tree marked. We used low thinning to remove most pole-sized trees (ladder fuels), improvement cutting to remove most shade-tolerant Douglas-fir from the mid and upper canopy, and selection cutting to reduce overall stand density enough to induce regeneration of shade-intolerant ponderosa pine. Harvests were conducted by private contractors in the winter of 2000/2001 on frozen, snow-covered soil. A cut-to-length system was used to cut and limb trees on site, leaving non-merchantable materials in place as a buffer between logging equipment and the soil. Merchantable timber was transported from the stand to a landing area, using forwarders.

Similar to the cutting treatments, the goal of prescribed burning was to reduce fuel loads and to move existing stand structure and composition toward historical conditions. Thus some tree mortality was desired as an objective of the treatment. We conducted six separate prescribed broadcast burns in the spring of 2002, with the first fire occurring on May 1 and the last fire occurring on June 25. Fires were initiated with drip torches along transects in each 9 ha unit. While all burns were conducted using the same methodology, unavoidable variation in weather conditions among burn days and the volume and spatial distribution of fuels within each nine ha unit led to variation in fire behavior among the six burns (Table 1).

Target basal areas were achieved to the nearest 0.1 m^2/ha on all six units receiving restoration cutting. Mortality due to burning was about 10 percent in the BURN treatment and about 15 percent in the THIN/BURN. Treatment significantly increased

38

basal area composition of seral species on the THIN and THIN/BURN treatments (from about 60 to 75 %), but not on the BURN or CONTROL. Post-treatment, the CONTROL had about 2000 saplings(1-10 cm diameter)/ha remaining, the THIN and BURN treatments about 1000/ha, and the THIN/BURN about 100/ha.

Field Methods

Within each 9 ha treatment unit, we established a 6 x 6 grid, yielding 36 permanent reference points. We randomly chose ten of these points as permanent center points for ten 20 x 50 m plots. We sub-sampled at each of these 10 plots by collecting soil samples from two opposing corners to form a single composite sample per plot. These 10 composite samples were independently analyzed and averaged, yielding a single datum from each treatment replicate (n=3).

We collected mineral soil samples from a depth of 0-10 cm, using a standard 2.5 cm soil probe. We transported all soil samples to the lab in a cooler, where they were refrigerated overnight prior to extraction and analysis. We measured net N mineralization, ammonification, and nitrification via the buried bag method (Eno 1960). Under this method, soil samples are collected and homogenized, a portion is analyzed immediately, and a portion is placed in a polyethylene bag and returned to the soil for a one-month period. Differences in NH_4^+ and NO_3^- measured at the beginning and the end of this *in situ* incubation reflect net ammonification and nitrification. These incubations were initiated in June and ended in July of each year the analysis was conducted. We also collected and analyzed samples for phospholipid fatty acid (PLFA) profiles and microbial biomass. Due to the high cost of this analysis, the 10 subsamples from each

39

unit were homogenized and analyzed as a single sample. These samples were handled with latex gloves to avoid contamination of samples with non soil-borne PLFA, sifted through a 2 mm sieve in the field, and immediately placed in a cooler with dry ice. Samples were transported to the lab immediately upon collection, where they were stored in a subzero freezer until analysis. We measured decomposition by placing two tongue depressors horizontally between the surface of the mineral soil and the organic horizon at each plot. Depressors were oven-dried (100 °C), weighed, and placed in the field in November 2002. The first set of depressors was collected in May 2003. The second set was collected in June 2004. Upon collection, depressors were oven-dried (100° C), brushed clean, and weighed. Decomposition is reported as % mass lost during the entire incubation period.

We also measured soil physical properties, including exposed mineral soil, bulk density and organic horizon thickness. Exposed mineral soil was measured throughout each 20 x 50 m plot. Soil bulk density and organic horizon thickness were sub-sampled on the corners of each 20 x 50 m plot, as described above. Bulk density was measured using a standard bulk density slide hammer. Bulk density cores were returned to the lab, oven-dried (105° C), weighed, and density calculated as mass per core volume. Organic horizon thickness was measured from the top of the O_i horizon to the bottom of the O_a horizon. Complete organic horizon (O_i, O_e, and O_a) samples were collected within a 15.2 cm diameter ring. Samples were oven-dried (65° C) and weighed. The mass of each sample per ring area was used to report total C and N data on an area basis.

We estimated surface fuels in all units before and after treatment implementation by randomly placing two 15.2 m long transects at the 36 permanent sampling points in

40

each treatment unit. Fuel loads (< 7.6 cm) along each transect were estimated following the protocol of Brown et al. (1982). We defined fine fuels as consisting of 100-hr fuels (2.5 – 7.6 cm), 10-hr fuels (0.6 – 2.5 cm), 1-hr fuels (0 – 2.5 cm), and litter (Oi). We estimated duff (Oe and Oa) depth reduction from burning by placing four eight-inch spikes around the 36 reference points in each unit. We pushed spikes level with the top of the duff layer prior to burning. Duff thickness in reference to the top of this spike was measured following burning.

Laboratory Methods

Exchangeable Ca^{+2}, Mg^{+2}, Na+, K+, extractable P, soil pH, and total C and N were measured on oven-dried soil (65° C). Exchangeable Ca^{+2}, Mg^{+2}, Na^{+}, and K^{+} were extracted by placing 10 g of dry soil into 50 ml of 1 M NH_4Cl solution. Soil suspensions were shaken for 1 hour and extracted as described above. Concentrations of Ca^{+2}, Mg^{+2}, Na^{+}, and K^{+} in extracts were analyzed via inductively coupled plasma spectrophotometery (Thermo Elemental Corp.). Available P was estimated by extracting 5 g of dry soil in 1 M NH_4F as described above. Phosphate in these extracts was measured on a segmented flow analyzer (Auto Analyzer II) using the method described by Murphy and Riley (1962). Soil pH was measured on a 2:1 suspension of 0.01 M $CaCl_2$:soil. Total C and N of both soil and O horizon were measured by dry combustion analysis on a Fissions Elemental Analyzer (Milano, Italy).

Net N mineralization, nitrification, amino N, and microbial respiration were measured on fresh, field moist soils, one day after soil collection. Extractable NH_4^{+} and NO_3^{-} were extracted by shaking 25 g (dry weight equivalent) of soil in 50 ml of 2 M KCL

41

followed by filtration through Whatman # 42 filter paper. Extracts were analyzed on a

segmented flow analyzer (Auto Analyzer III, Bran Luebbe, Chicago, IL) using the

Berthelot reaction (Willis et al. 1993) and cadmium reduction method (Willis and Gentry

1987), respectively. Amino N was measured on these same extracts using the ninhydrin

method (Moore 1968) and polyphenols by the Prussian Blue method (Stern et al. 1996).

Basal soil respiration was measured by incubating 50 g dry weight equivalent soil in a

sealed container with 20 ml 1 M NaOH traps for three days (Zibilske 1994).

Soil microbial community structure was assessed by phospholipid fatty acid

(PLFA) analysis according to the methods of White and Ringelberg (1998).

Phospholipid fatty acids were extracted from three grams of soil from composite samples

into a buffered methanol/chloroform solution. Phospholipids were separated from other

lipids by silicic acid chromatography and derivatized to fatty acid methyl esters (FAMEs)

for quantification by gas chromatography. FAMEs were quantified on an HP 6890 series

gas chromatograph and verified by GC-MS and by using columns of differing polarity.

The 28 most abundant PLFAs served as continuous variables for principal components

(PC) analysis. PC axes generated from the PLFA profiles indicate relative differences in

microbial community structure. Changes in populations of groups of microorganisms

were tracked using specific fatty acids as biomarkers. The fungal PLFA 18:2ω6 was

used to estimate the contribution of fungi (Frostegård and Bååth 1996). The ratio of

18:2ω6 to the bacterial PLFAs (i15:0, a15:0, 15:0, i16:0, 16:1ω9, 16:1ω7t, i17:0, a17:0,

17:0, cy17:0, 18:1ωt and cy19:0) was used to estimate the relative contributions of fungi

and bacteria (Frostegård and Bååth 1996). The 10- methyl branched fatty acids

(10me16:0, 10me17:0, and 10me18:0) were used to track actinomycetes (Kroppenstedt

42

1985). Total PLFA (nMol/g) extracted from samples was used as an estimate of microbial biomass.

Statistical Analysis

We performed all statistical analyses in SPSS version 11.0. We first determined whether each variable met parametric assumptions. When all these assumptions were adequately met, a blocked ANOVA was performed under the general linear model, where treatment was entered as a fixed factor and block was entered as a random factor. When block was not a significant factor at an alpha of 0.05, the comparison was repeated using a one-factor ANOVA. When data did not meet parametric assumptions, or could not be transformed to meet these assumptions, a non-parametric Kruskal-Wallis test was performed. Using Pearson's correlation coefficients, we further investigated whether any simple linear relationships existed between fire severity (measured as fine fuels consumed) and each response variable, which could explain some of the notable variation among the six burned units.

Results and Discussion

Soil Carbon and Nitrogen

The total C capital in the organic horizon was significantly higher in the THIN treatment and lower in both the BURN and THIN/BURN treatments relative to the CONTROL during the first year (Table 2). This difference was a function of differences in O horizon depth among the treatments, and not differences in C or N concentration, which did not differ among treatments (Table 2). Despite no detectable differences in

total C and N concentration in the O horizon, both the BURN and THIN/BURN

treatments resulted in a diminished C:N ratio during the first year (Table 2). The

differences observed during the first year became non-significant by year three. This is

likely the result of scorched canopy inputs in both burn treatments and decomposition of

logging residues in the THIN treatment that made treatments more similar to one another.

No significant differences were detected for total C, N, or C:N in the mineral soil during

year one or three (Table 2).

While no differences could be detected in the soil or organic horizon total N pool,

substantial changes occurred within the inorganic fraction of this pool (Table 3). The

most notable treatment effects were detected during weeks one and 4-5, were substantial

increases in NH_4^+ occurred following fire in both the O horizon and mineral soil. This

difference was greatest immediately following fire in the O horizon, and diminished over

time. The high NH_4^+ concentration in the O horizon appeared to cause a delayed pulse of

NH_4^+ in the mineral soil, which remained elevated through year 3 in the THIN/BURN.

All treatments showed an increase in NO_3^- levels at week 16-18, which corresponded

with the beginning of the wet season. Soil NO_3^- levels were significantly higher in the

THIN/BURN treatment at week 4-5, corresponding with peak levels of NH_4^+ in the

mineral soil. The greater response in the THIN/BURN relative to the BURN is likely a

function of greater fuel consumption in this treatment.

This data is supported by several other studies that have reported that NH_4^+

concentration following prescribed fire increased between two to 20 times that of

reference plots (White 1986, Covington and Sackett 1992b, Covington and Sackett

1992a, Monleon et al. 1997, Kaye and Hart 1998, DeLuca and Zouhar 2000). The

44

magnitude increase of O horizon and mineral soil NH_4^+ we observed following prescribed fire are within the range reported among these studies.

To determine whether the increase in inorganic N we detected in both burn treatments was partially the result of post-fire mineralization and not simply residual accumulation, we conducted a one-month *in situ* soil incubation to measure net N mineralization and nitrification rates. This analysis demonstrated that both net N mineralization and nitrification were higher in the THIN/BURN treatment relative to the other treatments (Table 4) during year 1, with net nitrification remaining elevated through year 3. This suggests that the large inorganic N pool found in the THIN/BURN treatment was not only the direct result of burning, but also the product of post-fire mineralization. Additionally, the significantly higher rates of net nitrification we detected one and three years following the THIN/BURN treatment, combined with only a minimal increase in soil NO_3^- detected during the same period, suggests that NO_3^- is rapidly removed from the soil solution through mechanisms such as plant uptake, immobilization, leaching, or denitrification.

Several factors could explain why the higher net N mineralization and nitrification rates occurred in the THIN/BURN treatment and not the BURN treatment, relative to the control. One possibility is that the more severe fires experienced in the THIN/BURN treatment relative to the BURN treatment (Table 1) resulted in a greater alteration of organic substrates ultimately affecting microbial transformation of soil organic N. In support of this idea, research has demonstrated that soil heating can alter organic substrate fractions, ultimately affecting mineralization rates (White 1994, Fernandez et al. 1997, Pietikainen et al. 2000a). One way in which fire may act to

45

stimulate mineralization and nitrification is to degrade recalcitrant organic N substrates into labile organic N substrates, such as amino groups. It has been demonstrated that amino N is a highly labile N pool, the magnitude of which is equal to the total inorganic N pool in grassland and forest ecosystems (Jones et al. 2005). The amino N pool reflects the balance between microbial uptake, ammonification, and enzymatic or thermal degradation of proteins (DeLuca and Keeney 1993). An additional mechanism by which fire could stimulate N mineralization and nitrification is by altering or eliminating soluble phenols, which have been shown to increase during secondary succession (MacKenzie et al. 2004). Phenols may diminish net N mineralization and nitrification through a variety of mechanisms, including stimulation of N immobilization, complexation of inorganic N, or microbial inhibition (White 1994, Northrup et al. 1995, Aerts 1999, Hattenschwiler and Vitousek 2000).

To explore these hypotheses, we measured soluble amino N concentration as a measure of labile organic N and total soluble phenol concentrations as a measure of bioavailable C. These analyses revealed no significant treatment differences in amino N concentration across all times in either the O horizon or the mineral soil; whereas total phenols in the O horizon were lowest in the THIN/BURN treatment relative to all other treatments (Table 3). This decline in total phenols may not have occurred in the BURN treatment because loss of phenols during combustion in this treatment may have been offset by a substantial deposition of scorched needles shortly after burning, replenishing the O horizon with fresh organic material rich in phenols. In contrast, much of the canopy fuels in the THIN/BURN treatment were transferred to the O horizon prior to burning during the harvest operation and were likely oxidized more completely. Other

46

factors we did not measure, such as soil moisture and temperature, likely have a greater effect on net mineralization and nitrification patterns.

Decomposition, another microbe-mediated transformation, occurred significantly faster in the BURN and THIN/BURN treatments relative to the THIN and CONTROL (Table 4) during the first year; whereas, decomposition in the THIN/BURN treatment exceeded all other treatments by year three. The tongue depressors used to measure decomposition had a C:N greater than 200:1; thus, decomposition was likely influenced heavily by nitrogen availability in the soil. The higher inorganic N concentrations in the BURN and THIN/BURN treatments likely enhanced the ability of the soil microbial community to decompose this high C:N substrate. Differences in soil moisture and temperature may also have contributed to the higher rate of decomposition in the BURN and THIN/BURN treatments.

Because decomposition and nutrient mineralization are dependent on the soil microbial community, we compared microbial community structure, biomass, and basal respiration rates among the treatments (Table 5). Restoration treatments may alter the soil microbial community structure and activity directly through heating, or indirectly by changing the physiochemical environment or the availability of substrates. Immediately following burning, O horizon respiration significantly differed among treatments, with the highest respiration occurring in the THIN treatment, and the lowest respiration occurring in the BURN treatment. Differences in O horizon respiration were only temporary, with no significant differences found in later sampling periods. No differences in soil microbial biomass were detected during the first year, however, higher

47

microbial biomass was found in the Control compared to all other treatments during year three. Soil respiration did not differ among treatments at any sampling time.

One and 4-5 weeks after the prescribed fire, no treatment effects were detected in the PLFA data, suggesting restoration treatments did not exert a strong direct effect on soil microbial community composition. In contrast, PLFA PC 2 was significantly different among treatments at week 16-18 (Table 5). This PC axis was heavily influenced by differences in actinomycete markers (10me16:0, 10me17:0 and 10me:18:0), which were significantly higher in the THIN/BURN treatment (Table 5). By year three, the phospholipid fatty acid data no longer demonstrated any significant differences in microbial community composition. Many studies have shown that soil heating results in a substantial short-term loss of microbial biomass, activity, or a shift in community structure (Pietikainen and Fritze 1993, 1995, Choromanska and DeLuca 2002, Korb et al. 2003). It is likely that the low-severity prescribed burning experienced in this study did not transfer enough energy into the soil to cause a direct restructuring of the microbial community.

Soil Chemical Properties

Restoration treatments had no significant effects on concentrations of soil exchangeable Ca^{+2}, Mg^{+2}, K^+, Na^+, extractable P, or pH (Table 6) during year one and three. These results were unexpected because it has been documented that the return of several elements from vegetation to soil is enhanced by both fire and harvesting. Fire is known to leave behind an ash rich in Ca, Mg, K, and P because C and N volatilize at much lower temperatures (Neary et al. 1999). Additionally, condensation of alkali metals

48

following fire is known to increase soil pH in many systems (Fisher and Binkley 2000).

Likewise, a rapid return of several nutrients from litter following harvest has been documented, with K showing the greatest mobility and Ca the least mobility (Klemmedson et al. 1985, Entry et al. 1991, Klemmedson 1992).

Several factors may explain why we did not find elevated concentrations of these soil chemical properties following treatments. Substantial patchiness existed throughout the 9 ha treatment units as a result of heterogeneous fuel distribution and irregular fire behavior. Treatment averages, therefore, may not reflect the range of processes that occurred within patches. The prescribed fires experienced in this study may not have been severe enough on average to generate sufficient ash necessary to increase soil ion concentrations. Additionally, increased concentrations of these ions in the upper surface of the soil may have been diluted by analyzing composite samples of the 0-10 cm depth.

Soil Physical Characteristics

Restoration treatments had a pronounced effect on the depth of the organic horizon (O_i, O_e, and O_a), where both BURN and THIN/BURN treatments resulted in a diminished depth, and the THIN treatment resulted in a thicker O horizon, relative to the CONTROL (Table 7). By year 3, O horizon thickness remained diminished in both burn treatments; whereas, the O horizon in the THIN treatment did not differ significantly from the CONTROL, suggesting that substantial decomposition and settling of logging residues had occurred. These differences can be attributed primarily to combustion of, or addition to the O_i horizon, with only minor changes in the O_e and O_a horizons. Both BURN and THIN/BURN treatments resulted in significantly more exposed mineral soil

49

relative to the other treatments during year one. By year 3 these differences were no longer detectable, primarily as a result of new litter inputs. Increased soil exposure in these treatments might lead to increased erosion potential; however, this exposed area was a very small fraction of each treatment unit area. We found no differences in soil bulk density among treatments. Higher bulk densities resulting from harvest operations in the THIN and THIN/BURN treatments were likely avoided because harvests were conducted on frozen and snow-covered soil using harvesting techniques designed to minimize soil compaction.

Prescribed Burn Severity

A unique aspect of this research is that treatments were both replicated and implemented at a scale more relevant than previous studies to real restoration projects. As a result, observed burn patterns and severities were more a function of factors such as fuel quantity and continuity, and weather conditions during the burn. In contrast, burn severities in studies using small plot sizes may not accurately reflect large scale operations because the entire plot may be effectively ignited, reducing the influence of weather and fuel distribution on burn severity. The scale and design used in this study allowed us to evaluate how variation in fire severity, expected in any large scale operation, was related to soil response variables. Most notably, we observed substantial variation in mean net mineralization and net nitrification in the BURN and THIN/BURN units, which we hypothesized was the result of fire severity. Regression analysis revealed that net nitrification rate (Figure 1), and net N mineralization (Figure 2) showed strong positive correlations with the mean fine fuels consumed within each treatment

50

unit. The mechanism responsible for these relationships could be the direct result of fire severity, or the indirect result of soil climate conditions that likely co-vary as a result of fire severity. These relationships suggest that unavoidable variation in restoration treatments can have a substantial influence on mean soil N transformation. It is not clear whether the relationships between net nitrification and net N mineralization and fire severity remain linear beyond the fire severity experienced in this study.

Conclusions

Numerous soil physical, chemical, and biological properties differed among treatments. The most significant included net N mineralization, nitrification, NH_4^+ availability, and decomposition rates, which were higher in both BURN and THIN/BURN treatments, with the most pronounced increase in the THIN/BURN treatment. Higher nitrate levels were also found in the THIN/BURN during week 4-5, whereas no other treatments resulted in elevated nitrate levels. Both burn treatments also demonstrated a significant loss of the organic horizon, which resulted in reduced organic horizon C and a reduced C:N ratio. Very few differences in the soil microbial community were detected; however, the THIN/BURN treatment resulted in a higher concentration of PLFA markers for actinomycetes 16 -18 weeks after burning. The only differences found in the THIN treatment were a thicker O horizon and higher respiration rates than other treatments.

These differences in N cycling and availability among treatments may influence the composition of the biotic community that establishes following treatment. It is recognized that plant species possess different ecological strategies for regeneration and survival that involve numerous growth and allocation tradeoffs, of which a plant's N

51

requirement is a central component. The importance of N as a structuring component of the plant community should be particularly strong in systems limited by N, such as ponderosa pine ecosystems (Mandzak and Moore 1994). The native grass species that reportedly dominated the understory of historical ponderosa pine forests likely relied on rapid nitrogen cycling that was promoted by periodic fire. Differences in short term N cycling rates among restoration treatments may lead to substantial differences in site productivity and plant community composition. In addition to differences among restoration treatments, nitrogen cycling appears to have a positive linear relationship with fire severity within the severity range experienced in this study. This relationship, paired with a better understanding of the nitrogen strategies of target plant species, may allow land managers to more effectively use prescribed fire as a tool in restoring the ponderosa pine understory community.

Acknowledgments

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Table 1: Summary of wind speed, relative humidity, temperature, fine fuels, and duff consumed on six separate prescribed fires at Lubrecht Experimental Forest, 2002.

	Burn 1	Burn 2	Burn 3	Burn 4	Burn 5	Burn 6
Date	5/1/2002	5/15/2002	5/31/2002	6/6/2002	6/14/2002	6/25/2002
Treatment	Thin/Burn	Burn	Thin/Burn	Thin/Burn	Burn	Burn
Wind Speed (km h^{-1})	3.2 – 8.0	1.6 - 4.8	4.8 - 9.7	6.4 - 12.9	1.6 - 4.8	1.6 - 4.8
R.H. Range (%)	28 – 42	35 - 46	20 – 29	27 - 45	20 - 41	26 - 48
Temp range (°C)	11.6 - 13.3	8.9 - 11.6	17.8 - 26.7	13.9 - 17.8	19.4 - 28.3	19.4 - 29.4
Fine Fuels Consumed (Mg ha^{-1})	14.67	3.00	22.24	14.99	1.66	4.35
Duff (O_e, O_a) Consumed (%)	14.2	17.4	14.4	10.4	2.0	13.3

60

Table 2: Total C and N (mean (SE), n=3) in the organic horizon and mineral soil (0-10 cm) following restoration cutting (THIN), burning (BURN), and restoration cutting followed by burning (THIN/BURN), at Lubrecht Experimental Forest, MT.

Variable		Control		Thin		Burn		Thin/Burn		p[1]
O Horizon										
C Mg ha^{-1}	[2]Year 1	19.9	(4.2)	28.4	(1.4)	16.9	(4.1)	14.6	(1.4)	*
	[2]Year 3	19.2	(1.3)	20.7	(2.7)	14.3	(1.2)	14.4	(3.6)	ns
N Mg ha^{-1}	[2]Year 1	0.48	(0.10)	0.61	(0.03)	0.43	(0.09)	0.42	(0.05)	ns
	[2]Year 3	0.43	(0.03)	0.49	(0.01)	0.34	(0.03)	0.35	(0.10)	ns
C g kg^{-1}	[2]Year 1	351	(28)	392	(7)	363	(19)	353	(30)	ns
	[2]Year 3	426	(17)	366	(44)	412	(18)	375	(9)	ns
N g kg^{-1}	[2]Year 1	9	(0.6)	9	(0.1)	10	(0.2)	10	(0.7)	ns
	[2]Year 3	10	(0.6)	9	(0.2)	10	(0.5)	9	(0.6)	ns
C:N	[3]Year 1	40.8	(0.4)	45.3	(1.1)	37.7	(2.8)	34.4	(0.7)	**
	[2]Year 3	45.2	(0.9)	42.1	(5.9)	42.9	(3.3)	42.4	(3.9)	ns
Mineral Soil										
C g kg^{-1}	[2]Year 1	23.1	(4.0)	18.9	(1.3)	18.6	(1.7)	24.0	(2.5)	ns
	[3]Year 3	24.9	(1.0)	16.9	(1.4)	21.6	(0.4)	20.6	(3.6)	ns
N g kg^{-1}	[2]Year 1	1.0	(0.1)	0.9	(0.0)	0.8	(0.0)	1.1	(0.1)	ns
	[2]Year 3	0.9	(0.2)	0.5	(0.1)	0.8	(0.0)	0.8	(0.1)	ns
C:N	[2]Year 1	21.0	(1.0)	20.3	(0.6)	20.9	(0.3)	21.1	(0.1)	ns
	[2]Year 3	29.8	(3.6)	30.3	(3.9)	25.6	(1.2)	28.1	(3.2)	ns

[1]p-value: ns > 0.1, * < 0.1, ** < 0.05
[2]One-factor ANOVA
[3]Kruskal-Wallis

61

Table 3: O horizon and mineral soil (0-10 cm) NH_4^+, NO_3^-, amino N, and total phenols (mean ± SE, n=3) 1, 4-6, and 16-18 weeks following prescribed fire at Lubrecht Experimental Forest, MT.

		Control		Thin		Burn		Thin/Burn		[1]p
		O horizon								
NH_4^+-N	[3]Week 1	6.0	(0.8)	6.0	(4.6)	45.9	(9.9)	88.3	(4.6)	****
	[3]Week 4-5	1.0	(0.6)	4.4	(1.9)	8.9	(2.5)	9.9	(1.8)	**
	[3]Week 16-18	2.0	(1.6)	5.1	(3.4)	6.3	(2.0)	9.3	(4.7)	ns
NO_3^--N	[5]Week 1	0.0	(0.0)	0.0	(0.0)	0.0	(0.0)	0.0	(0.0)	ns
	[5] Week 4-5	0.0	(0.0)	0.0	(0.0)	0.1	(0.1)	0.6	(0.4)	ns
	[5] Week 16-18	1.2	(1.2)	0.7	(0.3)	1.0	(0.0)	2.1	(1.1)	ns
Amino N	[5]Week 1	8.9	(8.7)	2.9	(1.9)	8.4	(8.4)	0.0	(0.0)	ns
	[4] Week 4-5	15.7	(6.2)	13.3	(7.9)	1.9	(1.9)	1.6	(1.6)	ns
	[3] Week 16-18	9.9	(2.9)	6.5	(3.3)	4.3	(3.9)	4.7	(3.7)	ns
Phenols	[3]Year 1	47.2	(2.1)	51.2	(3.1)	53.3	(2.0)	44.7	(1.2)	*
		Mineral Soil								
NH_4^+-N	[3]Week 1	0.2	(0.1)	0.9	(0.3)	3.1	(1.2)	3.4	(0.8)	**
	[3] Week 4-5	0.9	(0.1)	0.8	(0.1)	2.3	(0.3)	5.3	(1.0)	****
	[3] Week 16-18	0.4	(0.1)	0.7	(0.4)	1.9	(1.3)	2.4	(1.0)	ns
	[3]Year 3	1.1	(0.3)	1.0	(0.2)	1.3	(0.2)	1.9	(0.2)	*
NO_3^--N	[3]Week 1	0.00	(0.00)	0.00	(0.00)	0.00	(0.00)	0.00	(0.00)	ns
	[5] Week 4-5	0.00	(0.00)	0.01	(0.01)	0.03	(0.02)	0.17	(0.09)	*
	[3] Week 16-18	0.31	(0.31)	0.83	(0.02)	0.86	(0.04)	0.57	(0.28)	ns
	[3]Year 3	0.11	(0.04)	0.16	(0.04)	0.23	(0.02)	0.13	(0.03)	ns
Amino N	[3]Week 1	11.0	(1.4)	11.0	(2.8)	6.0	(1.4)	13.4	(4.6)	ns
	[3] Week 4-5	4.6	(1.4)	5.9	(0.2)	9.4	(3.0)	1.3	(1.1)	ns
	[4] Week 16-18	12.2	(5.3)	6.8	(1.4)	10.1	(2.9)	8.2	(5.8)	ns
Phenols	[3]Year 1	18.7	(0.7)	20.1	(0.8)	18.7	(0.3)	18.4	(0.5)	ns
	[3]Year 3	21.5	(7.3)	12.6	(1.2)	9.3	(4.5)	16.3	(3.74)	ns

[1]mg kg^{-1} dry soil
[2]p-value: ns > 0.1, * < 0.1, ** < 0.05, *** < 0.01, **** < 0.001
[3]One-factor ANOVA
[4]Blocked ANOVA
[5]Kruskal-Wallis

Table 4: Net N mineralization, ammonification, nitrification, and decomposition (mean ±

SE, n=3) following prescribed fire at Lubrecht Experimental Forest, MT. Decomposition

is reported as % mass lost during an *in situ* incubation.

		Control		Thin		Burn		Thin/Burn		[2]p
[1]Net N Mineralization	[4]Year 1	2.3	(0.6)	3.6	(2.1)	3.2	(0.8)	6.9	(0.6)	*
	[3]Year 3	5.7	(1.7)	5.0	(0.9)	7.2	(2.3)	8.0	(0.8)	ns
[1]Net Ammonification	[3]Year 1	2.0	(0.7)	2.5	(1.1)	2.8	(0.9)	3.6	(0.7)	ns
	[3]Year 3	3.9	(0.9)	3.2	(0.7)	5.1	(2.2)	5.1	(0.9)	ns
[1]Net Nitrification	[5]Year 1	0.3	(0.1)	1.1	(0.9)	0.4	(0.2)	3.2	(0.7)	**
	[3]Year 3	0.2	(0.1)	0.0	(0.0)	0.3	(0.2)	0.7	(0.1)	*
Decomposition (%)	[3]Year 1	24.3	(0.4)	24.9	(0.2)	30.3	(1.4)	28.4	(1.2)	***
	[3]Year 3	32.0	(1.6)	32.7	(1.7)	34.7	(3.6)	42.3	(5.3)	*

[1]$\mu g \ g^{-1} \ 30 \ d^{-1}$
[2]p-value: ns > 0.1, * < 0.1, ** < 0.05, *** < 0.01
[3]One-factor ANOVA
[4]Blocked ANOVA
[5]Square-root transformed; non-transformed values reported

63

Table 5: Soil microbial properties (mean $\pm$ SE, n=3) following prescribed fire at Lubrecht Experimental Forest, MT. PLFA principle components have no units, with PC 1 accounting for 35%, 39%, and 27%, and PC 2 accounting for 15%, 14%, and 21% of the variation in PLFA data during week 1, 4-5, and 16-18 respectively.

		Control		Thin		Burn		Thin/Burn		[1]p
Duff Respiration	[2]Week 1	3.5	(0.1)	3.8	(0.1)	3.0	(0.1)	3.4	(0.1)	***
(mg CO_2 $g^{-1}day^{-1}$)	[2]Week 4-5	3.4	(0.1)	3.2	(0.3)	2.9	(0.5)	3.6	(0.2)	ns
	[2]Week 16-18	3.2	(0.4)	4.0	(0.3)	3.0	(0.3)	2.2	(1.1)	ns
Soil Respiration	[2]Week 1	0.6	(0.0)	0.5	(0.6)	0.6	(0.3)	0.6	(0.2)	ns
(mg CO_2 $g^{-1}day^{-1}$)	[4] Week 4-5	0.7	(0.00)	0.8	(0.23)	0.6	(0.01)	0.7	(0.07)	ns
	[4] Week 16-18	0.6	(0.04)	0.6	(0.03)	0.5	(0.16)	0.6	(0.08)	ns
	[4]Year 3	0.6	(0.01)	0/6	(0.13)	0.6	(0.03)	0.5	(0.02)	ns
Microbial Biomass	[2]Week 1	315.7	(45.1)	295.2	(56.2)	296.1	(41.1)	269.0	(21.5)	ns
(nMol PLFA g^{-1})	[2] Week 4-5	281.0	(17.8)	320.9	(19.2)	282.4	(21.5)	312.8	(43.0)	ns
	[2] Week 16-18	395.9	(22.4)	333.1	(47.7)	289.9	(26.7)	305.2	(49.0)	ns
	[2]Year 3	397.2	(40.5)	336.2	(19.7)	257.9	(16.8)	311.3	(38.0)	*
PLFA PC 1	[2]Week 1	0.38	(0.44)	0.29	(1.01)	-0.77	(0.44)	0.10	(0.11)	ns
	[2] Week 4-5	0.08	(0.15)	-0.26	(0.93)	-0.05	(0.73)	0.24	(0.58)	ns
	[2] Week 16-18	-0.36	(0.57)	0.01	(0.66)	0.36	(0.56)	-0.01	(0.79)	ns
	[2]Year 3	-0.93	(0.41)	0.08	(0.42)	0.11	(0.79)	0.75	(0.38)	ns
PLFA PC 2	[2]Week 1	0.66	(0.01)	0.24	(0.72)	-0.30	(0.50)	-0.60	(0.40)	ns
	[2] Week 4-5	0.07	(0.15)	-0.26	(0.93)	-0.05	(0.73)	0.24	(0.58)	ns
	[2] Week 16-18	-1.01	(0.59)	0.28	(0.06)	-0.35	(0.26)	1.09	(0.47)	**
	[2]Year 3	-0.14	(0.90)	-0.26	(0.57)	0.33	(0.09)	0.08	(0.77)	ns
Actinomycetes	[2]Week 1	8.17	(0.37)	8.23	(0.52)	8.13	(0.38)	8.70	(0.63)	ns
(Mol %)	[2] Week 4-5	8.61	(0.19)	7.82	(0.81)	7.75	(0.58)	7.59	(0.29)	ns
	[2] Week 16-18	7.30	(0.10)	8.02	(0.26)	7.99	(0.31)	8.85	(0.35)	**
	[2]Year 3	7.47	(0.24)	8.12	(0.09)	8.27	(0.26)	8.76	(0.54)	ns

[1]p-value: ns > 0.1, * < 0.1, ** < 0.05, *** < 0.01
[2]One-factor ANOVA
[3]Blocked ANOVA
[4]Kruskal-Wallis
[5]Square-root transformed; non-transformed values reported

64

Table 6: Soil chemical variables (mean ± SE, n=3) in response to restoration cutting (THIN), burning (BURN), and restoration cutting followed by burning (THIN/BURN), at Lubrecht Experimental Forest, MT.

		Control		Thin		Burn		Thin/Burn		^2p
Ca	[3]Year 1	1588.9	(255.9)	1351.4	(146.1)	1410.5	(115.3)	1491.1	(185.7)	ns
	[3]Year 3	1055.3	(100.9)	1487.2	(257.9)	1268.3	(109.4)	1270.1	(125.3)	ns
Mg	[3]Year 1	174.3	(30.4)	137.6	(8.9)	144.3	(12.5)	163.0	(22.4)	ns
	[3]Year 3	164.7	(28.8)	119.4	(10.03)	131.4	(14.6)	149.3	(17.2)	ns
Na	[4]Year 1	7.2	(0.32)	6.2	(0.47)	6.7	(0.98)	7.1	(0.76)	ns
	[3]Year 3	15.7	(2.9)	13.5	(2.1)	12.3	(1.4)	14.3	(1.9)	ns
K	[4]Year 1	403.6	(58.6)	303.7	(37.2)	360.3	(30.9)	376.4	(39.8)	ns
	[3]Year 3	428.2	(38.8)	343.4	(41.4)	355.6	(63.5)	347.6	(32.8)	ns
P	[3]Year 1	0.46	(0.04)	0.48	(0.04)	0.50	(0.04)	0.48	(0.09)	ns
	[3]Year 3	1.66	(0.27)	2.37	(0.46)	1.7	(0.15)	2.14	(0.28)	ns
pH	[3]Year 1	5.0	(0.10)	5.1	(0.12)	5.3	(0.07)	5.4	(0.13)	ns
	[3]Year 3	4.7	(0.21)	5.0	(0.10)	5.1	(0.14)	5.1	(0.08)	ns

[1]All variables except pH are expressed mg kg^{-1} of dry soil
[2]p-value: ns > 0.1
[3]One-factor ANOVA
[4]Blocked ANOVA

Table 7: Soil physical characteristics (mean ± SE, n=3) in response to restoration cutting (THIN), burning (BURN), and restoration cutting followed by burning (THIN/BURN), at Lubrecht Experimental Forest, MT.

		Control		Thin		Burn		Thin/Burn		[1]p
O Depth (cm)	[2]Year 1	4.4	(0.5)	5.0	(0.3)	2.6	(0.5)	2.5	(0.3)	***
	[2]Year 3	3.8	(0.4)	3.5	(0.3)	2.2	(0.4)	1.6	(0.1)	***
Db (g cm^{-3})	[2]Year 1	0.91	(0.01)	0.95	(0.05)	0.90	(0.02)	0.90	(0.04)	ns
	[2]Year 3	0.92	(0.04)	0.98	(0.05)	0.95	(0.04)	0.96	(0.04)	ns
[4]Exposed Soil (m^2)	[3]Year 1	0.05	(0.03)	0.8	(0.29)	2.6	(0.78)	3.6	(0.04)	**
	[3]Year 3	0.04	(0.04)	0.03	(0.02)	0.5	(0.33)	0.3	(0.20)	ns

[1]p-value: ns > 0.1, * < 0.1, ** < 0.05, *** < 0.01
[2]One-factor ANOVA
[3]Kruskal-Wallis
[4]Measurements were made within 1000 m^2 plots

66
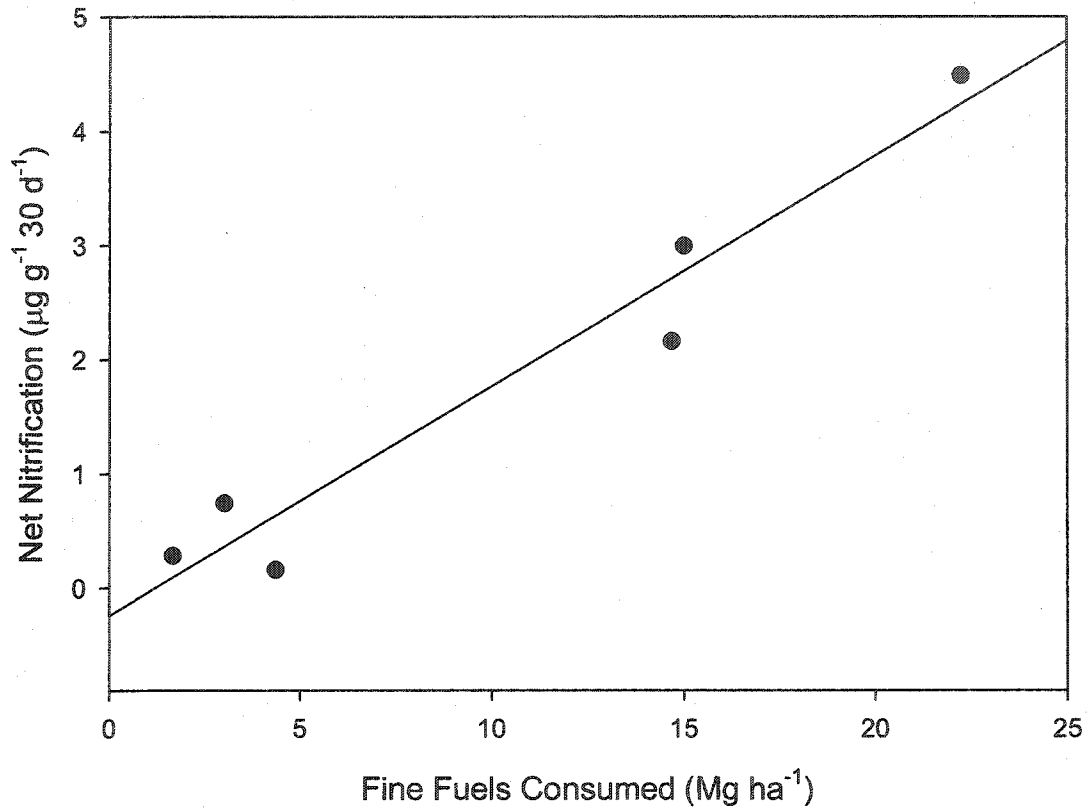
Figure 1: The linear regression of mean net nitrification against fine fuels consumed (diameter < 7.6 cm) from six separate 9 ha prescribed burns at Lubrecht Experimental Forest, MT (r^2=0.97, p=0.001).
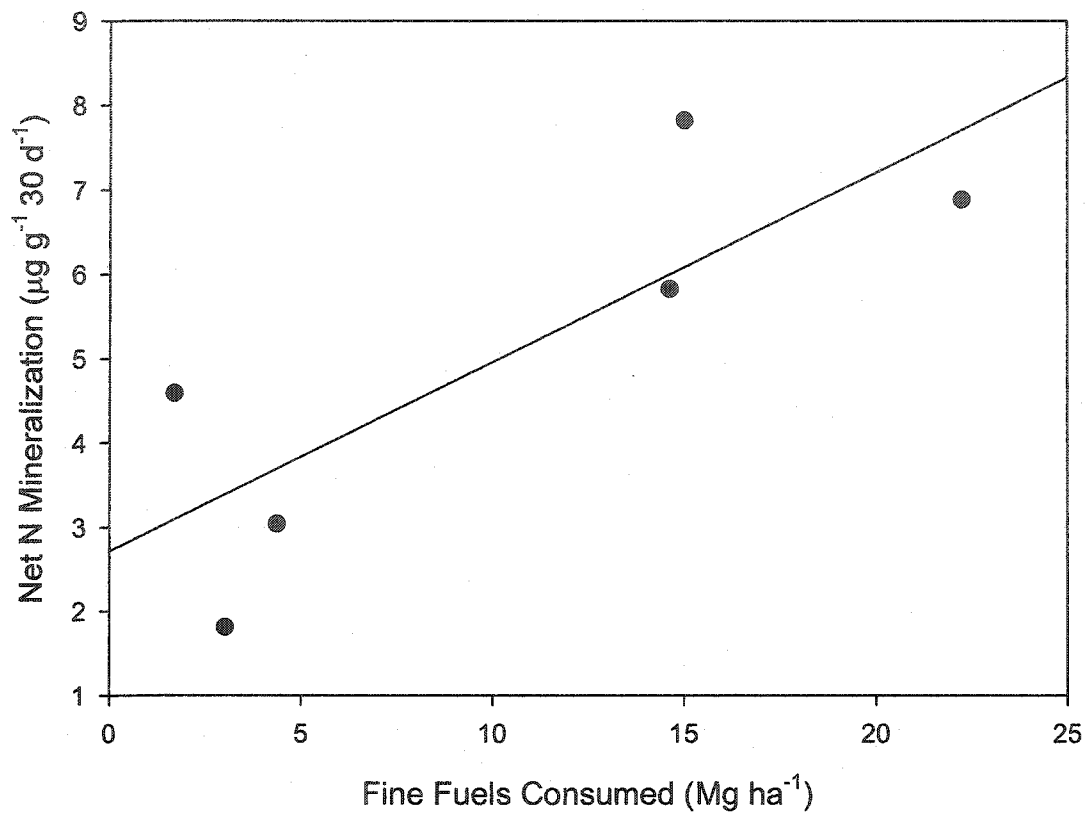
Figure 2: The linear regression of mean net N mineralization against fine fuels consumed (diameter < 7.6 cm) from six separate 9 ha prescribed burns at Lubrecht Experimental Forest, MT (r^2=0.814, p=0.049).

Chapter 3

Nitrogen Spatial Heterogeneity Influences Diversity Following
Restoration Treatments in a Ponderosa Pine/Douglas-fir Forest, Montana

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Abstract

The resource heterogeneity hypothesis (RHH) is frequently cited in the ecological

literature as an important mechanism for maintaining species diversity. The RHH has

rarely been evaluated in the context of restoration ecology where a commonly cited goal

is to restore diversity. In this study we focus on the spatial heterogeneity of total

inorganic nitrogen (TIN) following restoration treatments in a ponderosa pine (*Pinus*

ponderosa)/Douglas-fir (*Pseudotsuga menziesii*) forest in western Montana. Our

objective was to evaluate relationships between understory species richness and TIN

heterogeneity following mechanical thinning (Thin-only), prescribed burning (Burn-

only), and mechanical thinning with prescribed burning (Thin/burn) to discern the

ecological and management implications of these restoration approaches. We employed

a randomized block design, with three 9 ha replicates of each treatment and an untreated

control. Within each replicate, we randomly established a 20 x 50 m (1000 m^2) plot in

which we measured species richness across the entire plot, and in 12 one m^2 quadrats

randomly placed within each larger plot. Additionally, we measured TIN from a grid

consisting of 112 soil samples (0-5 cm) in each plot, and computed standard deviations as

69

a measure of heterogeneity. We found a correlation between the net increase in species richness and the TIN standard deviations one and two years following restoration treatments, supporting RHH. Using NMS ordination and chi-squared analysis, we found that high and low TIN quadrats contained different understory communities in 2003 and 2004, further supporting RHH. A comparison of restoration treatments demonstrated that Thin/burn and Burn-only treatments created higher N heterogeneity relative to the Control. We also found that within prescribed burn treatments, TIN heterogeneity was positively correlated with fine fuel consumption, a variable reflecting burn severity. These findings may lead to more informed restoration decisions that consider treatment effects on understory diversity in ponderosa pine/Douglas-fir ecosystems.

Introduction

A central focus in ecology is to understand the underlying mechanisms that regulate and maintain patterns of diversity. Strong diversity patterns such as an increase in species with area (Gleason 1922, MacArthur and Wilson 1967), a decrease in species with latitude (Pianka 1966), the regional hump-shaped relationship between productivity and diversity (Grime 1973, Rosenzweig and Abramsky 1993, Mittelbach et al. 2001), and the localized decrease in diversity with productivity following nutrient addition (Tilman 1982) have been thoroughly described. Ecologists have attempted to develop unifying theories that simultaneously explain these patterns. One such hypothesis, the resource heterogeneity hypothesis (RHH), suggests that diversity is a function of habitat or resource heterogeneity (Ricklefs 1977, Tilman 1984, Rozenzweig and Abramsky 1993, Tilman and Pacala 1993), which may promote species coexistence at any scale.

70

The basic premise of RHH is that species have sufficiently different niches from one another (MacArthur and Levins 1967) that each species is able to avoid displacement (Hardin 1960) under the unique set of resource conditions that complements its niche. A variety of resource conditions, therefore, promotes the coexistence of a variety of species. The fundamental prediction of RHH is that an area with heterogeneous resource availability will be more diverse than an equal area with homogeneous resource availability.

As with most hypotheses explaining diversity, RHH remains a viable hypothesis that is frequently referenced in the ecological literature, and is supported by some experimental evidence (Fitter 1982, Inouye and Tilman 1995, Burnett et al. 1997, Reynolds et al. 1997, Vivian-Smith 1997, Nichols et al. 1998, Sulkava and Huhta 1998). However, RHH has only rarely been considered in the applied field of restoration ecology, where a common goal of most restoration projects is to re-establish a diverse assemblage of vegetation that has been diminished through some degradation process. An understanding of how restoration treatments create or destroy heterogeneity of limiting resources may help predict the potential for restoration to re-establish or maintain diversity.

In the ponderosa pine ecosystems of the western United States, fire exclusion over the last century has led to an increase in forest biomass, an increase in shade-tolerant species, and a loss of understory diversity (Arno 1980, Arno et al. 1995a, Fule et al. 1997, Mast et al. 1999, Moore et al. 1999). It is likely that a corresponding change in nutrient availability, particularly N, has occurred due to the immobilizing environment created by the accumulation of C-rich litter (Covington and Sackett 1984, Kaye and Hart 1998,

71

MacKenzie et al. 2004). The frequent, low-intensity burning that reportedly dominated the disturbance regime prior to Euro-American settlement of the West likely created and maintained substantial heterogeneity of resources such as nitrogen. Furthermore, it is likely that the reduced N cycling believed to correspond with advancing secondary succession (MacKenzie et al. 2004) has led to greater spatial homogeneity of available N, although these ideas remain untested.

Restoration is increasingly used in pine-dominated ecosystems to move stand structure toward historical conditions, and reduce the risk of stand replacing crown fires. Silvicultural methods, such as mechanical thinning, prescribed burning, and prescribed fire and mechanical thinning used together, are tools frequently used by land managers to accomplish these goals. While these treatments may effectively reduce fuel densities and continuity (Pollet and Omi 2002, Fiedler et al. 2003), they may also influence spatial patterns of N, and therefore lead to different levels of understory diversity.

The purpose of this study is to explicitly examine variability of N availability following four restoration alternatives in a ponderosa pine/Douglas-fir forest in western Montana, and determine whether this variation has any relationship with understory richness, a fundamental component of diversity. We focus on N because it has been cited as a limiting nutrient in this system (Vitousek and Howarth 1991, Mandzak and Moore 1994), and has been shown to rapidly respond to restoration treatments (Covington and Sackett 1990, 1992a, Monleon et al. 1997, DeLuca and Zouhar 2000, Gundale et al. 2005), whereas other soil nutrients appear less responsive.

Our first hypothesis is that an underlying relationship between species richness and N heterogeneity will exist across all treatments, where plots with high N variability

72

will contain a greater number of species than plots with low heterogeneity. Second, because harvesting activities often lead to clumped distributions of fuels, and fire is known to result in an accumulation of inorganic N, we hypothesize that the greatest degree of N heterogeneity will be found in the treatment including both mechanical thinning and prescribed burning. Furthermore, we hypothesize that all treatments will show a higher degree of N heterogeneity than the untreated control, where we anticipate relatively low levels of N heterogeneity.

Materials and Methods

Our study site is part of the Fire and Fire Surrogates (FFS) national study network, which includes 13 research sites utilizing similar experimental designs and sampling protocols. The FFS study is a multiyear interdisciplinary study investigating the effectiveness of cutting and burning treatments for reducing wildfire hazard. It is also examining treatment effects on vegetation, soils, insects, diseases, birds and small mammals, and wood utilization. Separate manuscripts from our study site describe treatment effects on soil physical, chemical, and biological properties (Gundale et al. 2005), and understory vegetation (Metlen and Fiedler In prep).

We implemented our study in an approximately 100-year-old second-growth ponderosa pine/Douglas-fir forest at the University of Montana's Lubrecht Experimental Forest in western Montana. Mean annual air temperature is 7 °C and mean annual precipitation is 50 cm, with 44% falling as snow (Nimlos 1986). We used a blocked experimental design consisting of three 36-ha blocks. Soil in block one is a clayey-skeletal, mixed Eutric Haplocryalfs. Soil within block two is a loamy-skeletal, mixed,

73

frigid Typic Dystrocryepts. Soil in block three is a fine-silty, mixed Eutric Haplocryalfs.

Experimental units ranged in elevation from 1230 m to 1388 m. Each block was

quartered into square nine ha units, and assigned one of four treatments (Control, Burn-

only, Thin-only, and Thin/burn). We could not randomly assign one burn treatment in

two of the blocks because positioning required consideration of preexisting firebreaks.

All other treatments were randomly assigned.

Restoration Treatments

Restoration treatment prescriptions were designed to initiate the long-term

transition toward historical ranges of stand density, structure, and species composition,

and to reduce hazard of stand-replacing wildfire. Restoration targets for these stand

characteristics were based on early stand descriptions (Anderson 1933), studies of relict

old-growth ponderosa pine stands in Montana (Arno et al. 1995b, Fiedler 2000), and

ongoing uneven-aged silvicultural research (Fiedler 1995, 1999).

Pretreatment basal area densities ranged from 20.6 to 23.8 m^2/ha (trees >10 cm

diameter) among the four treatments. The basal area of pretreatment stands was

comprised of approximately 60% ponderosa pine and 40% Douglas-fir, with individual

tree diameters ranging from 10 to 70 cm.

The restoration cutting prescription set a post-treatment basal area target of 11

m^2/ha (trees >10 cm diameter), with a long-term goal of one-half to two-thirds of the

basal area in trees ≥ 50 cm diameter. The long-term species composition target is $\geq 90\%$

ponderosa pine. Restoration cutting was conducted in the winter of 2000/2001 on frozen,

snow-covered soil. A cut-to-length harvest system was used to cut and limb trees in the

woods. Logging slash (tops and limbs) and non-merchantable materials were left onsite to serve as a buffer between logging equipment and the soil. A low-impact log forwarder transported merchantable timber to a landing area outside each treatment unit.

The goal of prescribed burning was to reduce fuel loads and to move existing stand structure and species composition toward historical conditions. The six units assigned the burn treatment received separate prescribed broadcast burns in the spring of 2002. All burns were conducted using the same procedures, but variation in weather conditions among burn days and in the volume and distribution of fuels within each treatment unit led to variation in fire behavior and effects.

Field Methods

Within each nine-ha unit, a 6 x 6 grid was established, yielding 36 permanent reference points. One reference point within each replicate was randomly chosen to serve as a center point for a 1000 m^2 plot (20 x 50 m). This yielded a total of three 1000 m^2 plots per each treatment. Within each plot, soil samples were collected in a grid pattern consisting of 16 rows and seven columns, with each point separated from its nearest neighbor by three meters (Fig. 1).

We collected mineral soil samples (0-5 cm) at each grid location in June and July, 2002, and again in 2003, using a standard 2.5 cm soil probe. All treatments within a block were sampled before initiating sampling in the next block. This allowed us to evenly distribute any seasonal variation across all treatments. Soil samples were transported and stored under refrigeration, and extracted the following day. Extractable NH_4^+ and NO_3^- were extracted by shaking 25 g (dry weight equivalent) of soil in 50 ml of

75

2 M KCL followed by filtration through Whatman # 42 filter paper. Extracts were

analyzed on a segmented flow autoanalyzer using the Berthelot reaction (Willis et al.

1993) and cadmium reduction method (Willis and Gentry 1987), respectively. Hereafter

we report N as total inorganic nitrogen (TIN), which consists of the combined

concentrations of NH_4^+ and NO_3^-.

We used richness of understory vascular plants as a measure of diversity. We

sampled species richness at two spatial scales: 1000 m^2 plots (20 x 50 m) and twelve 1

m^2 quadrats (1 x 1 m) within each 1000 m^2 plot (Fig. 1). A modified random process was

used to disperse quadrats throughout each 1000 m^2 plot. The 1000 m^2 plots were first

subdivided into ten 10 m^2 sub-plots (10 x 10 m). Quadrats were then randomly placed in

one or two opposing corners of each 100 m^2 sub-plot, with at least one but no more than

two quadrats per sub-plot. We conducted pre-treatment vegetation sampling in the

summers of 2000/2001, and post-treatment sampling in the summers of 2003 and 2004.

We report richness as delta species richness, which is the net difference in richness

between pre-treatment and post-treatment measurements. This measure standardized the

richness response among plots, where preexisting variation in richness occurred. Species

were identified to the lowest taxonomic level possible using the taxonomy key of

Hitchcock and Cronquist (1973).

Statistical Analysis

The objective of our experimental design and statistical analyses was to evaluate

the relationship between TIN standard deviation and delta species richness at 1000 m^2

plot level, and determine whether species divergence on high and low N patches within

76

these plots was in part driving this relationship. An additional objective was to determine whether differences in TIN heterogeneity between treatments existed.

Total inorganic nitrogen standard deviations were computed for each 1000 m^2 plot using the 112 values of inorganic N collected within each plot's sample grid. We determined whether TIN standard deviation differed among restoration treatments using analysis of variance, followed by the Student-Newman-Keuls post hoc procedure. Distributional assumptions were graphically assessed and heteroscadasticity was tested using Levene's statistic. We computed correlation coefficients to evaluate the relationship between TIN standard deviation and species richness using all twelve 1000 m^2 plots (three per treatment). The relationship between TIN standard deviation and fine fuels consumed was also evaluated by computing correlation coefficients for data from the six 1000 m^2 plots treated with prescribed fire. All above analyses were performed using SPSS 12.0 Software (Chicago, Illinois).

Pretreatment species composition (presence/absence), and post-treatment species composition (2003 and 2004) within all quadrats (n=144) was ordinated using nonmetric multidimensional scaling (NMS) (Kruskal 1964, Mather 1976) with PC-ORD software (MjM Software Design, Gleneden Beach, Oregon). The total species pools used to construct ordination models consisted of 81, 106, and 118 species in pre-treatement, 2003, and 2004, respectively. A total of 120 species was detected across the three sampling years. Beginning with random start configurations, we produced three-dimensional ordination models to best represent species composition at all time periods. All ordinations used Euclidean distance and satisfied three criteria that indicated they satisfactorily represented the data in original multi-dimensional space (McCune and

Grace 2002). First, the cumulative coefficient of determination from each model indicated that it represented a large portion of the variation in the original data set (2000, $r^2 = 0.770$; 2003, $r^2 = 0.798$; 2004, $r^2 = 0.817$). Second, stress levels (S), which indicate a loss of separation between plots in ordination space relative to the original multidimensional space, remained below 20 (McCune and Grace 2002) for all ordinations (2000, $S = 18.77$; 2003, $S = 17.43$; 2004, $S = 16.08$). Lastly, Monte Carlo tests (n = 50) determined that there was a low probability that these final stress values occurred by chance ($p < 0.05$) and that the final ordination was stable (instability < 0.0005) for all models.

We used these ordination models to determine whether species composition differed in quadrats containing high or low TIN. Quadrat TIN concentrations were estimated from their closest gridpoint (<1 m). We defined quadrats with high and low TIN as falling above or below the median TIN concentration of all 144 quadrats sampled. Both high and low TIN quadrats were distributed among all treatments in 2002 and 2003 (Table 1). Student's t-tests were performed to see if NMS scores along model axes differed between high and low TIN quadrats. The ordination figures presented show two of the three ordination axes most strongly correlated with TIN.

We used Chi-square tests (Zar 1999) to identify species whose frequency increased between pre-treatment and post-treatment sampling periods. We conducted analyses for all four groupings of quadrats: low N 2002, low N 2003, high N 2002, and high N 2003. These analyses utilized 2 x 2 contingency tables (df = 1, $\alpha = 0.05$) with pre-treatment and post-treatment columns, and rows consisting of presence and absence counts for each column. Species too rare to satisfy the requirement that each expected

78

cell frequency be at least five (Zar 1999) were removed from the analysis. Any species whose frequency significantly increased in both high and low N quadrats during a given post-treatment year were also removed. Thus, we report only species positively increasing within high or low N quadrats (Table 2).

Both the NMS ordinations and chi-squared analyses were based on 144 quadrats, with 12 existing within each 1000 m^2 plot. These quadrats may exhibit some degree of spatial autocorrelation, meaning quadrats within 1000 m^2 plots are more similar to one-another than to quadrats in other 1000 m^2 plots. Despite the possible presence of spatial autocorrelation, these analyses are appropriate because they are secondary analyses to investigate the mechanisms driving the observed positive relationship between species richness and N heterogeneity. The potential influence of spatial autocorrelation suggests caution in extrapolating the identified response of individual species beyond our study.

Results and Discussion

Nitrogen Heterogeneity and Understory Species Diversity

Forest restoration treatments induced a notable increase in N heterogeneity that appears to be closely correlated with species diversity throughout the study area. Evidence for this relationship is provided by a relationship between species richness and N heterogeneity at the 1000 m^2 plot level, and divergence in species composition in high and low N patches (quadrats) within 1000 m^2 plots.

At the 1000 m^2 plot level, the data show a positive relationship ($r^2 = 0.40$; $p < 0.05$) between delta species richness in 2003 and TIN heterogeneity in 2002 (Fig. 2a), indicating that plots with the greatest TIN heterogeneity resulted in the largest net

79

increase in species the following year. This relationship weakened ($r^2 = 0.26$; $p < 0.10$), but remained positive one year later, when delta species richness in 2004 was correlated with TIN heterogeneity 2003 (Fig 2b). The weaker relationship found during the second sampling period (Fig. 2b) was primarily due to the influence of an outlier. This plot was located under an unusually dense patch of Douglas-fir that experienced little mortality during the prescribed fire. Therefore, understory diversity may have been decoupled from TIN heterogeneity in this plot due to exceptional overstory competition before and after restoration treatment.

A factor potentially confounding the interpretation of these correlations (Fig. 2) is the positive relationship expected between TIN heterogeneity and mean TIN within a plot. In other words, plots with higher TIN standard deviations also have higher TIN means (SD vs. Mean; 2002, $r^2 = 0.68$, $p < 0.05$; 2003, $r^2 = 0.43$, $p < 0.05$). Thus, it is difficult to distinguish whether increased species richness results from increased plot productivity, caused by higher N availability, or a divergence of species in high and low N patches. To evaluate whether community divergence had occurred between high and low N quadrats, we used ordination models of all quadrats (n = 144).

We first determined whether pre-treatment species composition could be distinguished in 2002 high and low TIN quadrats. This analysis indicated that no preexisting differences in species composition between these groups occurred prior to restoration (Fig. 3a). Following restoration treatments, high and low TIN quadrats in 2002 significantly differed from one another along the second axis in 2003 (df = 143, $f = 4.27$, $p = 0.04$), indicating a divergence in species composition (Fig. 3b). Furthermore, high and low TIN quadrats from 2003 were significantly separated along two axes in

80

2004 ordination space (Fig. 3c), which suggests increased patch-level divergence in species composition with time (df = 143; Axis 1, f = 7.27, p = 0.008; Axis 3, f = 3.81, p = 0.053).

Interestingly, high and low TIN quadrats showed greater differences in species composition in the following year (Fig. 3) compared to species composition during the same year (data not shown). Likewise, delta species richness in 2002 was not significantly related with TIN standard deviations during 2002 (data not shown), whereas delta species richness in 2003 showed a significant positive relationship with TIN standard deviations during 2002 (Fig 2a). These results suggest that divergence in species composition at the quadrat-level, and overall delta richness at the 1000 m^2 level lag behind TIN heterogeneity. The time required for a plant to respond to changing resource availability likely accounts for some of this observed lag. The one-year lag between TIN and understory composition also suggests that the system is dynamic at the quadrat level, with some degree of species turnover occurring annually in response to increases or decreases in TIN. Quadrats may gain or lose N by movement along topographic gradients, plant uptake and turnover, or mineralization and immobilization, leading to a changed N status from one year to another. Quadrats that undergo an increase or decrease in TIN are likely to experience an altered species composition the following year. Compositional changes appear to be driven mostly by annual and biennial species that are well adapted to quickly respond to resource pulses (Table 2).

These data provide strong support for RHH. Heterogeneity of limiting soil resources is frequently cited in the literature as a mechanism maintaining plant diversity in many systems (Ricklefs 1977, Tilman 1984, Rosenzweig and Abramsky 1993, Tilman

and Pacala 1993); however, far fewer studies have provided evidence that such a relationship actually exists. The large effort required to adequately sample spatial variation of soil resources likely limits investigation of this hypothesis. Our data are consistent with several studies, however, that provide support for RHH by showing that species composition is dissimilar on different resource patches (Fitter 1982, Inouye and Tilman 1995, Burnett et al. 1997, Reynolds et al. 1997, Vivian-Smith 1997, Nichols et al. 1998, Sulkava and Huhta 1998). Other studies have demonstrated that small scale heterogeneity of geomorphological characteristics, known to strongly influence resource availability, is strongly correlated with diversity at a larger scale (Burnett et al. 1997, Vivian-Smith 1997, Nichols et al. 1998).

To our knowledge, only one previous study has investigated the influence of N heterogeneity on diversity in the context of restoration. In a Kansas grassland, Baer et al. (2004) manipulated soil N concentrations prior to restoration and found that heterogeneous N plots did not result in an increase in species relative to homogeneous plots shortly following restoration. There are several potential explanation for these disparate results. Baer et al. (2004) noted that no response to N heterogeneity in their study may have been due to a the dominance of a native grass in all plots. We had no dominant species such as this in our study. Additionally, our spatial scale was much great than used by Baer et al. (2004), which likely incorporated the response of a larger number of species and minimized the influence of individual species. These disparate results may also reflect more severe N limitation in ponderosa pine/Douglas-fir forests compared to disturbed grassland. We are unaware of any additional published analyses

82

of diversity and soil resource heterogeneity relationships in the context of forest restoration.

Our data provide strong support that increasing N heterogeneity corresponds with increasing plant diversity. We focus on TIN because of its likely limitation to plant growth in this system and its rapid increase following disturbance. Further, belowground competition has been documented as an important structuring component of the understory community in the ponderosa pine ecosystem (Riegel et al. 1992, Riegel et al. 1995). We acknowledge that resource limitations other than N likely exist and may exhibit spatial heterogeneity. In particular, light availability and soil water status are altered by restoration treatments and may exhibit spatial heterogeneity. Additionally, some variation in species richness is likely a function of the stochastic nature of dispersal, where individual plots or quadrats may differ because of differences in seed banks and propagule pressure. Factors such as these may account for a large portion of the unexplained variation in our data. It is also unclear whether the relationships described here extend throughout the ponderosa pine ecosystem. Abiotic stress or limitation of other resources may organize the understory to a greater degree at other sites or in other regions.

Species Attributes

We identified numerous species whose frequency significantly increased in high or low N quadrats. In high N quadrats, four species in 2003 and six species in 2004 were significantly more frequent relative to pretreatment (Table 2). Species that increased frequency in high N patches were primarily forbs with high growth rates and excellent

83

dispersal abilities, attributes that allow these species to respond quickly to resource pulses. *Epilobium angustifolium, Collomia linearis, Cirsium vulgare, Taraxacum officinale* and *Logfia arvensis* are prolific producers of wind-dispersed seeds that are known to establish well after disturbance (Stickney and Campbell 2000). One species, *Verbascum thapsus*, has long-lived seeds that remain dormant in the seed bank until disturbance events create favorable conditions for germination (Burnside et al. 1996).

In low N quadrats, only one species in 2003 and four species in 2004 were significantly more frequent relative to pretreatment (Table 2). All species in low N quadrats (Table 2) were native perennials, and all but one have underground storage structures, including corms, bulbs, or rhizomes. These structures likely facilitate nutrient retention and allow associated species to persist in low resource conditions. Their increased frequency likely does not reflect a response to low N *per se*, but rather a response to disturbance on sites where resources other than N were substantially altered, such as light availability.

Both high and low N quadrats were distributed among all restoration treatments during 2003 and 2004 (Table 1); suggesting that increased frequency of species is not necessarily a function of a single restoration treatment. High N quadrats likely exist for a variety of reasons across all treatments. A majority of high N quadrats occurred in the Burn-only and Thin/burn treatments, likely due to fuel consumption from burning that resulted in high levels of NH_4^+ accumulation. A surprisingly large number of high N quadrats also occurred in the Thin-only and Control, which may have resulted from soil disturbance or their location on moist microsites. Low N quadrats existed in all treatments, and thus also likely reflect varying degrees of disturbance. In addition to

84

undisturbed quadrats in the Control, low N quadrats likely occurred on unburned or low-severity burn patches burn treatments, and locations experiencing a range of disturbance severity in the Thin-only.

The analysis we used to determine which species responded in high or low N quadrats was based on a small area ($144\ m^2$), thus we likely identified only a portion of the species in this system that responded in high or low N patches. The identified species, however, exhibit several attributes that are likely to be advantageous in low or high N environments. It is recognized that the traits of successful competitors in nutrient-rich and nutrient-poor locations differ substantially (Aerts 1999). Low resource environments may favor species with characteristics that promote nutrient retention. These include features such as long leaf-lifespan and increased production of secondary metabolites within leaves, which in turn result in slow N mineralization when litter is deposited on the forest floor (Aerts 1997). Aerts (1999) suggested that species with nutrient retention characteristics create soil-plant feedbacks, where low nutrient mineralization from their own litter leads to self-dominance. Under these low resource conditions, high N demanding species perform poorly because the nutrient supply is too limited to sustain their high growth rates. The very characteristics that allow low N species to be successful competitors in low N sites make them poor competitors in high nutrient environments because they lack the rapid growth rates needed to quickly acquire resources and avoid shading by competitors. The species we found associated with high and low N quadrats appear to reflect this trade-off.

Restoration Implications

Because an underlying relationship between TIN heterogeneity and species diversity has been identified, a pertinent question is which restoration treatment generates the highest degree of TIN heterogeneity, and thus is likely to result in higher diversity. A comparison of TIN standard deviation among treatments showed that both burn treatments became more heterogeneous (df =11, $F = 6.977$, $p = 0.013$) relative to the Control in 2002 (Fig. 4). In 2003, differences became slightly non-significant (df = 11, $F = 2.019$, $p = 0.190$), primarily due to the large standard error in the second replicate of the Burn-only treatment. This large standard error resulted from a dramatic increase in TIN heterogeneity in a single Burn-only plot from 2002 to 2003. Unlike any other plot included in the analysis, this plot was heavily colonized by the liverwort *Marchantia polymorpha*. There is some evidence that members of the genus *Marchantia* associate with N fixing bacteria (Brassell et al. 1986), thus this plot may have had an unusually large N input that resulted in increased heterogeneity.

It has been suggested that fire historically maintained spatial heterogeneity of resources in many systems (Raison 1979), and that exclusion of fire has led to a decrease in heterogeneity (Bonnicksen and Stone 1982). Reduced N heterogeneity may occur over time through a variety of processes, including selective root foraging in high resource patches, gradual invasion and densification of trees in forest openings, mass flow and leaching, and an increase in C rich substrates that lead to increased N immobilization throughout the stand. The Control in our study is representative of ponderosa pine/Douglas-fir forests in western Montana lacking wildfire for nearly a century. It is

86

impossible to know what N spatial patterns existed prior to fire exclusion, which precludes us from evaluating our data in terms of reference conditions. However, the data clearly show that stands in later stages of secondary succession, as a result of fire exclusion, have a low degree of spatial heterogeneity of available N (Fig. 4). The data also provide evidence that the reintroduction of fire through prescribed burning results in increased heterogeneity of available N.

We also found that within the first year of prescribed burning, N heterogeneity was positively correlated with fine fuel consumption (Fig. 5), a variable that is a proxy for fire severity. Fuel consumption during prescribed burning is a function of the abundance and distribution of fuels and weather conditions during burn days. These data imply that the addition of fuels to the forest floor through thinning operations, or variation in weather conditions during burn days, can influence spatial heterogeneity of N, and thus indirectly influence species richness. An important objective for future research is to determine how N heterogeneity responds to fire severity across a greater range of fire severities. It is plausible that N heterogeneity would demonstrate a unimodal relationship with fire severity. Severe stand-replacing fires may create spatial homogeneity that in turn leads to low diversity.

An increase in diversity is generally considered desirable; however, our data suggest that high N patches are also more likely to be colonized by exotic species (Table 2). One of four species in 2003, and four of six species in 2004 that positively responded to high N quadrats are considered exotic in western coniferous forests. In contrast, no exotic species were associated with low N quadrats (Table 2). While not all exotic

87

species are necessarily invasive, *V. thapsus* and *C. Vulgare* have characteristics of invasive species (Gross and Werner 1982, Petryna et al. 2002).

Invasive species in many systems have been shown to have common physiological and morphological traits. One such characteristic is a higher specific leaf area relative to native vegetation (Baruch and Goldstein 1999, Smith and Knapp 2001), which closely corresponds with a high relative growth rate and a high nitrogen requirement (Lambers and Poorter 1992, Reich et al. 1997). Additionally, these species tend to have exceptional seed production and dispersal abilities (Stickney and Campbell 2000). Thus, it may be difficult in most restoration operations to promote native species richness without also providing opportunity for invasive species colonization. Despite initial colonization and dominance of exotics, their invasion may be limited in duration. The exotic species identified in this study have been shown to require continued disturbance in order to persist in forest environments (Gross and Werner 1982, Petryna et al. 2002). Further study is needed on the duration and impacts of these invasive species following restoration treatments.

Conclusions

Results of this study provide strong evidence that an underlying relationship exists between TIN heterogeneity and understory plant species diversity in ponderosa pine/Douglas-fir forests, and thus also provide support for RHH. This relationship is driven by divergence of species composition on high and low N patches. Our data also indicate that the untreated Control, which has not experienced fire in over a century, exhibits a low degree of TIN heterogeneity. Additionally, both prescribed burn

88

treatments resulted in higher levels of TIN heterogeneity relative to the Control; whereas, the Thin-only treatment did not result in increased heterogeneity relative to the Control. These data suggest that the restoration treatments land managers choose to restore fire excluded ponderosa pine forests can directly influence N heterogeneity and subsequently influence species diversity as well. It is unclear whether TIN heterogeneity produced by restoration treatments is representative of historical conditions. Presumably, the frequent low-intensity fires that historically dominated western Montana ponderosa pine forests created and maintained heterogeneity; however, it may be impossible to reconstruct this aspect of reference conditions. These findings demonstrate the influence that N heterogeneity has on diversity in the context of ponderosa pine/Douglas-fir forest restoration, and may lead to more informed restoration decisions that consider treatment effects on understory diversity.

Acknowledgments

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Table 1: The distribution of high and low total inorganic N (TIN) quadrats among

treatments during 2002 and 2003. Each high and low N group consisted of 72 quadrats.

		Control	Thin-only	Burn-only	Thin/burn
2002	High N	20	7	17	28
	Low N	16	29	19	8
2003	High N	16	16	18	22
	Low N	20	20	18	14

98

Table 2: Species that increased in frequency in high or low total inorganic nitrogen (TIN) quadrats compared to pre-treatment. High and low quadrats were identified as above or below the median TIN for 2002 or 2003.

Nitrogen group/year	Species	X^2	^1p	Origin	Longevity	Lifeform
	2003 Vegetation					
High/2002	*Chamerion angustifolium*	7.4	**	Native	Perennial	Forb
	Verbascum thapsus	7.4	**	Exotic	Biennial	Forb
	Carex geyeri	6.3	*	Native	Perennial	Graminoid
	Collomia linearis	5.2	*	Native	Annual	Forb
Low/2002	*Carex concinnoides*	6.1	*	Native	Perennial	Graminoid
	2004 Vegetation					
High/2003	*Cirsium vulgare*	11.9	***	Exotic	Biennial	Forb
	Collomia linearis	7.4	**	Native	Annual	Forb
	Logfia arvensis	7.4	**	Exotic	Annual	Forb
	Verbascum thapsus	7.4	**	Exotic	Biennial	Forb
	Taraxacum officinale	4.2	*	Exotic	Perennial	Forb
	^{2}*Penstemon spp.*	4.0	*	Native	Annual	Forb
Low/2003	*Erythronium grandiflorum*	3.9	*	Native	Perennial	Forb
	Viola adunca	3.9	*	Native	Perennial	Forb
	Lomatium triternatum	3.8	*	Native	Perennial	Forb
	Poa secunda	3.8	*	Native	Perennial	Forb

^{1}* = p-values<0.05; ** = p-values<0.01; *** = p-values<0.001
^{2}*Penstemon* spp. include *P. procerus*, *P. confertus*, and their hybrids

99
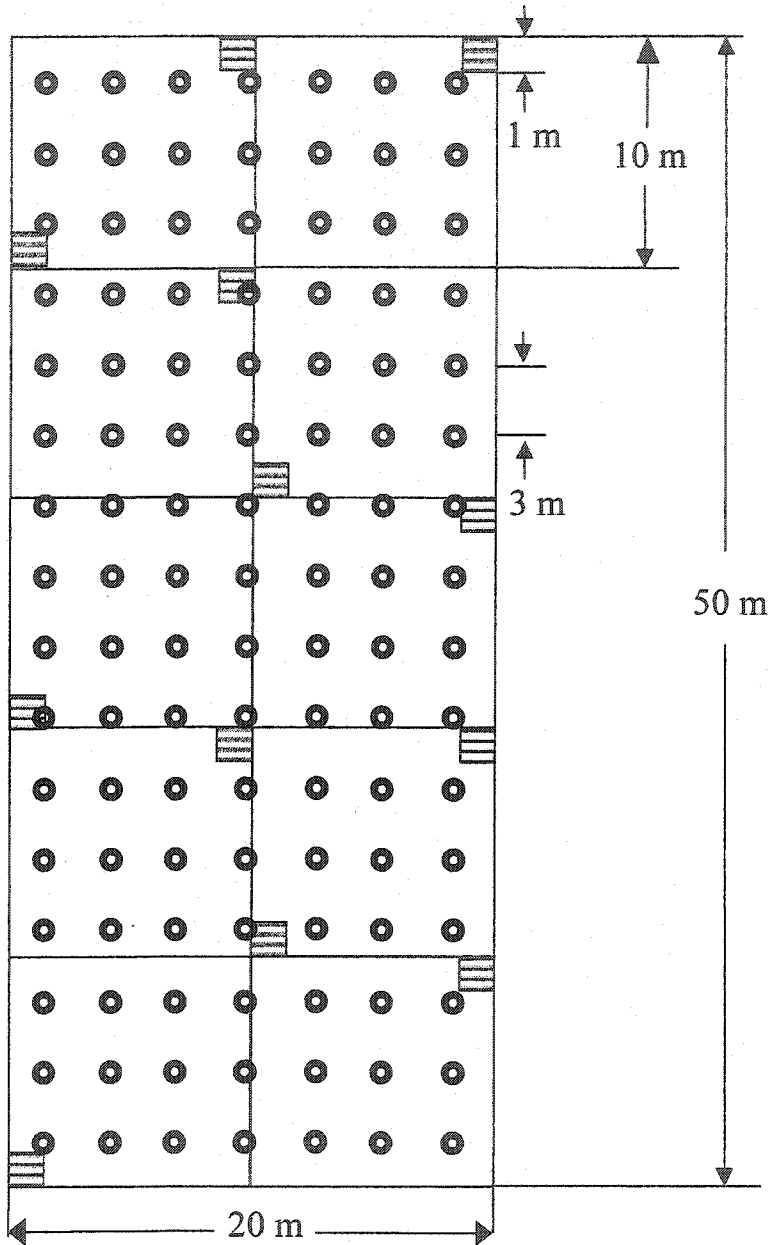
Figure 1: Sampling design for vegetation and soil N. Species diversity (presence/absence) was sampled at the 1000 m^2 plot level and at 12 1-m^2 quadrats within each plot. Soil samples (0-5 cm) were collected from a grid of 112 sample points (open circles) within each plot.
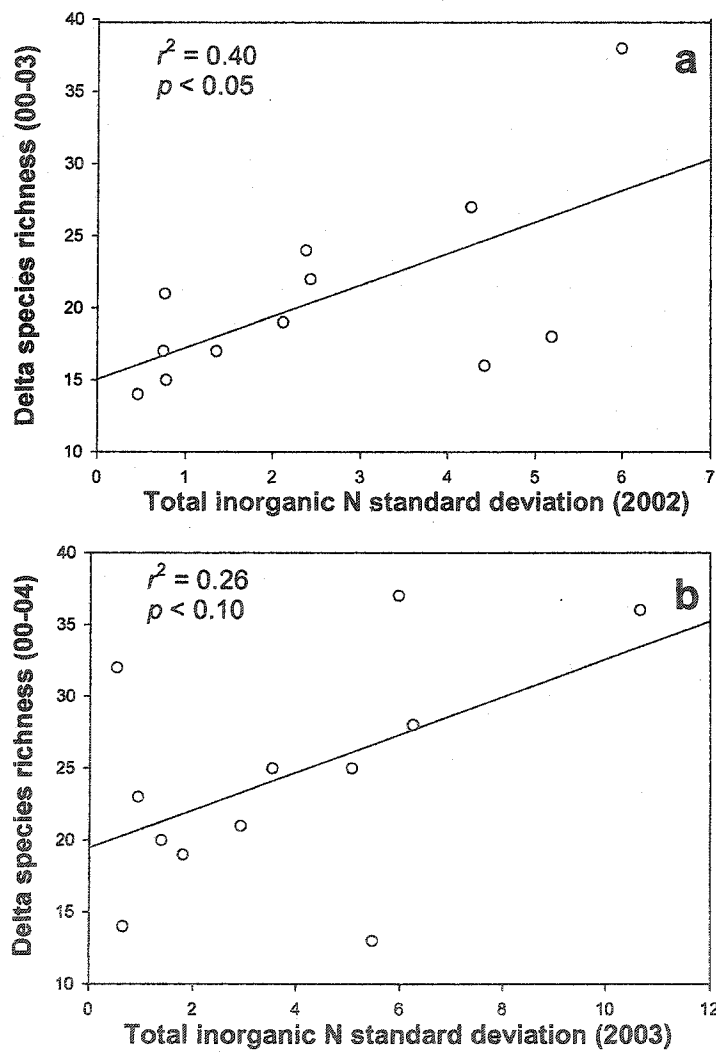
Figure 2: The correlation between delta species richness in 2003 (a) and 2004 (b), and

plot total inorganic N (TIN) standard deviation in 2002 and 2003, respectively.

101
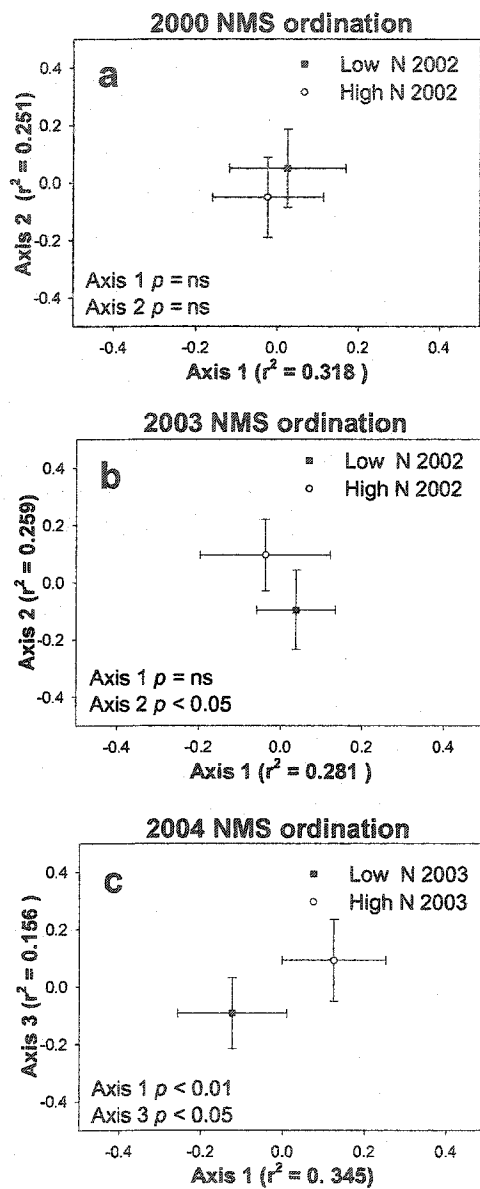
Figure 3: The location of high and low nitrogen centroids (± 95% confidence intervals) in NMS ordination space derived from species composition in 2000 (a), 2003 (b), and 2004 (c). The contribution of each axis (r^2) to the NMS model cumulative coefficient of determination is reported in parentheses for each axis.

103
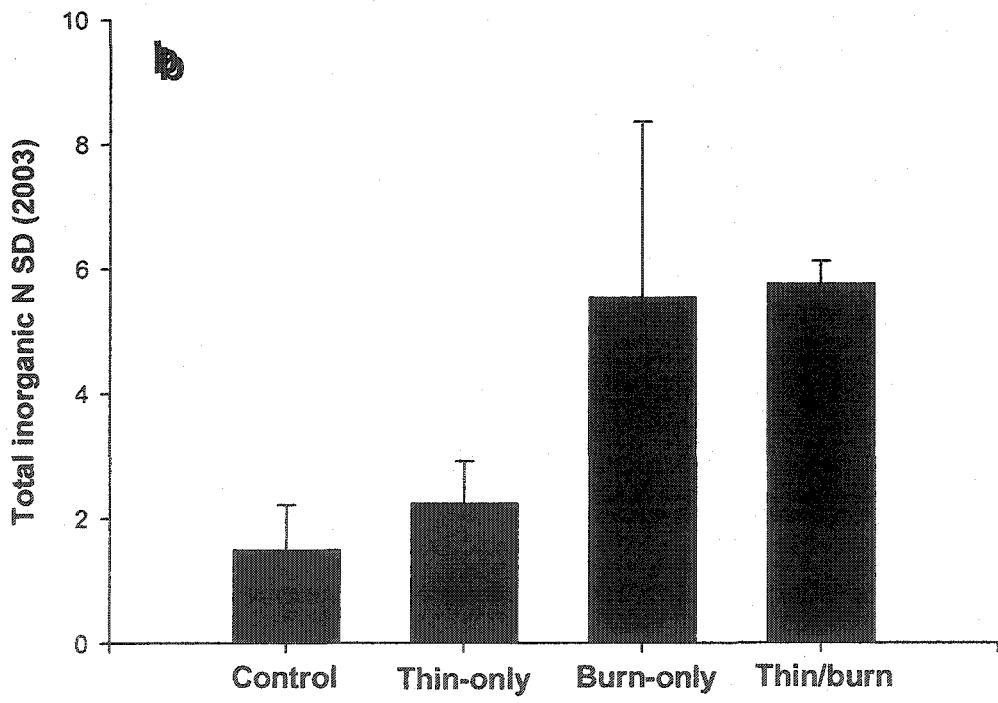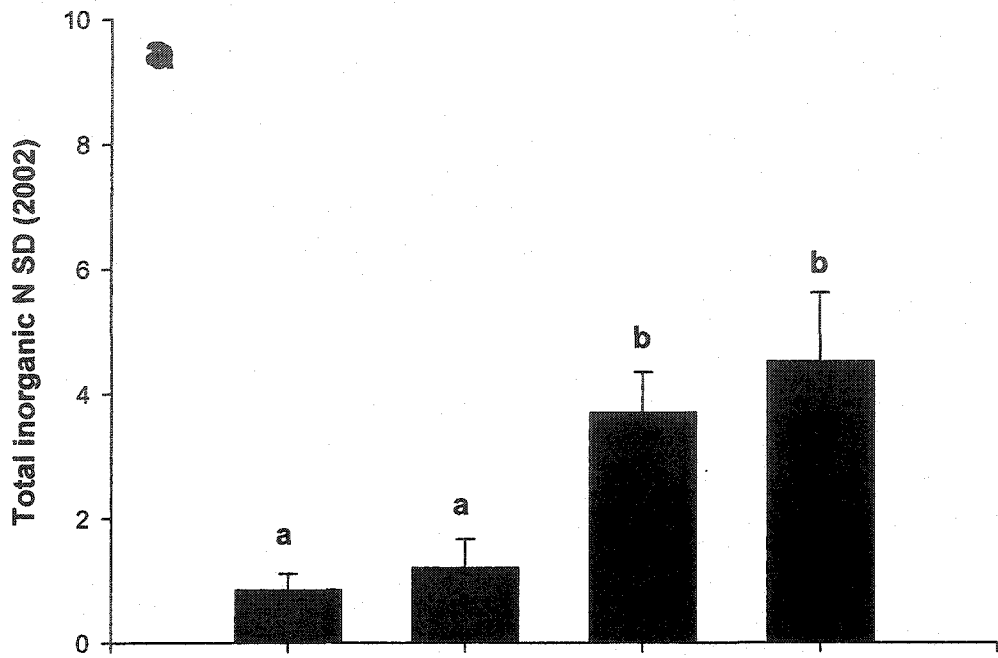
Figure 4: Mean standard deviations (SE) of inorganic N following four restoration treatments (Control, Thin-only, Burn-only, and Thin/burn). Letters above bars indicate post-hoc differences among treatments.
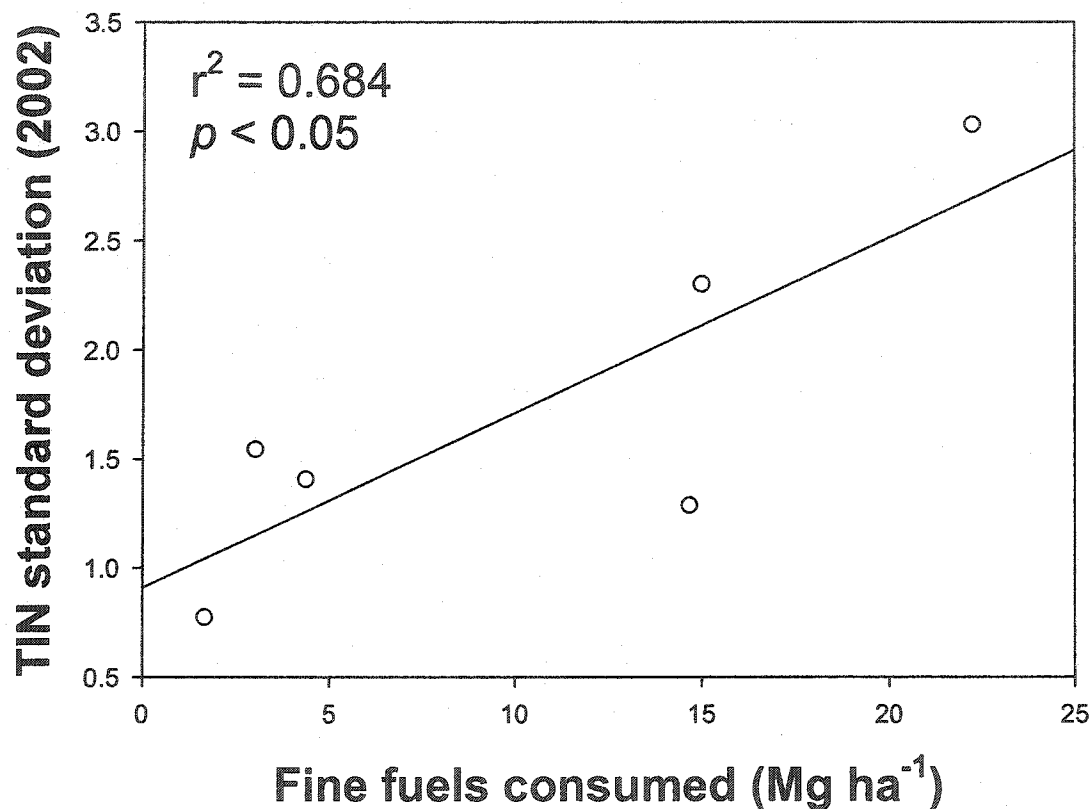
Figure 5: The correlation between standard deviation of total inorganic N (TIN) in 2002 and fine fuels consumed during prescribed burning of six 9 ha units.

105

Chapter 4

Charcoal and Litter Extracts Alter Soil Solution Chemistry and Growth of *Koeleria macrantha* in the Ponderosa Pine/Douglas-fir Ecosystem

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Abstract

Charcoal has been shown in natural systems to sorb litter extracts from late-successional species, increase nitrogen (N) cycling, and enhance growth of early-successional species. The potential role of charcoal on soil processes and plant growth in the ponderosa pine/Douglas-fir ecosystem has received little attention. We conducted a soil incubation experiment and two greenhouse experiments to determine whether charcoal derived from the ponderosa pine/Douglas-fir ecosystem may influence soil solution chemistry and growth of *Koeleria macrantha*, a perennial grass that thrives following fire. In our first experiment we incubated a forest soil with a factorial combination of low temperature (350 °C) Douglas-fir charcoal and extracts of *Arctostaphylos uva-ursi*. The experiment was repeated with the addition of glycine to eliminate any N substrate limitations. These experiments showed that low temperature charcoal increased N mineralization and nitrification when glycine was added, but reduced N mineralization and nitrification without the addition of glycine. Charcoal significantly removed soluble phenols added to solution from extracts, but may have contributed less soluble phenols to the soil. In our second experiment, we grew *K. macrantha* in soil amended with charcoal made at 300 °C from the bark of ponderosa

106

pine and Douglas-fir bark. Growth of *K. macrantha* was significantly diminished by both ponderosa pine and Douglas-fir bark relative to the control. In our third experiment, we grew *K. macrantha* in soil amended with six concentrations (0, 0.5, 1, 2, 5, and 10%) of charcoal collected from a wildfire. This experiment showed increasing growth of *K. macrantha* with charcoal addition. These results suggest some fundamental differences between laboratory generated charcoal and wildfire produced charcoal. Further, they suggest a need for a better understanding of how temperature and substrate influence the chemical properties of charcoal.

Introduction

It is well understood that fire alters nitrogen (N) cycling in the ponderosa pine/Douglas-fir (*Pinus ponderosa/Psuedotsuga menziesii*) ecosystem. Immediately following fire, a short-term increase in N availability has been shown to occur (Covington and Sackett 1990, 1992a, DeLuca and Zouhar 2000), which is likely the result of amine cleavage from organic N molecules during combustion, which releases a pulse of NH_4^+ into the soil (Christensen 1973, DeBano et al. 1979). Nitrogen availability has also been shown to remain elevated on the scale of months to years following fire as a result of enhanced mineralization (Covington and Sackett 1990, 1992a, Monleon et al. 1997, Kaye and Hart 1998, Gundale et al. In press). Numerous processes that influence N mineralization following fire have been identified, including improved substrate quality as a result of heating (White 1991, 1994, Fernandez et al. 1997, Pietikainen et al. 2000a), death of roots and soil organisms resulting in a large labile organic N pool (DeBano et al. 1979, Dunn et al. 1979, Diaz-Ravina et al. 1996, Neary et al. 1999), and a

reduction in C:N ratios due to preferential loss of C during combustion (Gundale et al. In press). A potentially overlooked factor that may also enhance N cycling following fire is the addition of charcoal to soils.

Several recent studies have shown that charcoal can greatly enhance soil fertility. In Amazonian forest soils, soils amended with charcoal and manure centuries ago sustain some of the highest biodiversity and productivity of any soils within the Amazon basin (Glaser et al. 2001, Glaser et al. 2002, Mann 2002). In boreal forest soils, charcoal has been shown to enhance N cycling by ameliorating the inhibitory effects of litter extracts from late-successional species, which in turn promotes growth of early-successional species (Zackrisson et al. 1996, Wardle et al. 1998, DeLuca et al. 2002, Berglund et al. 2004). The role of charcoal on N cycling in the ponderosa pine/Douglas-fir forest has received little attention.

Charcoal may increase N turnover through numerous mechanisms. It may act as a sorptive surface that selectively removes high C:N organic molecules from the soil solution (Zackrisson et al. 1996, Wardle et al. 1998, Glaser et al. 2002). The removal of these molecules may decrease microbial immobilization of N, leading to higher net mineralization and nitrification rates. Additionally, charcoal may remove specific groups of organic molecules, including plant secondary metabolites such as polyphenols, which may have direct negative effects on the microorganism driving soil N transformations (Rice and Pancholy 1972, Zackrisson et al. 1996, DeLuca et al. 2002, Berglund et al. 2004). Further, sorption of these organic molecules, along with the gradual breakdown of charcoal, may initiate humus formation and thus enhance long-term fertility (Glaser et al. 2002). In addition to these potential effects on soil solution chemistry, charcoal may

enhance soil fertility by creating habitat for microbes within its porous structure (Pietikainen et al. 2000b), or reduce soil bulk density thereby increasing macroporosity and gas exchange (Glaser et al. 2002). Changes in any of these soil properties may enhance N cycling following fire.

Unlike other factors that are know to influence N cycling following fire, charcoal is unique because of its high degree of recalcitrance. Thus, its effect on N cycling may last for decades and may influence successional processes. It has been reported that the exclusion of fire from ponderosa-pine/Douglas-fir forests leads to an inevitable "tightening" of nutrient cycles that is associated with increased dominance of slow-growing, highly nutrient-use efficient species (Covington and Sackett 1990, 1992a, Kaye and Hart 1998, MacKenzie et al. 2004). Litter of these late-successional species decomposes very slowly because of high leaf C:N ratios and an abundance of secondary metabolites (polyphenols), both characteristics that greatly enhance nutrient-use-efficiency. It has been reported that these leaf characteristics create a positive feedback where low nutrient mineralization from litter of these species enhances their own dominance (Aerts 1997, 1999). Charcoal may delay the onset of this feedback by altering solution chemistry and enhancing nutrient mineralization, thereby increasing the duration of the early-successional community.

Despite the many potential roles that charcoal may have in increasing soil fertility, its importance in the ponderosa pine/Douglas-fir ecosystem has received little attention. We conducted three separate experiments, using low-temperature charcoal from several substrates to investigate whether charcoal can influence soil solution chemistry, and growth of an early successional species. In our first experiment, our

objective was to determine whether charcoal had an influence on soil solution chemistry following addition of the leachate of a late-successional species, *Arctostaphylos uva-ursi,* via surface adsorption of organic compounds. We hypothesized that charcoal added to a ponderosa pine soil will effectively sorb the phenol fraction in litter extracts and thus enhance N cycling

In our second experiment, our objective was to compare the influence of charcoal made from the bark of two species, ponderosa pine and Douglas-fir, on *Koeleria macrantha,* a perennial grass species that thrives following fire disturbance in western Montana ponderosa pine/Douglas-fir forests. Bark charring during low intensity wildfire is a potentially significant source of charcoal in the ponderosa pine/Douglas-fir ecosystem. Charred bark may gradually slough from trees following fire and become incorporated in the soils surrounding trees. It is recognized that ponderosa pine is a more fire adapted species than Douglas-fir, thus an interesting hypothesis is that charred bark of the more fire adapted species will have a stronger effect on soil processes and plant growth. In our third experiment, our objective was to determine whether charcoal generated from a wildfire has any effect on *K. macrantha* growth, and to determine whether this relationship is dependent on soil charcoal concentration. In these experiments, we hypothesized that charcoal will improve solution chemistry by sorbing phenols and enhancing N cycling. Further, we hypothesized that *K. macrantha* will demonstrate enhanced growth in soils amended with wildfire and laboratory produced charcoal.

110

Methods

All three experiments were conducted using the same soil, which was collected from the subsurface horizon of a forest soil associated with low elevation (1,100 m) ponderosa pine/Douglas-fir vegetation in western Montana. The soil is described as a Sandy-skeletal, mixed, frigid Typic Dystrustepts. The soil was sieved (4mm), and one part sand was added to three parts soil, by mass, to create a soil with low fertility and high gas exchange. This sand amended soil had a pH of 6.8, electrical conductance of 91.2 μS/m, and had a textural distribution of 71 % sand, 21 % Silt and 8 % clay.

Experiment 1: Charcoal Sorption Potential

We conducted a laboratory incubation study using the soil described above, where Douglas-fir charcoal and extract of *Arctostaphylos uva-ursi* were added in a factorial combination yielding four treatments (Charcoal/Extract, Charcoal/No Extract, No Charcoal/Extract, and No Charcoal/No Extract). Each treatment was replicated five times. Treatments were established by adding 300 g of soil to mason jars. Charcoal treatments received 300 g of soil containing two percent charcoal (20 g/kg). Charcoal was generated in a muffle furnace by submerging Douglas-fir wood in sand, and heating to 350 °C for 2 hours. Charcoal was ground through an apple grinder, producing a variety of size classes, and sieved through a 4.75 mm sieve to remove all fragments larger than this diameter. *A. uva-ursi* extract was made by extracting 100 g of *A. uva-ursi* leaves in deionized water for 24 hours and filtering this extract through Whatman number 42 filters. The total phenol concentration of this extract was measured at 267.5 mg/l. Leachate treatments received 25 ml of this extract. No-extract treatments received an

111

equivalent volume of de-ionized water. This addition brought the soil in each mason jar to a water content of approximately 40%. Mason jars were dark incubated for 14 days upon which a portion of the soil was extracted and analyzed. This entire experiment was repeated exactly as described above but with glycine added to all mason jars to add a large source of highly labile organic N to stimulate more dramatic nitrogen cycling responses. Glycine is a simple amino acid that is readily mineralized to NH_4^+. Glycine was added to each mason jar at a rate of 75 mg/jar (250 mg/kg of soil). These two experiments will hereafter be referred to as the glycine and no-glycine trials.

Experiment 2: Effects of Bark Charcoal on Plant Growth

We conducted a greenhouse experiment consisting of three treatments (Douglas-fir charcoal, ponderosa pine charcoal, and a control) using the soil described above to evaluate the influence of low temperature charcoal, made from ponderosa pine and Douglas-fir bark, on growth of an early-successional species, *K. macrantha*. Each treatment consisted of 20 replicate pots, where each pot received 1.5 kg of soil. Both charcoal treatments received a 2% (by mass) charcoal addition. One percent of this charcoal was mixed in the soil, and was included in the 1.5 kg soil mass measurement. The other one percent was evenly distributed on the soil surface. We made charcoal from Douglas-fir and ponderosa pine in the laboratory by burying bark of each species in silica sand and heating to 350 °C in a muffle furnace for 2 hours. Charcoal was ground through a 1 mm sieve using a Whiley mill. Organic horizons were added to the surface of each pot to add an additional and substantial mineralizable pool of plant essential nutrients, and to provide a source of bio-available organic C that may influence soil nutrient

112

transformations. This organic material included O_i, O_e, and O_a horizons collected through a randomized process from a ponderosa pine/Douglas-fir forest that had not been exposed to fire for approximately 100 years. This organic material originated from numerous species, including understory and overstory species, but appeared to be primarily composed of ponderosa pine and Douglas-fir litter at various stages of decomposition. The organic material was homogenized and 100 g was allocated to the surface of each pot. A mixed bed ionic resin capsule (Unibest Inc., Bozeman, MT) was placed in the center of each pot to sorb nutrients throughout the duration of the experiment.

K. macrantha was grown in these pots between October 2004 and March 2005 under ambient light conditions. An average greenhouse temperature of 21 °C was maintained. K. macrantha seeds (Western Native Seeds, Coaldale, CO) were germinated in a separate soil medium and transplanted when shoots were approximately 2-3 cm long. Pots were watered three days a week throughout the duration of the experiment. At the end of the experiment, resin capsules were recovered and soil was rinsed from roots. Plants were oven dried at 65 °C and above and belowground mass was measured.

Experiment 3: Effect of Wildfire Charcoal on Plant Growth

We conducted a greenhouse experiment consisting of six treatments (0, 0.5, 1, 2, 5, and 10 % charcoal addition) using the soil described above to determine whether an increase in soil charcoal content has any influence on growth of K. macrantha. Treatments were established by adding 1.0 kg of each soil to ten replicate pots per treatment. The charcoal used in this experiment differed from both previous experiments

113

in that charcoal was collected following a wildfire rather than generated in the laboratory. Charcoal was collected in the spring of 2004 from the Black Mountain Fire (August 2003), Missoula, MT, from the soil surface. It was impossible to decipher the species origin of this charcoal. Additionally, collected charcoal was pulverized, producing fragments across a range of size classes. No attempt was made to discriminate against any size class in attempt to simulate the range of charcoal particle sizes likely incorporated into the soil under natural conditions. Organic material (50 g) was collected and added to the surface of each pot as described earlier. All other experimental conditions were run identically to experiment number two.

Laboratory Analyses

At the end of experiment one, 30 g of soil was extracted with 2 M KCl and analyzed for NH_4^+ and NO_3^- on a segmented flow analyzer (Auto Analyzer III, Bran Luebbe, Chicago, IL) using the Berthelot reaction (Willis et al. 1993) and cadmium reduction method (Willis and Gentry 1987), respectively. Amino N was measured on these same extracts using the ninhydrin method (Moore 1968). Soluble phenols were extracted by shaking 30 g of soil for 1 hour with 50 ml of deionized water followed by filtration. Insoluble phenols were extracted from these same samples by shaking with 50 percent methanol for 24 hrs followed by filtration. Phenols in these extracts were measured using the Prussian blue method (Stern et al. 1996). Respiration was measured at the end of the incubation by incubating 50 g dry weight equivalent soil in a sealed container with 20 ml 1 M NaOH traps for three days (Zibilske 1994).

114

Mixed bed ionic resin capsules (Unibest Inc., Bozeman, MT) were used in experiments two and three to determine solution NH_4^+, NO_3^- and PO_4^- throughout the duration of the experiments. Capsules were extracted with three consecutive 30 minute rinsings of 2 M KCl. Resin sorbed NH_4^+ and NO_3^- were analyzed from these extracted as described earlier. Phosphate in these extracts was measured on a segmented flow analyzer (Auto Analyzer II) using the molybdate method described by Murphy and Riley (1962).

Statistical Analyses

Our objective in experiment was to determine the effect of charcoal, extract and the interaction of these factors on several soil solution properties. Data in experiment one meeting assumptions of normality and homoscedasticity were analyzed using two-factor analysis of variance (ANOVA), where leachate and charcoal were entered as fixed factors under the general linear model. Variables not meeting these assumptions were analyzed using a Kruskal-Wallis test (K-W test). This analysis tests for differences among treatments but does not evaluate the significance of individual factors or interaction between factors.

Our objective in experiments two and three were to determine whether a single factor, charcoal source or charcoal concentration, respectively, had an effect on growth of *K. macrantha*. Data in experiment two and three were analyzed using one-factor ANOVA's followed by the Student-Newman-Keuls post-hoc procedure. Different letters are used to display post hoc difference in Table 3 and 4. Data not meeting assumption of normality and homoscedasticity were compared using K-W tests, which were not

115

followed by post-hoc procedures. All analyses were conducted using SPSS 12.0 software.

Results and Discussion

Experiment 1: Low Temperature Charcoal Sorption Potential

Both charcoal and extract significantly influenced numerous soil processes (Figure 1-3, Table 1). In both glycine and no-glycine trials, extracts negatively affected extractable NO_3^- concentration (Figure 1). The negative influence of *A. uva-ursi* on NO_3^- reported here is consistent with studies that have shown extracts from late-successional boreal species, such as the Ericaceous shrub *Empetrum hermaphroditum*, diminishes net nitrification (DeLuca et al. 2002, Berglund et al. 2004). Low temperature charcoal had an unexpected negative effect on NO_3^- in the no-glycine trial. In contrast, the effect of charcoal on NO_3^- appeared highly positive in the glycine trial. These result may be a function of the charcoal we used in this study, which was generated at a low temperature (350 °C). This low temperature charcoal likely contains a higher concentration of bio-available C than higher temperature charcoals, and thus may have caused NO_3^- immobilization in the no-glycine trial. This immobilization effect may not have reduced NO_3^- in the glycine trial because N limitations were drastically reduced with glycine addition.

Extract had a strong positive effect on NH_4^+ in both no-glycine and glycine trials (Figure 1, Table 1). This is likely a function of direct NH_4^+ addition from the extract as well as addition of substrates that are rapidly mineralized to NH_4^+. Charcoal had a strong negative effect on NH_4^+ in both no-glycine and glycine trials. The mechanisms for this

116

pattern may differ between the two trials. In the no-glycine trial, the most likely explanation for reduced NH_4^+ is the same immobilization mechanisms that may influence NO_3^-. In the glycine trial, it is likely that increased nitrification associated with charcoal contributed to reduced NH_4^+ concentrations.

Both charcoal and extract significantly influenced amino N concentrations Figure 1, Table 1). Amino N represents a highly labile fraction of organic N that is readily mineralized. Glycine, which is the simplest amino acid, allows for exceptionally rapid rates of mineralization to occur. The glycine trial resulted in substantially higher amino N concentrations than the no-glycine trial, which suggests that the added glycine was not completely utilized, and that substrate limitation did not occur during this trial. In both glycine and no-glycine trials, extract appeared to add a significant amount of amino N to soils. The effect of charcoal on amino N, however, differed in glycine and no-glycine trials. In the no-glycine trial, charcoal significantly increased amino N concentrations. Furthermore, this positive charcoal effect on amino N significantly interacted with the effect of extract on amino N. This may reflect that charcoal stimulated the activity of heterotrophic bacteria, an effect that was enhanced by the added C source in the extract. Enhanced microbial activity may increase soil amino N as a function of population turnover (DeLuca and Keeney 1993). In contrast to the no-glycine trial, charcoal had a negative effect on amino N in the glycine trial. Glycine was likely the most substantial contribution to the amino N pool in this trial. Reduced amino N in the presence of charcoal in this trial may reflect enhanced microbial utilization of glycine in the presence of charcoal, or sorption of glycine to charcoal, either directly or as part of the formation of phenol protein complexes (Hattenschwiler and Vitousek 2000).

117

As expected, *A. uva-ursi* extract significantly increased both soluble and insoluble phenols (Figure 2, Table 1). The addition of charcoal to soil significantly diminished the soluble phenol concentration. This result is consistent with several studies in the boreal forest that have demonstrated a high capacity of charcoal to adsorb phenolic compounds (Zackrisson et al. 1996, Wardle et al. 1998, DeLuca et al. 2002, Berglund et al. 2004). This phenol fraction is likely chemically different from the insoluble phenol fraction. Soluble phenols are likely more bio-available, and thus likely have a large potential to affect soil microbes and soil processes (Harborne 1997). In contrast to the soluble phenol fraction, insoluble phenols were significantly higher in charcoal treatments of both trials, suggesting that charcoal sorbed soluble phenols from solution making them less bio-available.

Soil respiration showed little response to charcoal in glycine or no-glycine trials (Figure 3, Table 1). In the no-glycine trial, extract significantly increased soil respiration. Extract and charcoal had no individual effect on soil respiration in the glycine trial, however, the interaction between charcoal and extract showed a significant effect. This interaction demonstrated that if either charcoal or leachate were added alone, they negatively influenced respiration, but when they were added together, respiration was high.

These data demonstrate that low temperature charcoal removed soluble phenols from solution, which increased the pool of sorbed phenols measured through methanol extraction. This corresponded with increased nitrification in the Glycine trial, as seen in higher NO_3^- and lower NH_4^+ pools. In the low N environment of the no-Glycine trial, this change in phenol fractions did not correspond with enhanced nitrification. In

118

contrast, charcoal in this trial appeared to cause N immobilization. This may be due to the presence of bio-available C that is present on low temperature charcoal.

These results are consistent with Bergland et al. (2004) and DeLuca et al. (2002) who demonstrated an interactive effect between charcoal and labile N addition, where the effect of charcoal on N nitrification only occurred when a labile N source was also present. These studies are also consistent with the Terra preta phenomenon reported in the Amazonian basin, where charcoal and manure (labile N source) were historically incorporated into the soil. Today these soils maintain the highest fertility in the region which is likely a function of the interactive effect of charcoal and manure.

Experiment 2: Effects of Bark Charcoal on Plant Growth

In this experiment, we found that charcoal from both species diminished growth of *K. macrantha* relative to the control (Table 3). Reduced total mass in the charcoal treatments was a function of both reduced aboveground and belowground growth. *K. macrantha* growing in pots with Douglas-fir charcoal had a significantly higher root to shoot ratio than the other treatments which appeared to be primarily driven by low aboveground biomass.

We found that resin sorbed NH_4^+ and NO_3^- were significantly higher in the Douglas-fir charcoal treatment relative to ponderosa pine charcoal treatment and control. Resin sorbed PO_4^- was significantly higher in both Douglas-fir and ponderosa pine charcoal treatments. These results may be interpreted in several ways. First, they may indicate higher mineralization and nitrification rates in the presence of charcoal. If higher mineralization occurred in the presence of charcoal, it is unclear why a corresponding

119

increase in plant growth did not occur. It is possible that the low temperature charcoal used in this study contributed a pool of insoluble phenols, as demonstrated in the first experiment, which inhibited root growth of *K. macrantha,* despite a positive effect on nutrient availability. This fraction of total phenols may not exist in higher temperature charcoals, and further experimentation should be done to evaluate this hypothesis. Another explanation for lower nutrient sorption to resin capsules in the control may be that higher nutrient uptake occurred in these pots, leaving a diminished nutrient pool for sorption. A third possibility is that charcoal may have enhanced macroporosity, allowing more soil solution to pass through capsules.

Experiment 3: Effect of Wildfire Charcoal on Plant Growth

Unlike experiment two, natural charcoal collected from a wildfire showed a positive effect on growth of *K. macrantha.* Both total mass and aboveground mass were significantly higher in pots amended with 5% and 10% charcoal addition than the control. Pots with lower charcoal content (0.5% - 2%) showed an intermediate growth response. No significant shift in allocation to above or belowground structures was detected across the charcoal gradient.

As in experiment two, resin sorbed NO_3^-, and PO_4^- decreased as plant growth increased. These results suggest that these measurements do not reflect any direct effect charcoal may have on nutrient cycling, but rather are indicative of the solution nutrient concentration as influenced by plant uptake. No difference in resin sorbed NH_4^+ occurred across the charcoal gradient.

120

The different response of *K. macrantha* to charcoal in experiments one and three suggest that charcoal produced in a laboratory may be greatly different from charcoal generated during wildfire. Differences in temperature and oxygen availability during formation may influence the chemical and structural nature of charcoal, and therefore change its influence on soil solution chemistry. Additionally, in a natural setting soil charcoal may be colonized by organisms that were not present in laboratory generated charcoal. A third difference between the charcoal used in this experiment from the previous two experiments was the range of size classes added. This experiment incorporated charcoal ranging from large (1-2 cm) to microscopic fractions. We noted substantial root penetration into large charcoal particles at the end of this greenhouse experiment, which suggests that some resource is more available inside charcoal particles. It is possible that grinding charcoal to a smaller size class in some way changes the influence of charcoal on resource availability.

Conclusions

It is clear that charcoal has the potential to significantly alter soil solution chemistry and growth of *K. macrantha*. Charcoal did not appear to stimulate N cycling in a low nutrient setting, but when glycine was added to soil, charcoal greatly enhanced N mineralization and nitrification, where a significantly higher accumulation of NO_3^- occurred. This result may indicate that low temperature charcoal contributes bio-available or bio-inhibitory carbon that has an antagonistic influence on nutrient cycling

121

under low nutrient conditions. As hypothesized, charcoal effectively adsorbed soluble phenols from solution. This may effectively reduce the influence of extract on soil organisms, plants and processes. This same charcoal also appeared to add a significant pool of less soluble phenols to the soil. It is unclear whether this relatively insoluble phenol pool is bio-available, and thus influences biological activity, but it may act as a C substrate that causes nutrient immobilization to occur or may directly inhibit roots or microbial activity. Low-temperature laboratory-generated charcoal had a negative effect on growth of *K. macrantha*. This may be the result of an inhibitory effect that insoluble phenols in this charcoal have on root growth. In contrast, charcoal created during a wildfire had a positive effect on growth of *K. macrantha*, suggesting laboratory charcoal may not have adequately represented field collected charcoal.

Further research is needed to understand how the many properties of charcoal change when charcoal formation is done under different temperatures and from different substrates. It is likely that formation of charcoal at higher temperatures increases its sorptive capacity, and thus increases its influence on N cycling. Additionally, it is likely that more complete charring occurs at higher temperatures, leaving less bio-available carbon to potentially interfere with nutrient cycling or plant growth.

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123

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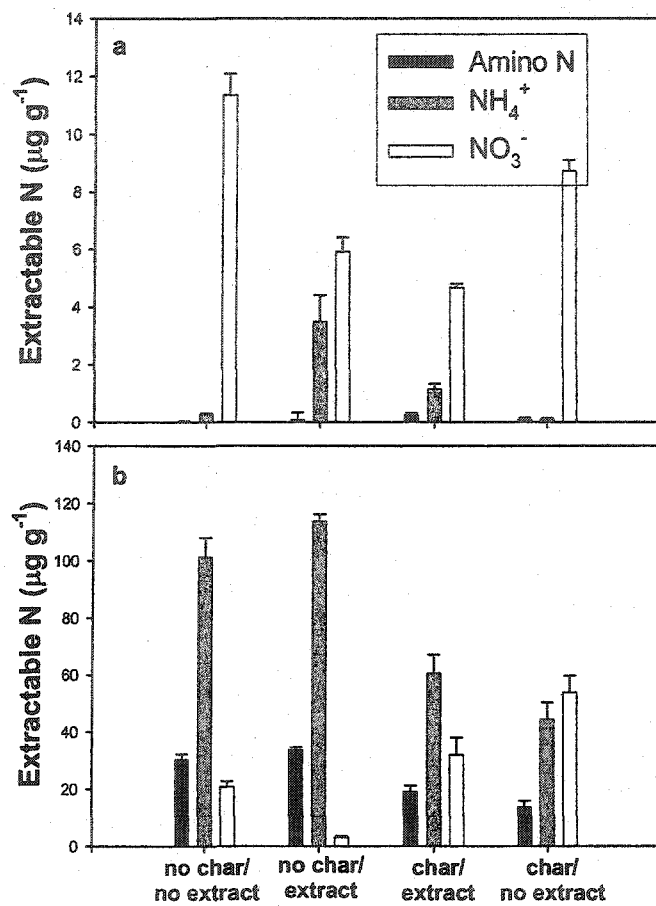
Figure 1: Extractable amino N, NH_4^+, and NO_3^- (mean (SE) from a soil incubation experiment (14 days) without (a) and with (b) glycine addition. Soils were amended with a factorial combination of charcoal and extracts from *Arctostaphylos uva-ursi* leaves.

127

Figure 2: Soluble and sorbed phenols (mean (SE)) from a soil incubation experiment (14 days) without (a) and with (b) glycine addition. Soils were amended with a factorial combination of charcoal and extracts from *Arctostaphylos uva-ursi* leaves.

Figure 3: Basal soil respiration (mean (SE)) from a soil incubation experiment (14 days) without (a) and with (b) glycine addition. Soils were amended with a factorial combination of charcoal and extracts from *Arctostaphylos uva-ursi* leaves.
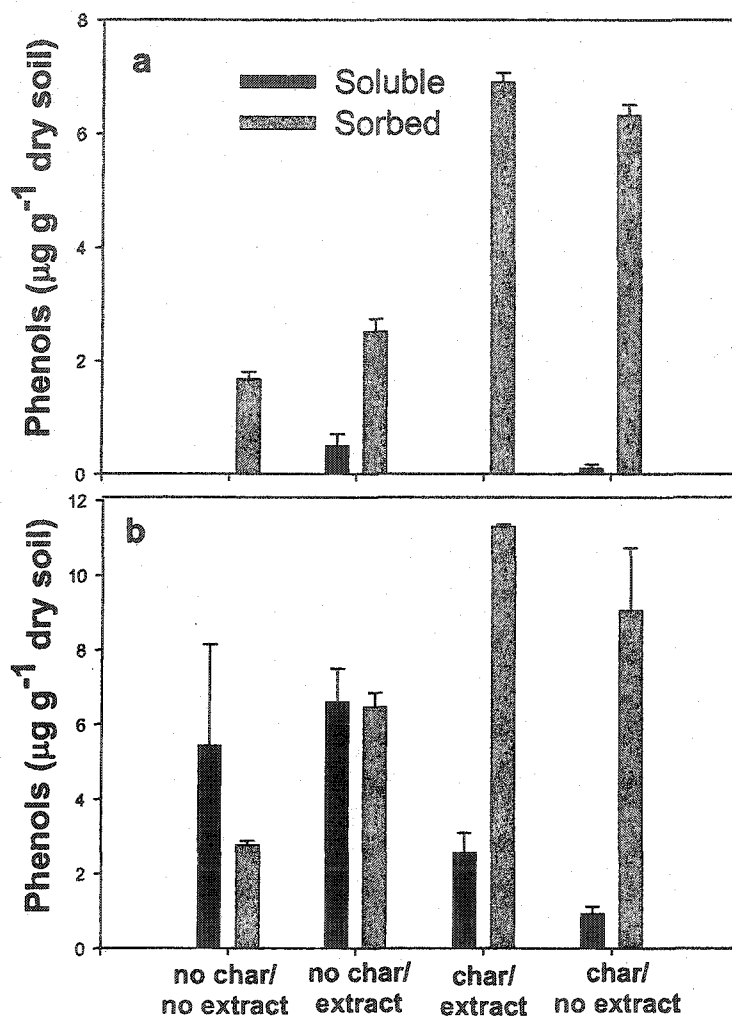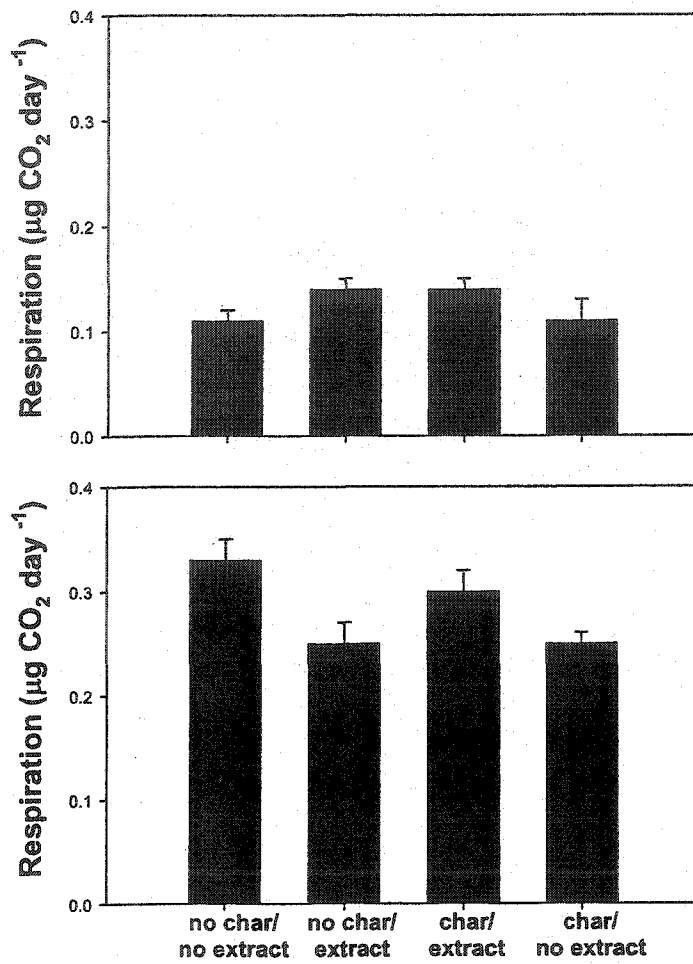
Table 1: Statistical analysis of an incubation study where soil was amended with charcoal made from Douglas-fir wood at 400 degrees.

	NO$_3^-$		NH$_4^+$		Amino N		Soluble Phenols		Total Phenols		Respiration	
	Test statistic	p-value	Test statistic	p-value	Test statistic	p-value	Test statistic	p-value	Test statistic	p-value	Test statistic	p-value
No Glycine												
2 x 2 ANOVA												
Charcoal	5.96	***	-	-	5.15	*	-	-	650.3	***	-	ns
Extract	94.94	***	-	-	18.93	***	-	-	16.6	***	5.23	*
Charcoal*Extract	1.96	*	-	-	9.85	**	-	-	-	ns	-	ns
K-W test	-	-	17.31	***	-	-	14.74	**	-	-	-	-
Glycine												
2 x 2 ANOVA												
Charcoal	-	-	96.5	***	83.87	***	-	-	37.05	***	-	ns
Extract	-	-	6.54	**	6.58	*	-	-	10.58	**	-	ns
Charcoal*Extract	-	-	-	ns	-	Ns	-	-	-	ns	12.69	**
K-W test	15.6	***	-	-	-	-	9.70	0.021	-	-	-	-

[1]Test statistics are F-values from a two-factor ANOVA, where significance was tested for Charcoal, Extract and Charcoal*Extract interaction. Data that did not meet parametric assumptions of normality or homoscedasticity were analyzed using the Kruskal-Wallis test.
[2]p-value: ns > 0.1, * < 0.05, ** < 0.01, *** < 0.001

130

Table 2: Plant mass and resin sorbed nutrients (Mean ±SE, n=20) from a greenhouse experiment where soil was amended with 2% charcoal made from Douglas-fir and ponderosa pine bark at 350 °C. Letters indicate differences using the Student-Newman-Keuls post-hoc procedure.

	df charcoal	pp charcoal	no charcoal	p-value[1]
Total Mass (g)	a1.55 (0.18)	A1.91 (0.14)	b2.54 (0.98)	***
Root Mass (g)	a0.82 (0.09)	A0.89 (0.08)	b1.20 (0.13)	*
Aboveground Mass (g)	a0.74 (0.10)	B1.02 (0.07)	c1.34 (0.11)	***
Root:Shoot	a1.20 (0.08)	B0.87 (0.06)	b0.92 (0.07)	0**
NH_4^+ µg resin capsule^{-1}	a4.59 (0.87)	B1.42 (0.64)	b2.20 (0.79)	*
NO_3^- µg resin capsule^{-1}	a1770.8 (286.2)	b935.8 (241.9)	b581.5 (211.8)	**
PO_4^- µg resin capsule^{-1}	a5.13 (1.43)	A5.60 (0.85)	b0.48 (1.22)	*

p-value: *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$

131

Table 3: Plant mass and resin sorbed nutrients (Mean ±SE, n=10) from a greenhouse experiment where soil was amended with 0, 0.5, 1, 2, 5, and 10% charcoal collected from a wildfire. Letters indicate differences using the Student–Newman–Keuls post-hoc procedure.

| | Percent Charcoal | | | | | | [1]p |
	0%	0.5%	1%	2%	5%	10%	
Total mass (g)	a0.6 (0.2)	ab1.0 (0.3)	ab1.1 (0.2)	ab1.1 (0.2)	b1.3 (0.1)	b1.3 (0.1)	*
Root mass (g)	0.3 (0.2)	0.6 (0.2)	0.7 (0.1)	0.7 (0.1)	0.8 (0.1)	0.8 (0.1)	ns
Aboveground mass (g)	a0.2 (0.1)	ab0.4 (0.1)	ab0.4 (0.1)	ab0.4 (0.1)	b0.5 (0.1)	b0.6 (0.1)	**
Root:Shoot	1.3 (0.2)	1.5 (0.3)	1.7 (0.2)	1.6 (0.3)	1.7 (0.2)	1.3 (0.1)	ns
[3]NH_4^+	55.6 (4.0)	49.8 (4.6)	36.9 (6.0)	42.7 (2.2)	43.0 (1.3)	44.4 (2.9)	[2]*
[3]NO_3^-	a1539.8 (463.4)	b947.9 (128.4)	bc552.3 (116.3)	bc556.1 (93.4)	bc561.8 (278.7)	c248.6 (29.8)	***
[3]PO_4^-	a10.1 (1.4)	a8.8 (2.1)	ab5.8 (1.2)	ab6.5 (1.7)	bc1.7 (1.0)	c0.0 (1.2)	***

[1] All p-values are for one-way ANOVAS, unless otherwise noted. p-value: ns, $p>0.05$; *, $p<0.05$; **, $p<0.01$, ***, $p<0.001$

[2] Kruskal–Wallis test p-value

[3] μg resin

132

Chapter 5

Temperature and Substrate Influence the Chemical Properties of Charcoal in the
Ponderosa Pine/Douglas-fir Ecosystem

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Abstract

Charcoal is a recalcitrant component of soils and has recently been shown to
enhance soil fertility in several ecosystems. Charcoal likely has a positive effect on soil
fertility because of several key properties, including its ability to sorb dissolved organic
molecules, contribute soluble nutrients such as NH_4^+ and NO_3^-, and stimulate activity of
autotrophic nitrifiers. Little is known about how these and other potentially important
properties vary among charcoals made at different temperatures and from different
substrates, two formation factors that are likely to vary in forest ecosystems exposed to
fire. We evaluated variation in charcoal properties caused by temperature and substrate
in the ponderosa pine/Douglas-fir (*Pinus ponderosa/Psuedotsuga menziesii*) ecosystem
by generating charcoal from ponderosa pine and Douglas-fir bark and wood in the
laboratory. As char generation temperature increased, density, NH_4^+ concentration,
soluble and total phenols, and the influence on nitrifier activity decreased. Conversely,
total C content, NO_3^- concentration, and catechin sorption increased with temperature.
For most variables, the magnitude difference caused by substrate was smaller than
differences caused by temperature. Comparisons of properties among substrates
suggested that charcoal made from Douglas-fir bark was the most dissimilar compared to

133

other charcoals. Charcoal made from Douglas-fir bark had a significantly lower density (350 and 800 °C), total C content (800 °C), soluble (300 and 800 °C) and total phenol (300 °C) concentration than other substrates. Further, charcoal made from Douglas-fir bark had significantly higher NH_4^+ and NO_3^- concentrations (300 and 800 °C), and stimulated higher nitrifier activity (300 and 800 °C) than other charcoals. We measured these same variables on charcoal collected following a wildfire and compared these estimates to the range of estimates measured on laboratory charcoal. Wildfire charcoal appeared to be more similar to low than high temperature charcoal generated in the laboratory. Additionally, all variables measured on wildfire charcoal showed a higher degree of variation among replicates, suggesting that a range of charcoal types exist in natural conditions.

Introduction

Several recent studies have demonstrated that charcoal, a highly recalcitrant byproduct of fire, can have significant long-term effects on soil fertility and ecosystem function. In the Amazonian basin, forest soils amended with charcoal and manure centuries ago retain some of the highest biodiversity and productivity of any soils in the region (Glaser et al. 2001, Glaser et al. 2002, Mann 2002). In boreal forest soils, charcoal has been shown to enhance N cycling for decades following fire by ameliorating the inhibitory effects of litter leachate from late-successional species, which in turn promotes the growth of early-successional species (Zackrisson et al. 1996, Wardle et al. 1998, DeLuca et al. 2002, Berglund et al. 2004). Recent studies have also shown that charcoal can have strong effects on N cycling and soil solution chemistry in the ponderosa pine/Douglas-fir (*Pinus ponderosa/Psuedotsuga menziesii*) ecosystem of the western

United States (DeLuca et al. 2005). Currently, we lack a thorough understanding of the numerous physical and chemical properties of charcoal that may influence soil fertility, and further, how these properties vary in natural systems.

Charcoal may contribute to enhanced soil fertility through a variety of mechanisms. One of the most well known functions of charcoal is its ability to sorb organic molecules, a function that has long been recognized in industrial, medical and pollution management fields (e.g. Ho et al. 2004, Limoto et al. 2004, Mizuta et al. 2004, Seger and Muelenbelt 2004). In forest ecosystems, sorption may directly influence soil solution chemistry by selectively removing high C:N organic molecules from the soil solution (Zackrisson et al. 1996, Wardle et al. 1998, Glaser et al. 2002), which in turn may enhance net mineralization and nitrification rates. It has been suggested that plant secondary metabolites may also have direct inhibitory effects on the soil microbial community (Rice and Pancholy 1972, Lodhi and Killingbeck 1980), thus it has been proposed that charcoal sorption may ameliorate this effect (Zackrisson et al. 1996, DeLuca et al. 2002, Berglund et al. 2004). In addition to these sorptive properties, charcoal may contain available forms of several essential nutrients, such as NH_4^+, NO_3^-, and PO_4^-, and thus increase nutrient availability.

These potentially important properties of charcoal likely demonstrate a high degree of variability in natural systems. Two factors that likely contribute greatly to variability in charcoal properties are formation temperature and the type of substrate from which charcoal formed (Glaser et al. 2002). Temperature may affect charcoal by volatilizing certain elements and organic molecules, while concentrating those that remain. Thus high temperature charcoal may have a greatly different chemical

135

composition than low temperature charcoal. Substrate may also influence properties of charcoal because lignified materials, such as wood and bark, likely have different chemistries and physical structures (Bold et al. 1987). For instance, a simple difference in tracheid diameter between the wood of two species may determine the surface area, and thus sorption capacity, of charcoal generated from these substrates.

Differences in charcoal properties caused by temperature and substrate may have important ecological significance, although this has never been investigated. In the ponderosa pine/Douglas-fir ecosystem of the inland western United States, it is recognized that fire frequency and intensity has significantly changed following Euro-American settlement of the West (Arno 1980, Barrett and Arno 1982, Arno et al. 1995a, Fule et al. 1997, Mast et al. 1999, Moore et al. 1999). Historically, fire return intervals were more frequent, less intense, and promoted dominance of large diameter ponderosa pine. This fire regime also prevented wide-spread dominance of Douglas-fir, a less fire tolerant species. Exclusion of fire from this system has led to increased stand densities, increased dominance of Douglas-fir, and an increased risk of high-intensity stand-replacing wildfire (Arno 1980, Barrett and Arno 1982, Arno et al. 1995, Fule et al. 1997, Mast et al. 1999, Moore et al. 1999). These differences between current and historical fire intensity and forest species composition may influence several characteristics of charcoal that enhance soil fertility following fire.

The objective of this study, therefore, is to evaluate whether temperature and substrate cause any differences in several charcoal properties that may contribute to soil fertility following fire. The substrates we evaluated include charcoal made from ponderosa pine and Douglas-fir bark and wood. These substrates were selected because

136

their relative contribution to the forest charcoal pool may have changed dramatically as a function of forest composition changes that have occurred in the last century. Further, we compare the influence of temperature on charcoal by generating charcoal at two temperatures, 350 °C and 800 °C, which represent lower and upper thresholds for charcoal formation. These temperatures may reflect differences in charcoal formation conditions during historical low-intensity wildfires and current high-intensity wildfires. Our last objective is to compare the properties of charcoal made in the laboratory with charcoal formed during a wildfire. This comparison will allow us to better understand the natural variability found in these charcoal properties in the ponderosa pine/Douglas-fir ecosystem.

Methods

We collected wood and bark samples from five ponderosa pine and Douglas-fir trees within a two ha area at Lubrecht Experimental Forest, western Montana. Samples were cut from stumps of trees between 50 to 100 years of age, following a recent harvest. We generated charcoal from each wood and bark sample using a muffle furnace (Barnstead Thermolyne 62730, Barnstead International, Dubuque, Iowa). Charcoal was generated by covering samples in silicate sand and heating to 350 °C or 800 °C for two hours. Wildfire charcoal was collected during spring 2004 from the Black Mountain fire, Missoula, MT, August 2003. Samples were collected and composited from the soil surface along five 100 m transects in burned ponderosa pine/Douglas-fir forest within this fire. Charcoal made in the laboratory from ponderosa pine wood, ponderosa pine bark,

137

Douglas-fir wood, Douglas-fir bark, and charcoal collected following a wildfire are hereafter referred to as PPW, PPB, DFW, DFB, and WFC, respectively.

A variety of analyses were conducted on both laboratory generated and wildfire charcoal. Density was measured by weighing charcoal pieces and submerging them in water to measure volume displacement. All other analyses were conducted on charcoal samples ground in a shatterbox. Total C and N were measured by dry combustion analysis on a Fissions Elemental Analyzer (Milano, Italy). Total N was below detection limits in all samples and thus is not reported. Extractable NH_4^+ and NO_3^- were extracted by shaking 1 g of charcoal in 20 ml of 2 M KCL followed by filtration through Whatman number 42 filter paper. Extracts were analyzed on a segmented flow analyzer (Auto Analyzer III, Bran Luebbe, Chicago, IL) using the Berthelot reaction (Willis et al. 1993) and cadmium reduction method (Willis and Gentry 1987), respectively. Available P was estimated by extracting 1 g of charcoal in 20 ml of 0.01 M $CaCl_2$. Phosphate in these extracts was measured on a segmented flow analyzer (Auto Analyzer II) using the method described by Murphy and Riley (1962).

Soluble phenols were extracted by shaking 1 g of charcoal in 20 ml of deionized water for one hour followed by filtration through Whatman 42 filter paper. Total phenols were extracted by shaking 1 g of charcoal in 20 ml of 50% methanol for 24 hours, followed by filtration. Phenols in these extracts were measured using the Prussian blue method (Stern et al. 1996). Phenol sorption potential was measured by adding 50 ml solution of the biphenol catechin, to 1 g of charcoal. Catechin solution was made by dissolving 0.2 g of catechin in 1 L of deionized water (200 ppm). Thus, effectively 10 mg of catechin was delivered to each gram of charcoal. This slurry was shaken for 24

138

hours, extracted and catechin concentration was analyzed using the Prussian blue method (Stern et al. 1996).

Nitrifier activity was measured using an aerated slurry assay (Hart et al. 1994), where 0.7 g of charcoal and 15 g of soil were incubated for 23 hours with 100 ml of nutrient solution (0.75 mM $(NH_4)_2SO_4$, 0.3 mM KH_2PO_4, and 0.7 mM K_2HPO_4) with the pH adjusted to 7.2, and the suspensions shaken to maintain aeration. Aliquots of 10 ml of agitated solution were removed at 0, 1, 2, 22, and 23 hrs, extracted with 10 ml of 4 M KCl, and analyzed for NO_3^--N using the method described above. We used the slope of the NO_3^- accumulation curve to estimate nitrifier activity (ng hr^{-1}). Nitrifier activity on a no-charcoal control was also analyzed (0.65 ±0.39 ng $NO_3^- h^{-1}$), and values above and below this control mean represent a positive and negative effect on nitrification, respectively. The soil used in this slurry was a sandy loam classified as a Dystric Haplocryepts, was collected from a ponderosa pine/Douglas-fir forest (1668 m) in the Bitterroot valley, western Montana, and had a pH of 5.3, and total C content of 6.2 g/kg.

The objective of statistical analyses was to detect differences between substrates at each temperatures, and to detect differences between temperatures within each substrate. Properties of each charcoal substrate within a temperature were statistically compared using a one-factor ANOVA. When data in this comparison did not meet assumptions of normality, data were compared using a Kruskal-Wallis test. Post-hoc analysis was conducted on these comparisons using the Student-Newman-Keuls procedure. Letters next to mean values (Table 1) indicate pair-wise differences within this comparison. Differences between 350 and 800 °C samples were analyzed for each substrate using Student's t-tests. When data in this comparison did not meet assumptions

139

of normality, they were analyzed using a Mann-Whitney U test. All statistical comparisons were conducted using SPSS version 11.0.

Results and Discussion

<u>Temperature and Substrate</u>

Both temperature and substrate caused highly significant differences in numerous charcoal properties. For most variables, the differences caused by temperature appeared to be much more substantial than those caused by substrate. One exception was charcoal density, where greater differences existed between substrates than between temperatures. At the low temperature, DFW had a higher density than PPW, whereas PPB had a significantly higher density than DFB (Figure 1). Unlike any other substrates, DFB appeared to rapidly swell immediately following heating, which appeared to create charcoal with a uniquely low density. The density of bark for both species remained constant between high and low temperatures. In contrast, wood density of both species significantly declined with temperature. Ponderosa pine wood was much more resistant to density loss relative to DFW. Density differences caused by substrate and temperature are potentially important because they may determine the amount of surface area per unit mass of charcoal, which may affect its activity.

The C content of charcoal was also greatly influenced by temperature (Figure 2). At the low temperature, all substrates had indistinguishable C contents. At the high temperature, the C content of all substrates increased by nearly 20 percent relative to the low temperature. Douglas-fir bark had a significantly lower C content than other substrates at the high temperature. The increase in C content between the two temperatures is likely due to volatilization of other elements. Nitrogen, phosphorous and

140

sulfur are three nutrients that begin to volatilize at temperatures below 800 °C (Wright

and Bailey 1982, Neary et al. 1999), and appear to volatilize at a higher efficiency than C.

Extractable P also demonstrated both temperature and substrate effects (Figure 3).

Extractable P declined with increasing temperature in DFW, DFB, and PPB, whereas it

increased with temperature in PPW. Phosphorous begins to volatilize around 600 °C

(Prichett and Fisher 1987). The increase in P availability in PPW with temperature may

indicate some degree of structural protection within this substrate that diminishes

volatilization. At the low temperature, DFW and DFB had significantly higher

extractable P than PPW and PPB. Except for a significant increase in extractable P in

PPW, this pattern persisted at the high temperature.

Both NH_4^+ and NO_3^- were significantly influenced by temperature and substrate

(Figure 4 & 5). At the low temperatures, relatively high concentrations of NH_4^+ were

extracted from all substrates except PPW. At the high temperature, significantly less

NH_4^+ was extracted from all substrates, except for PPW, which did not change relative to

the low temperature. No differences in NH_4^+ concentration were detected between

substrates at the high temperature. The relatively higher NH_4^+ concentrations detected at

the low temperature is likely the result of amine bond cleavage in organic N molecules

during heating. As temperature increases, the pool of organic N in each substrate likely

becomes depleted, and a greater portion of cleaved amine groups likely become oxidized

and volatilized.

At the high temperature, a portion of NH_4^+ appears to become oxidized and

remains on the substrates as NO_3^- (Figure 5). As evidence for this, NO_3^- content was

inversely related to NH_4^+ content. At the low temperature, low concentrations of NO_3^-

141

were extracted from all substrates. Both DFW and DFB showed significantly higher NO_3^- than PPW or PPB at this temperature. At the higher temperature, NO_3^- content increased significantly on all substrates, and remained significantly higher in DFW and DFB relative to PPW.

In addition to directly enhancing nutrient availability, charcoal may have antagonistic characteristics that reduce nutrient availability. One potentially influential property on nutrient availability is the potential contribution of bio-available C from charcoal. Polyphenols are a class of molecules that likely become available during degradation of lignified materials. Lignin is a polymer composed of phenol monomers. Thus heating may degrade polymers, yielding monomers that are more bio-available. Availability of phenols may inhibit microbe and root activity, or may provide microbes with a high C:N substrate that stimulates nutrient immobilization.

The data clearly show that temperature has a highly significant influence on both soluble and total phenol pools (Figure 6 & 7). Soluble phenols, which represent a highly bio-available C pool, decreased by an order of magnitude at the high temperature relative to the low temperature. At the high temperature, total phenols decreased by two orders of magnitude relative to the lower temperature. Substrate effects were also detected, but the difference was of a smaller magnitude than those caused by temperature. Ponderosa pine wood had a significantly higher, and DFW a significantly lower soluble phenol concentration than other substrates at both temperatures. Likewise, PPB had a significantly higher, and DFB had a significantly lower total phenol concentration at the low temperature; however, no differences in total phenol content could be detected between substrates at the high temperature. These data suggest that significant phenol

142

volatilization or degradation into non-phenol chemical structures occurs between 350 and 800 °C. The lower concentration of soluble and total phenols in high temperature charcoal, therefore, is less likely to have an antagonistic effect on soil nutrient cycling.

We measured relative sorption capacity by exposing charcoal samples to catechin, a simple polyphenol. This analysis demonstrated that charcoal made from all substrates and at both temperatures acted as an effective sorptive surface of this compound (Figure 8). Of the 10 mg (200 mg/l) of catechin exposed to each g of charcoal, the smallest amount of catechin removed by any charcoal sample was 76 percent. Temperature had a much larger effect on charcoal sorption than did substrate. At the high temperature, both PPB and PPW sorbed the entire 10 mg of catechin in solution, whereas DFW and DFB sorbed a slightly smaller, but significant, portion of catechin in solution. At the low temperature, PPW and PPB also demonstrated significantly higher catechin sorption relative to DFW and DFB. It is possible that the differences in sorption capacity between samples could be amplified if a higher quantity and concentration of catechin was available for charcoal samples to sorb. This is particularly true for high temperature samples, where sorption did not appear to be near saturation. It is also notable that the quantity of catechin sorbed by all charcoal samples was several orders of magnitude higher than the quantity of soluble and total phenols extracted from charcoal. This implies that if soil solution chemistry is dominated by high concentrations of dissolved organic matter the positive effects of charcoal on reducing solution C:N ratios is likely to outweigh its potential contribution of bio-available C. In contrast, if soil solution chemistry does not have high C:N ratios, the bio-available C supplied by charcoal, particularly low temperature charcoal, may have a negative effect on nutrient cycling.

143

Several studies have shown that charcoal can have a strong influence on nitrification, leading to accumulation of NO_3^- in the soil (Zackrisson et al. 1996, DeLuca et al. 2002, Berglund et al. 2004). Our data show that both substrate and temperature have a strong effect on nitrifier activity (Figure 9). At both temperatures, bark of both species resulted in higher nitrification rates relative to the no-charcoal control. In contrast, wood charcoal of both species resulted in a lower nitrification rate relative to the control, indicating an inhibitory effect on nitrification. The positive effect of PPB and DFB on nitrification remained positive, but declined at the high temperature. It is unclear what unique property of bark stimulated nitrification in this assay.

Wildfire Charcoal

Mean values for many of the charcoal properties were more similar to lower temperature charcoal than high temperature charcoal (Table 1). Mean WFC density was nearly identical to the low temperature DFW and DFB densities. Carbon content of WFC was approximately half–way between mean C content of laboratory-made charcoal at low and high temperatures. Extractable PO_4^- concentrations were also most similar to low temperature DFW and DFB charcoals.

In contrast, NH_4^+ and NO_3^- concentrations of WFC charcoal were over four times higher than all laboratory charcoal. This higher concentration of NH_4^+ and NO_3^- (38.3 and 4.4 µg/g, respectively) on WFC charcoal may occur because this charcoal likely forms in the presence of combusting organic matter that liberates NH_4^+. Further, we collected wildfire charcoal approximately six months following the wildfire, thus substantial nitrification may have occurred during this period.

144

Like most other properties, both soluble and total phenol concentration of WFC charcoal were more similar to low temperature charcoals than high temperature charcoal. Average catechin sorption of WFC charcoal was slightly higher than all low temperature laboratory charcoals. Wildfire charcoal had a positive effect on nitrification potential relative to the control, and this effect was of similar magnitude to PPB and DFB, which had the highest effect of nitrification potential of all laboratory generated charcoal we analyzed.

Another notable difference between wildfire and laboratory generated charcoal is the variation among replicates. Laboratory generated charcoal produced very small standard errors for all variables measured. This is typical of laboratory studies where experimental conditions can be closely regulated. In contrast, the standard errors of nearly all variables measured on WFC charcoal were one to two orders of magnitude higher than laboratory generated charcoal. This demonstrates that a high degree of variability in these charcoal properties occurs in natural systems.

The charcoal properties reported here are likely heterogeneously distributed in forest environments as a function of landscape variation in species composition, fuel quantity, as well as variation in weather conditions during fires. Charcoal may influence competitive interaction of plants by interfering with allelopathy or enhancing nutrient availability. Spatial heterogeneity of charcoal properties may promote coexistence of plant species by creating contrasting competitive environments adjacent to one-another. The influence of charcoal on plant competition and species co-existence has received very little attention, and is worthy of future research.

145

Conclusions

Both generation temperature and substrate type directly influenced numerous charcoal properties. As temperature increased, density, NH_4^+ concentration, soluble and total phenols, and nitrifier activity decreased. In contrast, total C content, NO_3^- concentration, and catechin sorption increased with temperature. For most variables, the magnitude difference caused by substrate was smaller than differences caused by temperature. Comparisons of properties among substrates suggested that DFB was the most dissimilar substrate. Douglas-fir bark had a much lower density (350 and 800°C), total C content (800°C), soluble (300 and 800°C) and total phenol (300°C) concentration than other substrates. Further, DFB had significantly higher NH_4^+ and NO_3^- concentrations (300 and 800°C), and stimulated higher nitrifier activity (300 and 800°C) than other charcoals. Wildfire charcoal appeared to be more similar to low than high temperature laboratory generated charcoal. Additionally, all variables measured on wildfire charcoal showed a higher degree of variation among replicates, suggesting that a range of charcoal types exists in natural conditions. Spatial heterogeneity of charcoal, and its numerous properties, may influence competitive interactions among plants and may be a mechanism that promotes diversity in systems such as the ponderosa pine/Douglas-fir ecosystems.

Acknowledgments

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147

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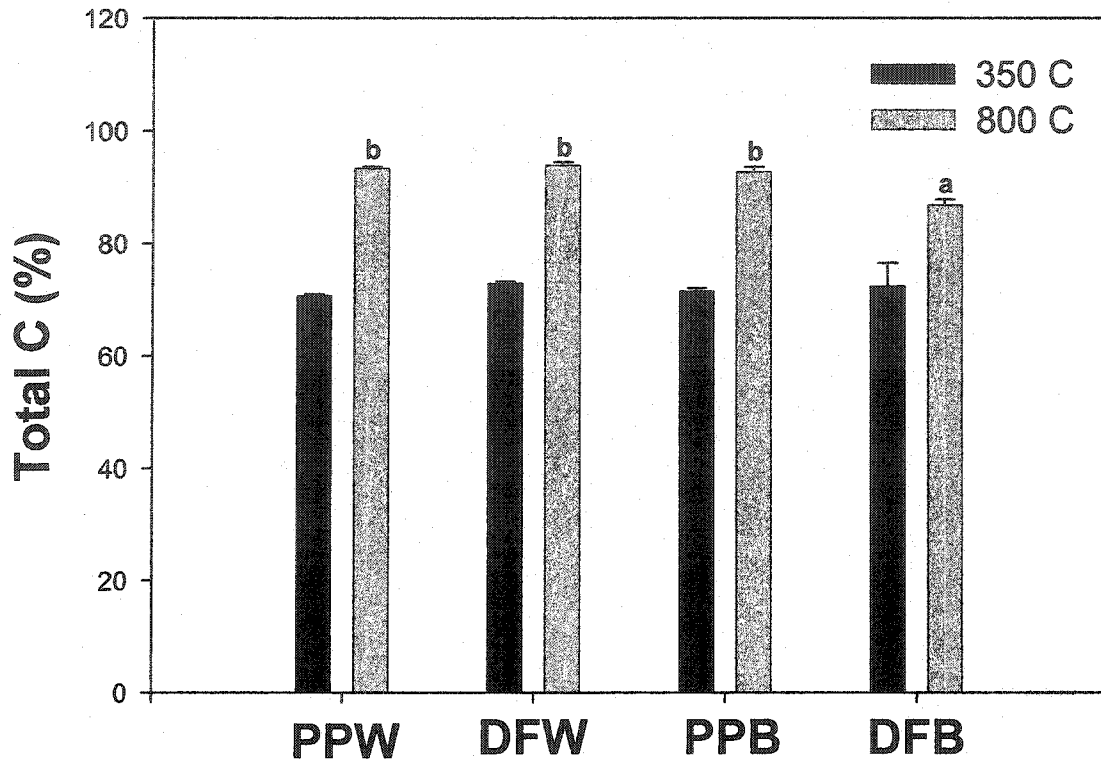charcoal from wildfire in the Boreal forest. Oikos 77:10-19.
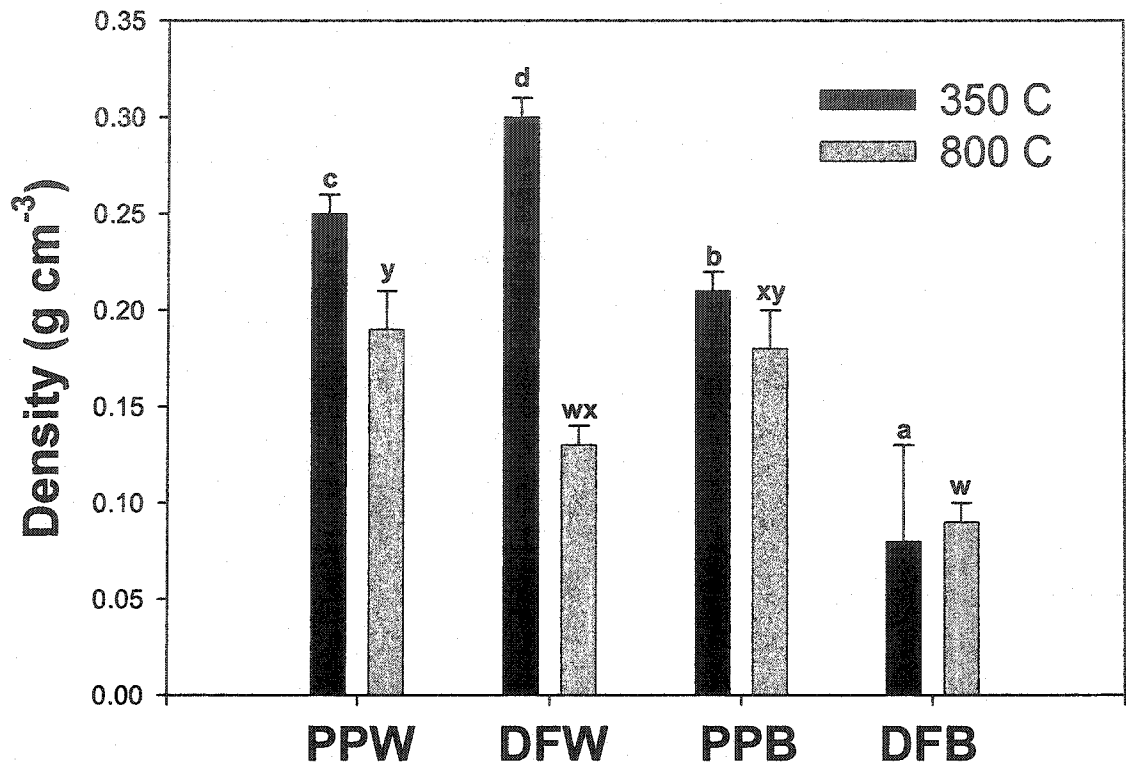
Figure 1: Density of charcoal (mean (SE), n=5) made at two formation temperatures (350 and 800 °C), and from four substrates. Substrates include Douglas-fir and ponderosa pine bark and wood (PPW= ponderosa pine wood; DFW= Douglas-fir wood; PPB=ponderosa pine bark; DFB=Douglas-fir bark). Differences within a temperature were conducted using a one-way ANOVA (350 °C: $p<0.001$; 800 °C : $p < 0.001$). Letters indicate post-hoc differences at 350 or 800 °C. Differences between temperatures for each substrate were compared using a Student's T-test (PPW, $p<0.05$; DFW, $p<0.001$, PPB, p=ns; DFB, p=ns).

Figure 2: Total C of charcoal (mean (SE), n=5) made at two formation temperatures (350 and 800 °C), and from four substrates. Substrates include Douglas-fir and ponderosa pine bark and wood (PPW= ponderosa pine wood; DFW= Douglas-fir wood; PPB=ponderosa pine bark; DFB=Douglas-fir bark). Differences within a temperature were conducted using a one-way ANOVA (350 °C: p=ns; 800 °C : p < 0.001). Letters indicate post-hoc differences at 350 or 800 °C. Differences between temperatures for each substrate were compared using a Student's T-test (PPW, p<0.001; DFW, p<0.001, PPB, p<0.001; DFB, p<0.001).
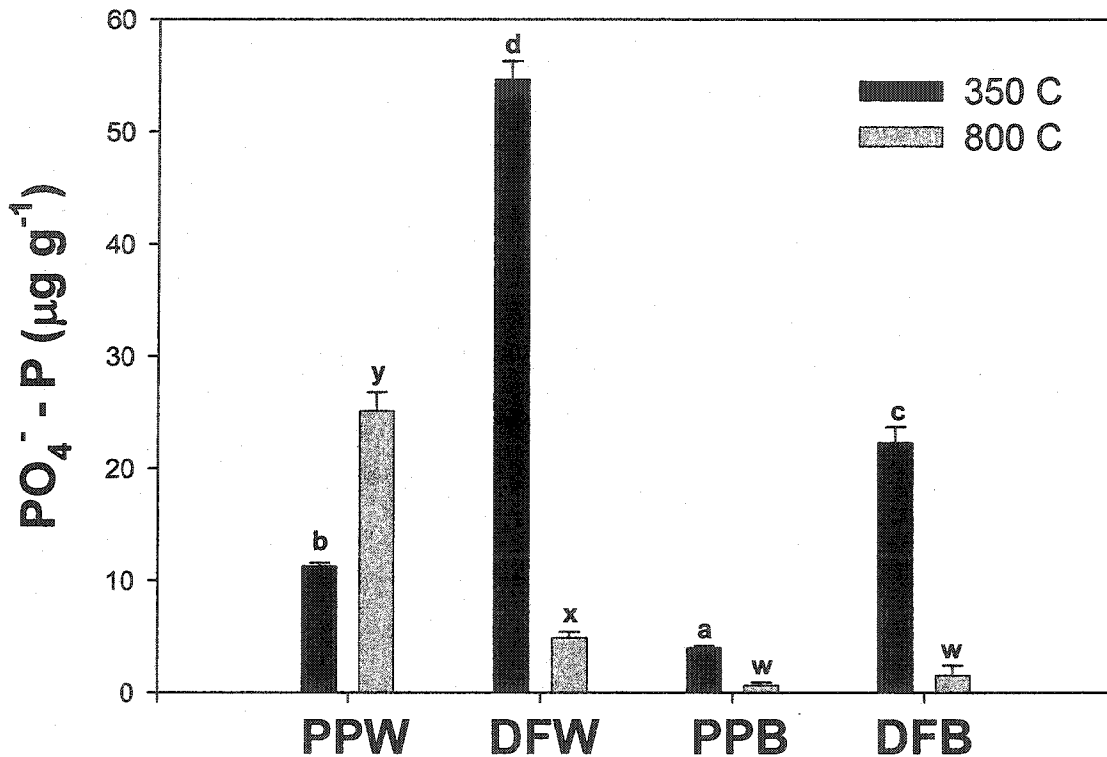
152

Figure 3: Phosphorous extracted from charcoal (mean (SE), n=5) made at two formation temperatures (350 and 800 °C), and from four substrates. Substrates include Douglas-fir and ponderosa pine bark and wood (PPW= ponderosa pine wood; DFW= Douglas-fir wood; PPB=ponderosa pine bark; DFB=Douglas-fir bark). Differences within a temperature were conducted using a one-way ANOVA (350 °C: $p<0.001$; 800 °C : $p < 0.001$). Letters indicate post-hoc differences at 350 or 800 °C. Differences between temperatures for each substrate were compared using a Student's T-test (PPW, $p<0.001$; DFW, $p<0.001$, PPB, $p<0.001$; DFB, $p<0.001$).
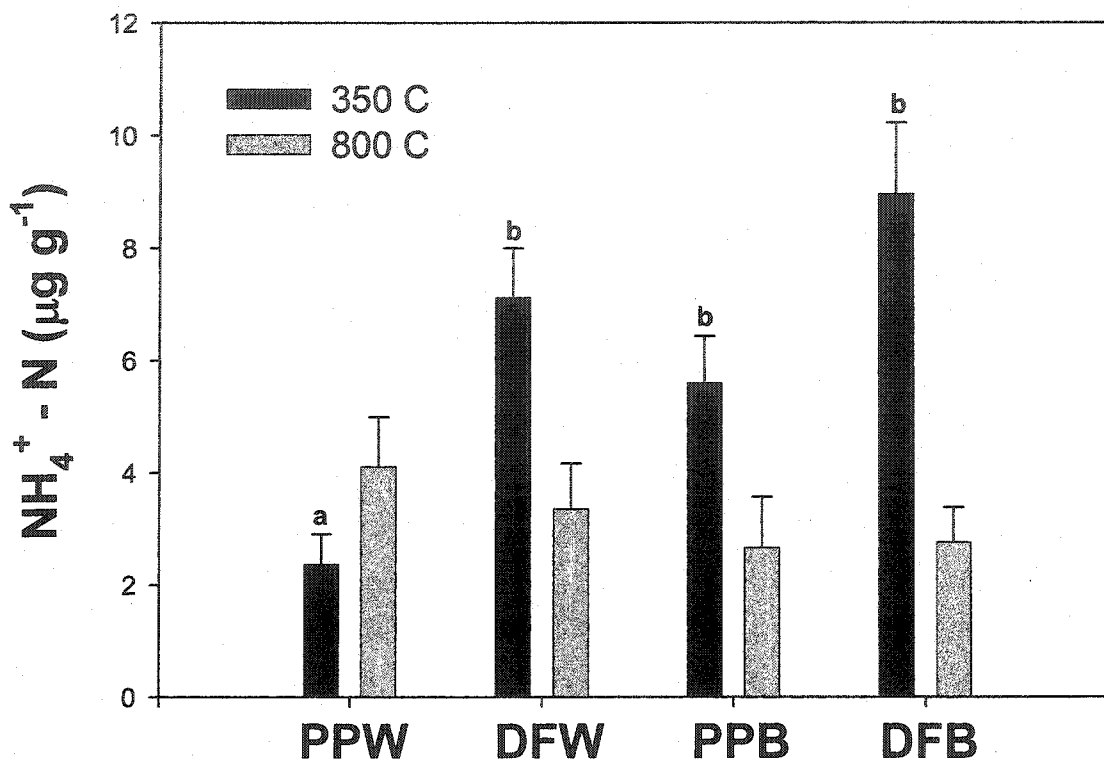
Figure 4: Ammonium extracted from charcoal (mean (SE), n=5) made at two formation temperatures (350 and 800 °C), and from four substrates. Substrates include Douglas-fir and ponderosa pine bark and wood (PPW= ponderosa pine wood; DFW= Douglas-fir wood; PPB=ponderosa pine bark; DFB=Douglas-fir bark). Differences within a temperature were conducted using a one-way ANOVA (350 °C: $p < 0.001$; 800 °C : p=ns). Letters indicate post-hoc differences at 350 or 800 °C. Differences between temperatures for each substrate were compared using a Student's T-test (PPW, p=ns; DFW, $p < 0.05$, PPB, $p < 0.05$; DFB, $p < 0.01$).
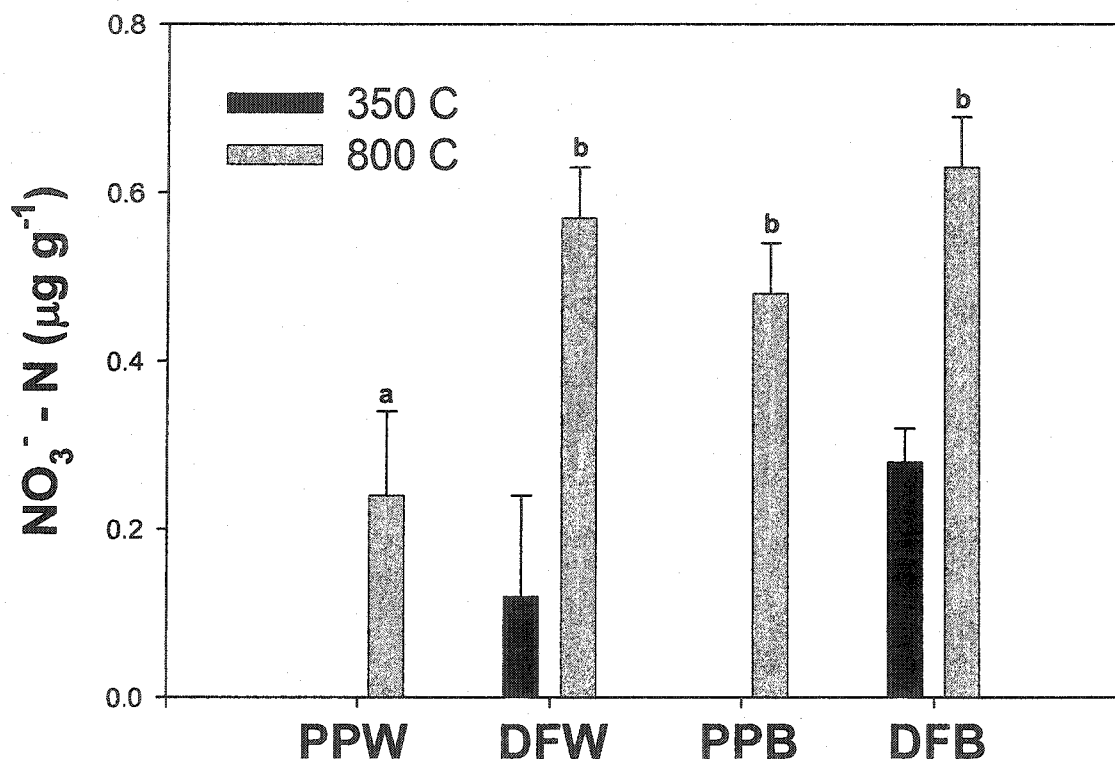
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Figure 5: Nitrate extracted from charcoal (mean (SE), n=5) made at two formation temperatures (350 and 800 °C), and from four substrates. Substrates include Douglas-fir and ponderosa pine bark and wood (PPW= ponderosa pine wood; DFW= Douglas-fir wood; PPB=ponderosa pine bark; DFB=Douglas-fir bark). Differences within a temperature were conducted using a one-way ANOVA and Kruskal-Walis (350 °C: $p<0.01$; 800 °C: $p < 0.01$, respectively). Letters indicate post-hoc differences at 350 or 800 °C. Differences between temperatures for each substrate were compared using a Student's T-test or Mann-Whitney U test (PPW, $p<0.05$; DFW, $p<0.01$, PPB, $p<0.001$; DFB, $p<0.001$).
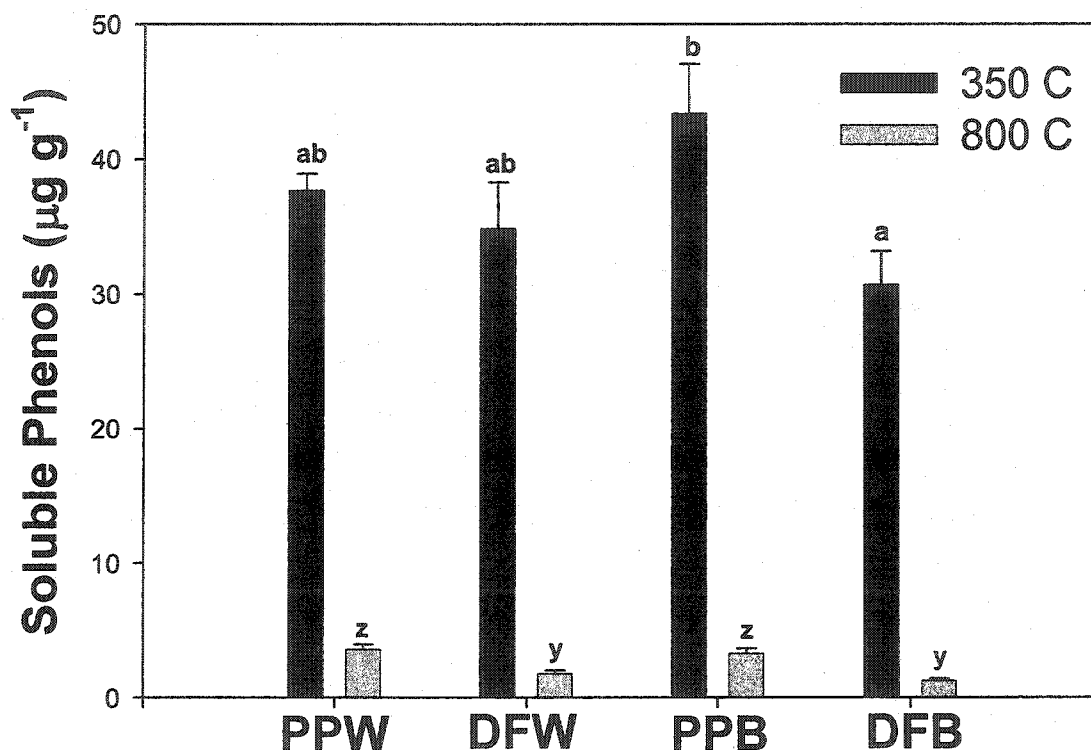
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Figure 6: Soluble phenols extracted from charcoal (mean (SE), n=5) made at two formation temperatures (350 and 800 °C), and from four substrates. Substrates include Douglas-fir and ponderosa pine bark and wood (PPW= ponderosa pine wood; DFW= Douglas-fir wood; PPB=ponderosa pine bark; DFB=Douglas-fir bark). Differences within a temperature were conducted using a one-way ANOVA (350 °C: $p<0.05$; 800 °C : $p < 0.001$). Letters indicate post-hoc differences at 350 or 800 °C. Differences between temperatures for each substrate were compared using a Man-Whitney U-test (PPW, $p<0.001$; DFW, $p<0.001$, PPB, $p<0.001$; DFB, $p<0.001$).
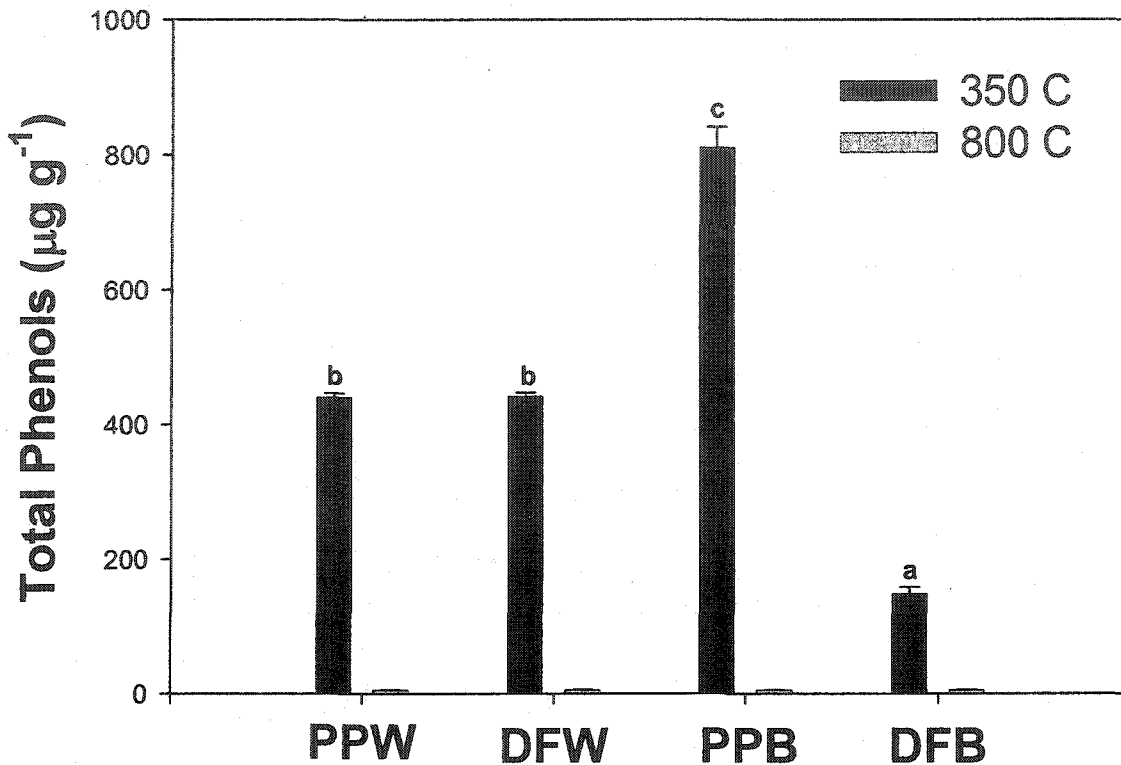
Figure 7: Total phenols extracted from charcoal (mean (SE), n=5) made at two formation temperatures (350 and 800 °C), and from four substrates. Substrates include Douglas-fir and ponderosa pine bark and wood (PPW= ponderosa pine wood; DFW= Douglas-fir wood; PPB=ponderosa pine bark; DFB=Douglas-fir bark). Differences within a temperature were conducted using a one-way ANOVA (350 °C: $p < 0.001$; 800 °C: p=ns). Letters indicate post-hoc differences at 350 or 800 °C. Differences between temperatures for each substrate were compared using a Man-Whitney U-test (PPW, $p < 0.001$; DFW, $p < 0.001$, PPB, $p < 0.001$; DFB, $p < 0.001$).
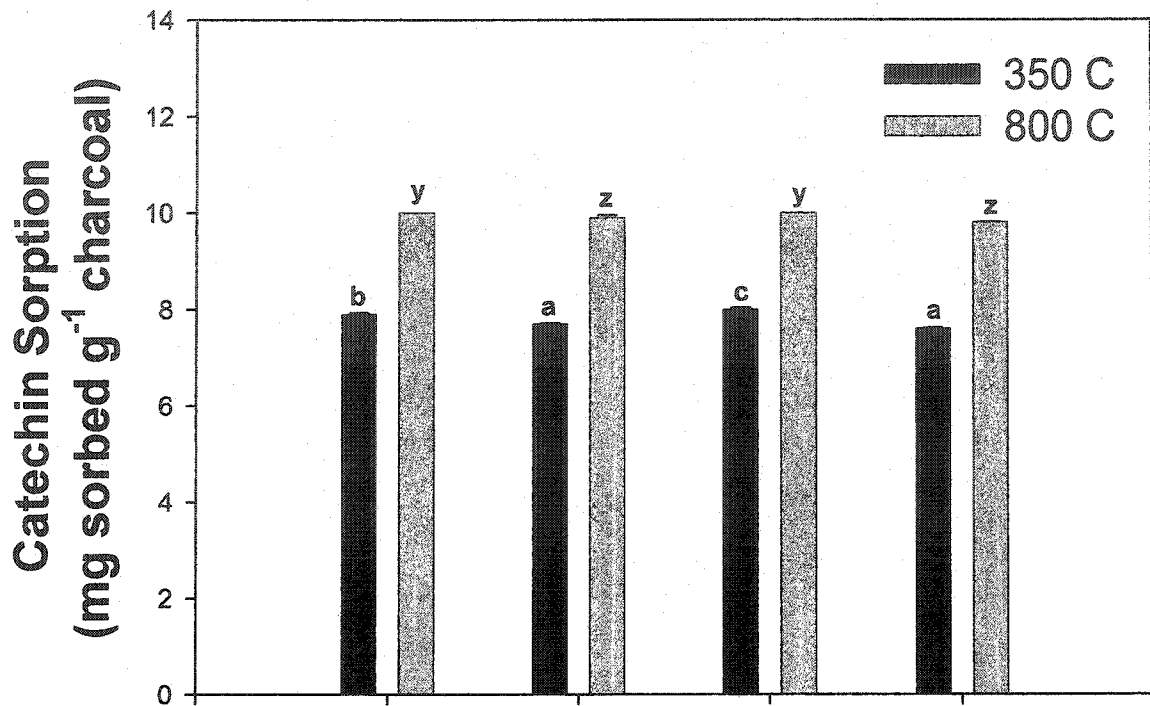
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Figure 8: Catechin sorption by charcoal (mean (SE), n=5) generated at two temperatures (350 and 800 °C), and four substrates. Substrates include Douglas-fir and ponderosa pine bark and wood (PPW= ponderosa pine wood; DFW= Douglas-fir wood; PPB=ponderosa pine bark; DFB=Douglas-fir bark). Differences within a temperature were conducted using a one-way ANOVA (350 °C: p<0.001; 800 °C: p < 0.001). Letters indicate post-hoc differences at 350 or 800 °C. Differences between temperatures for each substrate were compared using a Man-Whitney U-test (PPW, p<0.001; DFW, p<0.001, PPB, p<0.001; DFB, p<0.001).
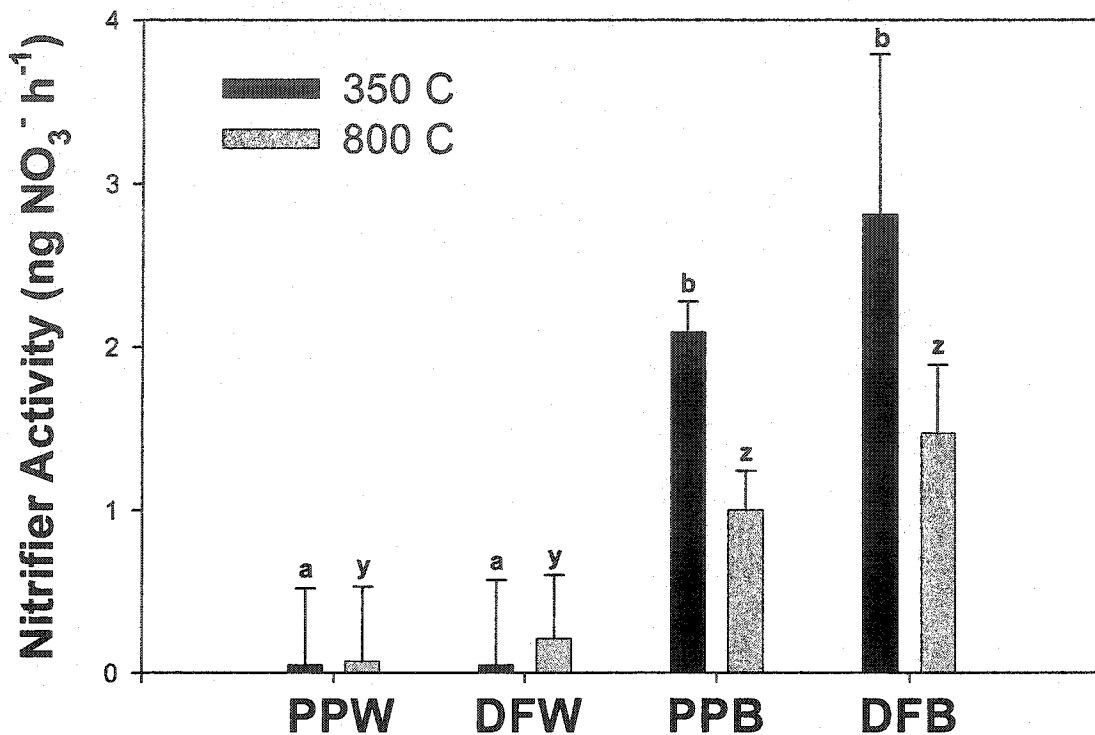
158

Figure 9: Nitrifier activity of soil amended with charcoal (mean (SE), n=5) influenced by formation temperatures (350 and 800 °C), and substrate. Substrates include Douglas-fir and ponderosa pine bark and wood (PPW= ponderosa pine wood; DFW= Douglas-fir wood; PPB=ponderosa pine bark; DFB=Douglas-fir bark. A no-charcoal control was also analyzed (0.65 (0.39) ng NO_3^- h^{-1}); thus, values above and below this control mean represent a positive and negative effect on nitrification, respectively. Differences within a temperature were conducted using a one-way ANOVA (350 °C: p<0.001; 800 °C: p < 0.001). Letters indicate post-hoc differences at 350 or 800 °C. Differences between temperatures for each substrate were compared using a Student's T-test (PPW, p=ns; DFW, p=ns, PPB, p<0.001; DFB, p<0.01).

159

Table 1: Properties of wildfire generated charcoal (mean (SE), n=5) collected from the Black Mountain Fire, Missoula, MT, August 2003.

Variable	Mean	SE
Density $(g\ cm^{-3})$	0.29	(0.4)
Total C (%)	82.3	(2.8)
PO_4^- $(\mu g\ g^{-1})$	41.6	(3.6)
NH_4^+ $(\mu g\ g^{-1})$	38.3	(1.1)
NO_3^- $(\mu g\ g^{-1})$	4.4	(0.1)
Soluble Phenols $(\mu g\ g^{-1})$	48.2	(4.8)
Total Phenols $(\mu g\ g^{-1})$	393.8	(29.3)
Catechin Sorption $(mg\ sorbed\ g^{-1}\ charcoal)$	8.6	(0.4)
Nitrifier Activity $(ng\ NO_3^-\ h^{-1})$	2.6	(0.1)

160

Chapter 6

Conclusions

Within this dissertation I presented four research manuscripts evaluating N cycling and availability in the context of forest succession and restoration. In the first manuscript I compared numerous soil chemical, biological and physical properties following four restoration treatments in the ponderosa pine/Douglas-fir ecosystem. The most significant differences between treatments included net N mineralization, nitrification, NH_4^+ availability, and decomposition rates, which were higher in both BURN and THIN/BURN treatments, with the most pronounced increase in the THIN/BURN treatment. Higher nitrate concentrations were also found in the THIN/BURN during week 4-5, whereas no other treatments resulted in elevated nitrate concentrations. Both burn treatments also demonstrated a significant loss of the organic horizon, which resulted in reduced organic horizon C and a reduced C:N ratio. Very few differences in the soil microbial community were detected; however, the THIN/BURN treatment resulted in a higher concentration of PLFA markers for actinomycetes 16 -18 weeks after burning. The only differences found in the THIN treatment were a thicker O horizon and higher respiration rates than other treatments.

Because N parameters, more than any other variables measured, differed among treatments, and N is thought to be a limiting nutrient in the ponderosa pine/Douglas-fir ecosystem, it is plausible that changes in N spatial patterns following restoration correspond with diversity patterns, as predicted by the resource heterogeneity hypothesis (RHH). This idea was evaluated in the second manuscript by evaluating N spatial heterogeneity within the experimental framework of the restoration study presented in the

161

first manuscript. Results of this study provided strong evidence that an underlying relationship exists between total inorganic N (TIN) heterogeneity and understory plant species diversity in ponderosa pine/Douglas-fir forests, and thus also provide support for RHH. This relationship was in part driven by divergence of species composition on high and low N patches. Our data also indicate that the untreated Control, which has not experienced fire in over a century, exhibits a low degree of TIN heterogeneity. Additionally, both prescribed burn treatments resulted in higher levels of TIN heterogeneity relative to the Control; whereas, the Thin-only treatment did not result in increased heterogeneity relative to the Control. These data suggest that the restoration treatments land managers choose to restore fire excluded ponderosa pine forests can directly influence N heterogeneity and subsequently influence species diversity as well. It is unclear whether TIN heterogeneity produced by restoration treatments is representative of historical conditions. Presumably, the frequent low-intensity fires that historically dominated western Montana ponderosa pine forests created and maintained heterogeneity; however, it may be impossible to reconstruct this aspect of reference conditions. These findings demonstrate the influence that N heterogeneity has on diversity in the context of ponderosa pine/Douglas-fir forest restoration, and may lead to more informed restoration decisions that consider treatment effects on understory diversity.

In the third manuscript I explored the role that charcoal may have in enhancing and maintaining N cycling following fire, which in turn may promote dominance of early successional species, such as *K. macrantha*. This was accomplished using a soil incubation experiment and two greenhouse experiments. In the soil incubation

162

experiment charcoal and extracts of *Arctostaphylos uva-ursi* were added in factorial combination, both of which had a large effect on N cycling. As hypothesized, charcoal effectively sorbed soluble phenols from solution. This may effectively reduce the influence of extract on soil organisms, plants and processes. This sorption of phenols by charcoal corresponded with increased nitrification in a high N environment created by glycine addition, but did not appear to stimulate N cycling in a low nutrient setting (no-glycine trial). This result may indicate that low temperature charcoal contributes bio-available or bio-inhibitory carbon that has an antagonistic influence on nutrient cycling under low nutrient conditions.

Low-temperature laboratory-generated charcoal had a negative effect on growth of *K. macrantha*. This may be the result of an inhibitory effect that insoluble phenols from this charcoal have on root growth, or an immobilization effect charcoal may have on inorganic N under low N conditions. In contrast, charcoal created during a wildfire had a positive effect on growth of *K. macrantha*, suggesting laboratory charcoal may not have adequately represented field collected charcoal.

The conflicting results of these two greenhouse experiments suggested that a better understanding of numerous charcoal properties was needed. In the final research manuscript I compare numerous charcoal properties among charcoal made in the laboratory at a high (800 C) and low (350 C) temperature and from different substrates, including Douglas-fir and ponderosa pine wood and bark.

This analysis demonstrated that both generation temperature and substrate type directly influenced numerous charcoal properties. As temperature increased, density, NH_4^+ concentration, soluble and total phenols, and nitrifier activity decreased. In

contrast, total C content, NO_3^- concentration, and catechin sorption increased with temperature. For most variables, the magnitude difference caused by substrate was smaller than differences caused by temperature. Comparisons of properties among substrates suggested that Douglas-for bark was the most different substrate. Douglas-fir bark had a much lower density (350 and 800°C), total C content (800°C), soluble (300 and 800°C) and total phenol (300°C) concentration than other substrates. Further, Douglas-fir bark had significantly higher NH_4^+ and NO_3^- concentrations (300 and 800°C), and stimulated higher nitrifier activity (300 and 800°C) than other charcoals.

These data may help explain why laboratory charcoal had a negative effect, and wildfire charcoal had a positive effect on growth of *K. macrantha*. Both soluble and total phenols extracted from wildfire charcoal fell between those extracted from Douglas-fir bark charcoal and ponderosa pine bark charcoal (Figure 1). Thus phenols, which may represent bio-available or bio-inhibitory carbon, do not provide a mechanism for why *K. macrantha* growth diminished in soils amended with laboratory charcoal and was enhanced in soils amended with wildfire charcoal. Likewise, the differences in catechin sorptivity among the charcoals, although statistically significant, do not appear to be of great enough difference in magnitude to provide a mechanisms for the contrasting results between greenhouse experiments. In contrast wildfire charcoal appears to have significantly higher concentrations of extractable P, NH_4^+, and NO_3^- relative to either Douglas-fir or Ponderosa pine bark charcoal (Fig. 1). The greater addition of these nutrients on wildfire charcoal may effectively overcome the nutrient immobilization effect that is created by the bio-available carbon present on low temperature charcoal, as described in research manuscript number three.

It is clear that N plays a central role in succession in the ponderosa pine/Douglas-fir ecosystem. The research manuscripts in this dissertation provide a better understanding of how N cycling changes following restoration in this system; and further, how the spatial heterogeneity of available N following restoration influences understory plant diversity. Further, the research presented in this dissertation identifies that charcoal, a byproduct of fire, has a great potential to enhance N cycling for decades following fire. This long term influence on N cycling, in turn, may extend the dominance of early successional species, such as *K. macrantha*.
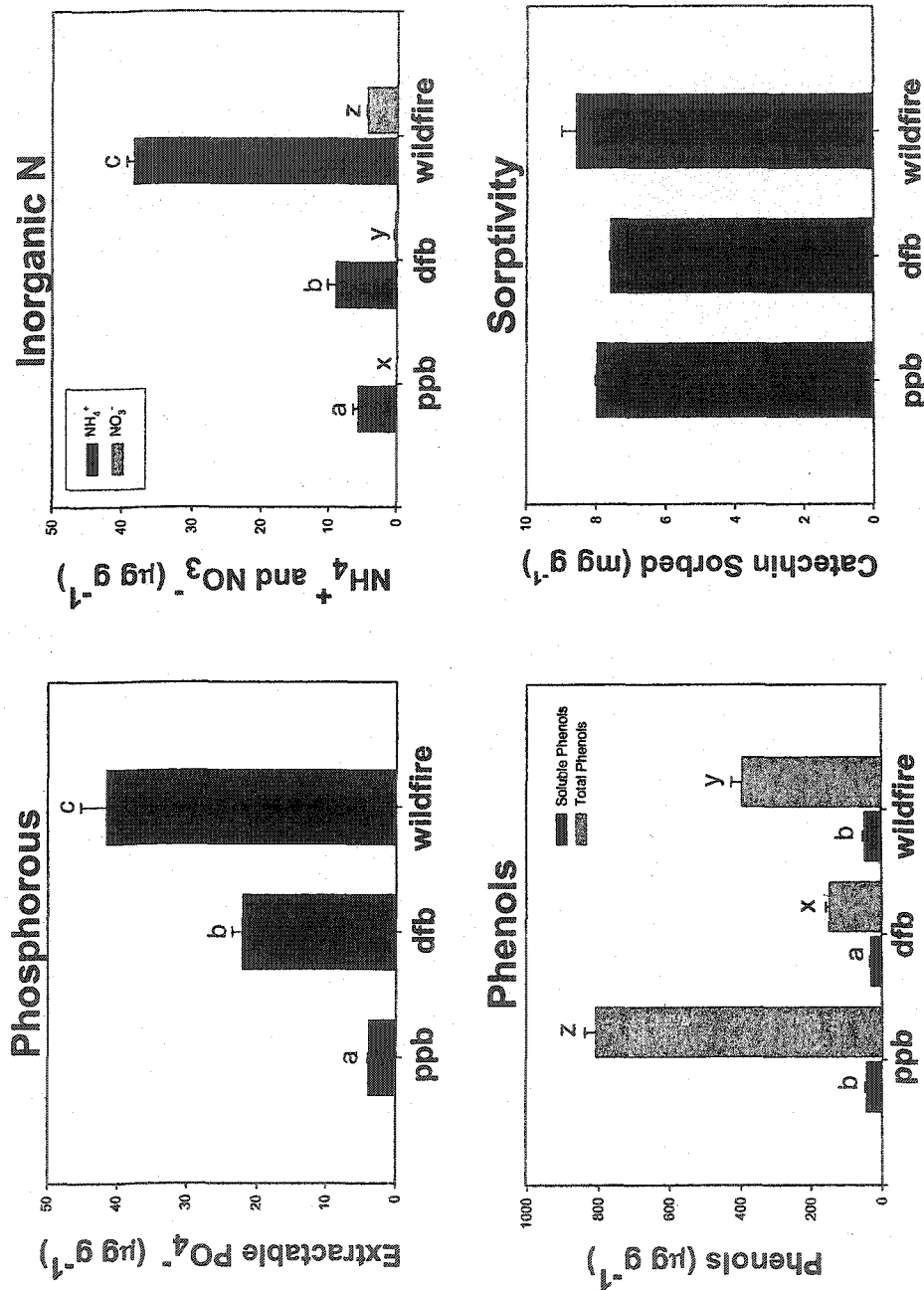
Figure 1. A comparison of extractable phosphorous, inorganic N (NH_4^+ and NO_3^-), soluble and total phenols, and catechin sorptivity of charcoal made from ponderosa pine bark (ppb), Douglas-fir bark (dfb), and wildfire collected charcoal. Data were compared using a one-factor ANOVA or Kruskal-Wallis test (P, Inorganic N, and Phenols: one-way ANOVA, n=5, df=14,2, $p<0.000$ Sorptivity: K-S test=6.50, df=2, $p=0.039$). Letters indicate post-hoc differences evaluated using S-N-K.

166