University of Nevada, Reno

The Effects of Pile Burning and Charcoal on Carbon and Nitrogen Transformations in Eastern Sierra Nevadan Forested Soils

A thesis submitted in partial fulfillment of the requirements for the degree of Masters of Science in Hydrology

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General Abstract

Over a century of fire prevention and suppression in the western United States has led to overstocked and diseased forests with increased fuel loading and vertical continuity of fuels, thus increasing the risk of severe and stand-replacing wildfire. Mechanical thinning followed by pile burning is a commonly used technique to reduce fuel loads. Prescribed pile burning and products of pile burning, such as ash and charcoal, may influence nutrient dynamics within forest soils. Specifically, charcoal-rich environments have been shown to enhance nitrogen cycling. Pile burning also generates a pulse of biologically available nutrients through the mineralization of organic matter and deposition of ash and charcoal. Lab incubations of soil amended with varying amounts of charcoal (0.5, 1, 2.5, and 5% by weight) were conducted to assess the importance of charcoal on microbial activity and nitrogen cycling in forest soils from Little Valley, approximately 30 km south of Reno, Nevada. Carbon respiration and potential nitrification rate, proxies for microbial activity and size of the nitrifying microbial community respectively, were monitored over a two month period. Nitrogen (N), phosphorous (P), and potassium(K), were also monitored in natural pile burns with ages ranging from three to eight years and at varying points within a pile burn between 0 to 10 cm of the surface horizon. In general, microbial respiration and potential nitrification were highest at 2.5% charcoal additions. Also, microbial respiration rates decreased but potential nitrification rates increased over time across most treatments. Polyphenol sorption onto charcoal-amended soil was also analyzed and showed that extractable polyphenol concentrations decreased with increasing amounts of charcoal which may partly explain patterns in potential nitrification rates. Pile burns displayed decreasing nutrient concentration with age and lower levels of N and K compared to unburned soils whereas P was greater within pile burns. Prescribed fire is a powerful tool at the disposal of forest managers and can be used to increase forest health and productivity. Managing prescribed fires for increased charcoal production can benefit soil microbial activity but may stimulate nitrogen losses through increased nitrate production.

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Chapter 1: General Introduction

Fire has been an integral part of North American semi-arid forests affecting forest composition, health and productivity, prevention of non-native species encroachment, and preservation of landscape heterogeneity (Hart *et al.,* 2005). Most pre-settlement fires in the Sierra Nevada region were caused by summer and fall lightning strikes resulting in a fire return interval between two and 25 years (Stephens and Collins, 2004; Carle, 2008). Pre-settlement fires were low to moderate in severity, and forest vegetation adapted to fire by producing thick bark, elevated crowns, buds protected by scales, rapid growth, serotinous cones, and/or prolific sprouting following fire (Moghaddas and Stephens, 2007; Carle, 2008). Fire prevention and suppression in Sierra Nevadan forests have altered forest composition resulting in an increased risk of disease, insect outbreak, and stand replacing fires (Tiedemann *et al*., 2000). Recent examples of these stand replacing wildfires include the Angora Fire in the Lake Tahoe basin in 2007 and the Rim fire near Yosemite National Park in 2013.

To lower the risk of wildfires, the amount of understory vegetation and ladder fuels are reduced using a variety of mechanical and fire-based fuel treatments. Mechanical treatments include whole-tree-harvesting, cut-to-length treatments, and grazing. Harvest materials can either be removed or left on site where materials can be masticated, spread across the forest floor followed by broadcast burning, or collected in piles followed by burning of these piles. While broadcast burning may resemble the natural fire regime in terms of severity and intensity, it is often expensive. In addition, restrictions on air quality limit the time window that broadcast burns can be executed. Finally, broadcast burns are sometimes hard to contain. Because of these concerns, pile burning of woody debris is becoming a common management option as smoke production is limited and burns are easier to control compared to broadcast burning. However, the fuel loads within burn piles are typically very high resulting in localized, high intensity fires that can dramatically impact soils.

Fire-based fuel reduction techniques, including pile burning, can have significant impacts on N, P and K cycling in forest soils, but effects are different for each specific nutrient. Most soil N is found in the O-horizon or upper mineral horizon and is predominantly present as organic N (Choromanska and DeLuca, 2001). A large fraction of organic N is typically lost through volatilization upon combustion of organic material (Johnson *et al.*, 2011). The pyrolysis of organic material also increases the amount of ammonium (NH_4^+) due to both chemical mineralization of organic material, the deposition of ash onto the forest floor, as well as lysis of soil microorganisms (Prieto-Fernandez *et al.*, 2004). Following an increase in NH_4^+, nitrification can be stimulated resulting in an increase in soil nitrate (NO_3^-). After this initial pulse of NH_4^+ and NO_3^-, soil NH_4^+ and NO_3^- can continue to increase through mineralization of N and subsequent formation of NO_3^- due to nitrification (Canfield *et al.*, 2010).

The persistence of N pulses in soils following fire can vary depending on climatic conditions, nutrient speciation, the depth of heat penetration into the soil, and the production of ash or charcoal (Prieto-Fernandez *et al.*, 2004). Microbial biomass N and potentially mineralizable N can increase immediately following a fire, but this increase is typically sustained less than a year (Prieto-Fernandez *et al*., 1998; DeLuca and Zouhar, 2000). Elevated NH_4^+ levels can last between two to five years depending on the frequency of fire events (Mackenzie *et al*., 2004), but other studies show that NH_4^+ concentrations return to pre-fire levels within a year after a fire (Choromanska and DeLuca, 2001). The persistence of elevated NO_3^- levels, which is more water-soluble and mobile than NH_4^+, depends on soil moisture, climate, and leaching pathways (Knicker, 2007).

Fire has different effects on P compared to N. Volatile losses of P are relatively small due to a higher volatilization temperature of P compared to N (Richter *et al*., 1982; Cade-Menun *et al.,* 2000). Inorganic orthophosphate availability can increase following a fire due to the mineralization of organic P and increases in soil pH, but this enhanced availability depends on the burn intensity and depth of heating (Cade-Menun *et al*., 2000). Typically, pH increases following a fire due to the denaturing of organic acids and the input of bases into the soil, especially if soil temperatures exceed 450^{o}C (Certini, 2005). Since P is most biologically available at a pH of 6.5, burning can increase the availability of P by increasing pH in relatively acid soils (Romanya *et al.,* 1994; Cade-Menun *et al.,* 2000). The duration of elevated P levels is highly variable and can last between 45 days and ten years (DeBano, 1988; Cade-Menun *et al.,* 2000). The fate of elevated P in burned soils largely depends on pH. Orthophosphate availability tends to decrease by binding to Al-, Fe-, and Mn-oxides under acidic conditions, while under more alkaline conditions P availability decreases due to formation of insoluble Ca-phosphates (Certini, 2005).

Similar to P, only a small portion of K in burned organic matter is released to the atmosphere because of its relatively high volatilization temperature (Richter *et al.,* 1982). Base cations, such as Ca^{2+}, Mg^{2+}, and K^{+} are deposited on the forest floor after burning, typically in the form of ash (Certini, 2005). Following a fire, K becomes biologically available once ash is assimilated in soils (Johnson *et al.,* 2011) but can later be lost from soils due to leaching (Richter *et al.,* 1982). Because of its high solubility, the duration of increased K levels following a fire is relatively short and ranges from three to six months (Adams and Boyle, 1980; Kutiel and Inbar, 1993).

Soil microorganisms are essential in mediating nutrient responses to fire. Soil heating may cause lethal temperatures for soil microorganisms, but temperatures depend on fire intensity and duration, O-horizon characteristics, soil porosity, heat conductivity, and soil water content (Hart *et al*., 2005; Knicker, 2007). Since the majority of soil microorganisms reside in the organic and mineral surface soil horizons, a large fraction of the soil microbiota undergo lysis during a soil heating event with fungi being more sensitive to heating than bacteria and algae (Knicker, 2007). Following a fire it may take up to 10 years for microbial biomass C and N to recover to pre-burn conditions (Fritze *et al.,* 1993) depending on the type of soil microorganism. In general, bacterial and algal species are able to recover faster than fungal species because of the lack in ability of fungi to recolonize a burned soil (Prieto-Fernandez *et al*., 1998).

Charcoal is an important byproduct of pile burning and is known to affect nutrient dynamics following fire. Pyrolysis of organic materials results in formation of charcoal upon incomplete combustion of woody material at temperatures ranging from 250°C to 500°C (Certini, 2005; Knicker, 2007). Charcoal is a C-rich substance with an aromatic structure that is capable of increasing water holding capacity, cation exchange, and retention of alkaline metals and PO_4^{3-} in soils (DeLuca and Aplet, 2008; Kolb *et al*., 2009). Because of its chemical properties, charcoal is highly resistant to decay and remains in soils for up to a century (Zackrisson *et al*., 1996). Typically, 70% of the total pool of soil charcoal can be found in the top 10 cm of the soil but it can be found as deep as one meter in the soil profile (DeLuca and Aplet, 2008).

The presence of charcoal in combination with physico-chemical changes to soil properties have the potential to stimulate microbial activity and increase bio-availability of N, P, and K (Certini, 2005; Kolb *et al*., 2009). Previous studies indicate that microbial biomass and activity increase with increasing amounts of charcoal present in the soil (Kolb *et al.,* 2009; Smith *et al*., 2010). A number of mechanisms have been proposed to explain the effect of charcoal on soil microbial processes. First, charcoal can host soil microorganisms in small pore spaces (<20 µm in diameter) avoiding predation by larger, predatory micro-arthropods (Zackrisson *et al*., 1996). Consequently, these protective capabilities increase with increasing amount and porosity of charcoal (Kolb *et al*., 2009). Wardle *et al.* (2008) observed that decomposition of native organic matter was stimulated by the presence of charcoal which may be due to the presence of a ‘protective cover’ for microorganisms. Second, charcoal has the ability to adsorb organic compounds that can be used as a C source for microorganisms (Zackrisson *et al.,* 1996). Third, charcoal itself may produce labile C that can be used as a substrate for soil microbes (Smith *et al*., 2010). In addition to stimulating heterotrophic activity, charcoal has been shown to positively affect autotrophic microbial activity, including nitrification. Several studies show that soil NO_3^- concentrations and (gross) nitrification rates increase with increasing amounts of charcoal present (DeLuca *et al*., 2002; Hart *et al*., 2005; Ball *et al*., 2010), potentially as a result of reduced predation in combination with the presence of an NH_4^+ pulse following a fire (DeLuca *et al.,* 2006). Charcoal may also affect nitrification by adsorbing N-complexing, nitrification-inhibiting compounds, such as polyphenols produced by plants (Wardle *et al*., 1998). Sorption of polyphenols by charcoal can reduce polyphenol concentrations in soils by up to 50% (DeLuca *et al*., 2002).

The work presented in this thesis focuses on the effects of pile burning on nutrient cycling in Sierra Nevada forest soils and particularly how charcoal in soils may mediate nutrient cycling in response to burning. While several studies have addressed the effect of charcoal on microbial processes, only a limited number of studies have tested charcoal application rates that are representative for the levels of charcoal found in soils following prescribed fires. In addition, few studies have measured if the effects of charcoal depend on the source of charcoal such as tree species and/or tree components. Previous studies used field collected charcoal (DeLuca *et al.,* 2006), activated C (Berglund *et al.,* 2004), grasses (Smith *et al.,* 2010), or biochar feedstock that may have contained manure (Kolb *et al.,* 2009). Potentially, differences in physical and chemical properties, including surface area, porosity, and surface charge, between charcoal types may differentially affect microbial activity to varying degrees. We are aware of only one study that looked at charcoal derived from different tree species in a temperate forest and no studies have been carried out in Sierra Nevada forests. Gundale and DeLuca (2006) observed that, in a Ponderosa pine forest, charcoal derived from Ponderosa pine material had a higher capacity to adsorb allelopathic substances than charcoal derived from Douglas-fir wood and bark material, which was attributed to differences in tracheid diameter of the wood. Additionally, lower net nitrification rates were recorded for charcoal generated from Ponderosa pine bark compared to other charcoal treatments. A second aspect of pile burning addressed in this thesis is the persistence of changes in nutrient dynamics following pile burning. While studies have been conducted on the effects of pile burning in Sierra Nevada forests, most of these studies have looked at short term (<2 yr) effects and little is known about long-term effects of pile burning on soil nutrients. Johnson *et al.* (2011) studied pile burns conducted in Sierra Nevada forests, but only addressed immediate effects of these burns. This study showed that burning did not affect NO_3^-, and increased K^+ in the center of pile burns occurring in meadows, while effects were inconsistent for NH_4^+ and P.

Chapter two of this thesis describes a lab incubation study in which lab-generated charcoal, originating from different vegetation types and tree components, was amended to soil at a range of application levels representative of natural charcoal levels following a prescribed pile burn. We specifically looked at the effects of charcoal additions on microbial respiration and potential nitrification. In addition, we compared microbial processes between unburned soils and soils within pile burn scars. In this study, we also quantified the effects of charcoal on the adsorption of polyphenolic substances as means to assess charcoal's ability to sorb C compounds that can either act as a C source for microbes or inhibit nitrification. Chapter three describes a field study that investigates the persistence of N, P, and K within pile burns three to eight years after ignition. This study follows up on work conducted by Johnson *et al.* (2011) who studied piles in the same study area 1-2 yr after ignition. We examined nutrient dynamics as a function of pile burn age and location within a pile burn using ion exchange resins installed in various locations within burn scars ranging in age from three and eight years. In Chapter 4 we summarize the conclusions from both chapters 2 and 3 and describe the potential implications of our study.

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CHAPTER 2

The Effects of Charcoal and Pile Burns on C and N Dynamics in Eastern Sierra Nevadan Forested Soils

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Abstract

Over a century of fire prevention and suppression in the western United States has led to overstocked and diseased forests with increased fuel loading and vertical continuity of fuels, thus increasing the risk of severe and stand-replacing wildfire. Mechanical thinning followed by pile burning is a commonly used technique to reduce fuel loads. Charcoal, a product of pile burning, can have major impacts on nutrient dynamics within forest soils. Specifically, charcoal-rich environments have been shown to enhance nitrogen cycling. In this study incubations of soil amended with varying amounts of charcoal (0.5, 1, 2.5, and 5% by weight) were conducted to assess the importance of charcoal in microbial activity and nitrogen cycling in forest soils in Little Valley, approximately 30 km south of Reno, Nevada. Carbon respiration and potential nitrification rate, proxies for microbial activity and size of the nitrifying microbial community respectively, were monitored over a two month period. In general, microbial respiration was highest at 2.5% charcoal additions, while nitrification was stimulated equally at all charcoal amendment levels by the end of the incubation. Also, microbial respiration rates decreased but potential nitrification rates increased over time across most treatments. Polyphenol sorption onto charcoal-amended soil was also analyzed and showed that extractable polyphenol concentrations decreased with increasing amounts of charcoal which may partly explain patterns in potential nitrification rates. The production of charcoal during prescribed fires may benefit soil microbial activity, but may also stimulate carbon and nitrogen losses through increased microbial activity.

Key Words: Prescribed fire, nutrient cycling, carbon respiration, microbial activity, forest soils, incubation.

Introduction

Fire suppression and prevention have been the dominant form of forest management for over a century in the western United States and has contributed to increases in stand density, the number of small trees, amount of understory shrub vegetation and accumulation of organic matter, increasing the risk of severe, stand-replacing wildfires (Stephens and Collins*,* 2004). It is estimated that severe wildfires currently threaten more than 72 million ha of federal land in the United States (Moghaddas and Stephens, 2007). To reduce fuel loads, several management options are available, including fire-based treatments such as broadcast and pile burning. Because of concerns about air quality, cost and containment associated with broadcast burning, pile burning has become a more frequently used technique to dispose of downed fuels. Still, pile burning can locally impact soils due to the high soil temperatures achieved upon ignition of piles that may impact soil nutrient dynamics and subsequent microbial recovery of burn scars (Jiménez Esquilín *et al.,* 2007).

Pile burning can influence soil nitrogen (N) dynamics in multiple ways. Total N pools typically decrease due to volatilization of organic N present in the O-horizon or upper mineral horizon (Johnson *et al.*, 2009). Ammonium (NH_4^+) and nitrate (NO_3^-), bio-available forms of N, can however increase following pile burning. Production of NH_4^+ is attributed to chemical mineralization of organic material, deposition of ash on the forest floor, and lysis of soil microorganisms (Prieto-Fernandez *et al.,* 2004). Elevated NH_4^+ levels can be sustained through mineralization of N. Following the increase in NH_4^+, nitrification can be stimulated resulting in an increase in soil NO_3^- (Canfield *et al.,* 2010). Microbial biomass typically decreases after a pile burn because the transfer of heat into soils is typically large enough to kill most organisms (Jiménez Esquilín *et al.,* 2007) and recovery of microbial communities to pre-burn conditions may take years (Switzer *et al.,* 2012).

Charcoal formed due to incomplete combustion of woody materials can potentially have a large impact on soil N cycling and microbial recovery (Certini, 2005). Charcoal is a carbon-rich substance with an aromatic structure that is capable of increasing water holding capacity in soils, increasing cation exchange, and retaining alkaline metals and nutrients (DeLuca and Aplet, 2008; Kolb *et al.,* 2009). Charcoal is highly resistant to decay and remains chemically active in soil for up to a century (Zackrisson *et al.,* 1996). Charcoal can be found as deep as one m into the soil profile, but typically 70% of charcoal particles reside in the top 10 cm of the soil profile (DeLuca and Aplet, 2008). Although soil heating due to fire initially has detrimental impacts on microbial biomass (DeLuca *et al.,* 2002), the deposition of charcoal may increase the recovery of microorganisms following a fire (Zackrisson *et al.,* 1996). Indeed, previous studies indicate that the presence of charcoal stimulates microbial biomass and activity (Kolb *et al.,* 2009; Smith *et al*., 2010), and presence of charcoal can increase decomposition rates of native organic C following fire (Wardle *et al.,* 2008).

Factors that may benefit microbial recolonization following fire include an increase in soil pH due to base cations deposited with ash and charcoal, adsorption of organic C onto the surface of charcoal particles, and an increase in soil available water and nutrients (Pietikäinen *et al.,* 2000; DeLuca *et al*., 2008; Smith *et al*., 2010). Charcoal may also promote restoration of the soil microbial biomass due to its ability to allow microorganisms to enter pore spaces in charcoal (Pietikäinen *et al.,* 2000; Wardle *et al.,* 2008). Pores that are less than 20 µm in diameter permit the access of bacteria, fungi, and other microorganisms but prevent access of predatory micro-arthropods, thus creating a 'safe-haven' for microorganisms (Zackrisson *et al.,* 1996; Kolb *et al*., 2009).

Charcoal may be particularly important for the nitrifying community (DeLuca *et al.,* 2002; Hart *et al.,* 2005). Previous studies have shown that NO_3^- concentrations and net nitrification rates increase with increasing charcoal concentration (DeLuca *et al*., 2002; 2006; Berglund *et al.,* 2004; Ball *et al.,* 2010). This increase may be due to the protective cover of charcoal in combination with the pulse of NH_4^+ following a fire creating an environment that favors nitrification (DeLuca *et al.,* 2006). In addition, nitrification can also be stimulated by sorption of nitrification-inhibiting compounds such as polyphenols (Wardle *et al.,* 1998; Berglund *et al.,* 2004). Sorption of polyphenols by charcoal can reduce free polyphenol concentrations in soils by up to 50% (DeLuca *et al.,* 2002).

The source of charcoal may be important in determining the ability of charcoal to stimulate the recovery of microorganisms, as physical and chemical properties are likely to depend on the composition of the substrate from which the charcoal is derived (Keech *et al.,* 2005). Previous studies focusing on the effects of charcoal or biochar on soil microbial activity used field-collected charcoal (DeLuca *et al.,* 2006), activated carbon (Berglund *et al.,* 2004), grasses (Smith *et al.,* 2010), or biochar feedstock that may have contained manure (Kolb *et al.,* 2009). We are aware of only one study however that compared charcoal derived from different tree species. Gundale and DeLuca (2006) observed that charcoal derived from Ponderosa pine wood and bark material had a higher capacity to adsorb allelopathic substances than charcoal derived from Douglas-fir wood and bark material due to differences in tracheid diameter of the wood. Additionally, lower net nitrification rates were recorded for Ponderosa pine bark charcoal compared to other charcoal treatments for reasons that were not entirely clear. Furthermore, only a limited number of studies have tested a variety of charcoal application ranges that are representative of the levels of charcoal found in soils following prescribed fires. Finally, most studies on microbial-charcoal interactions have been carried out in boreal biomes (Zackrisson *et al.,* 1996; Wardle *et al.,* 1998; 2008) and limited data are available for semi-arid forests.

The objective of our study is to assess the response of soil microbes to varying quantities of charcoal originating from different sources in semi-arid forests of the Sierra Nevada. We specifically tested the following hypotheses:

H1: Microbial respiration will increase with increasing amounts of charcoal potentially due to the presence of increased pore spaces and sorption of labile C compounds to the surface of charcoal particles.

H2: The effects of charcoal amendment on microbial activity will depend on the source of charcoal, i.e., tree species or tree component, with microbial activity being greater in soils amended with charcoal that has greater surface area and porosity.

H3: Potential nitrification rates will increase with increasing charcoal application, levels which may be explained by increased sorption of nitrification-inhibiting substances such as polyphenols.

H4: Microbial respiration will be greater in soils that experienced pile burning compared to unburned soils due to the presence of charcoal and increased C and N availability in soils exposed to pile burning.

To test our hypotheses, we added charcoal to unburned soils using a range of application levels found in pile burn scars. We added charcoal from two tree species commonly found in semi-arid western forests, Jeffrey pine (*P. jeffreyi*) and lodgepole pine (*P. contorta*) to assess if the source of charcoal affects microbial processes. We further separated the woody material into branch and bole material to assess the effect of tree component on microbial activity. We compared these results with results from an incubation of soils from existing pile burns allowing us to separate effects of charcoal on microbial processes from other fire-induced effects, including deposition of ash and combustion of soil organic matter. Finally, we evaluated the capacity of charcoal-amended soils to adsorb polyphenolic substances.

Methods

Field Site

The study area is located in Little Valley, Nevada, approximately 30 km south of Reno, Nevada. Little Valley is part of the Carson Range and elevation ranges from 1900 to 2500 m. The overstory vegetation is dominated by Jeffrey pine (*Pinus jeffreyi*) and lodgepole pine (*P. contorta*), while the understory is dominated by bitterbrush (*Purshia tridentata*) and manzanita (*Arctostaphylos patula*). Soils with a Jeffrey pine overstory were located on areas having a 10-15% slope with a sparse understory consisting of bitterbrush and manzanita. Soils belonged to the Marla series (loamy sand, mixed, frigid Aquic Dystroxerepts; USDA, 2014). Soils with a lodgepole pine overstory were located in a meadow and had an understory of mixed herbaceous species. These areas had slopes of less than 5% and soils belonged to the Marla series (loamy sand, mixed, frigid Aquic Dystroxerepts) and Toiyabe series (Mixed, frigid, shallow Typic Xeropsamments; USDA, 2014). The climate is semi-arid characterized by dry, warm summers and cold, wet winters. The mean annual precipitation is 87.5 cm and the mean annual air temperature is 5^{o}C. Approximately half of the precipitation in Little Valley occurs as snow, with snowmelt being a dominant hydrologic event (Johnson *et al*., 2011; http://environment.unr.edu/whittell/climate/index.html).

Soil Collection

We collected soil from pile burns having three burn ages in areas dominated by either Jeffrey pine (referred to as JP) or lodgepole pine (referred to as LP). We sampled soils with a hand trowel from the 0-10 cm layer within the burn area as well as unburned soils adjacent to pile burns (control). A total of three piles per vegetation type/age were sampled. Burned soils were collected from the center of each pile burn and were separated into three age classes representing the time since the pile was ignited: (1) JP and LP ignited in late May 2008; (2) LP ignited in February 2010; (3) JP estimated to have been ignited autumn of 2012 (S. VanderWall, personal communication). Unburned soils for each vegetation type were combined while samples from burned soils were combined for each vegetation/age combination. After sampling, we air-dried, sieved (2 mm mesh size), and homogenized the samples.

We amended unburned control JP and LP soils with charcoal using five amendment levels (0, 0.5, 1, 2.5, and 5% charcoal by weight of soil), two different charcoal origins (JP and LP trees), and two different tree components (branch and bole material). In addition, we incubated burned soils from the center of each pile. Soil moisture at field capacity was determined for burned, unburned, and unburned + amended soils by adding water to saturate the soils and letting water drain for 24-48 h. Moist samples were weighed prior to and after oven-drying at 105^{o}C for 24-48 h until a constant mass was reached.

The production of charcoal for this study followed a similar procedure as described by Zackrisson *et al*. (1996). Woody JP and LP branch and bole material was placed in a muffle furnace set to 450^{o}C and material was allowed to oxidize for 15 min. After 15 min, the charred material was removed from the furnace and allowed to cool. The charcoal was then crushed with a mortar and pestle until a fine homogenous texture was reached.

Microbial Respiration

To measure microbial respiration, we incubated amended and unamended soils and measured carbon dioxide (CO_2) production. For all incubations, we filled glass bottles (250 mL) that had a septum installed in the lids, with 25 g of soil. We added deionized water to the incubated soils to reach 60% of field capacity by weight (DeLuca *et al*., 2006; Kolb *et al*., 2009). At the start of the incubation, we weighed and capped the incubation bottles, and stored them in a dark incubator set at 25^{o}C. Microbial CO_2 production was measured once a week at time 0 (baseline) and again after 24 h. A gas sample was taken from the headspace with a 250 µl syringe through the septum in the lid, followed by analysis of the gas sample using an infrared gas analyzer (LiCOR LI-8100A). After each CO_2 measurement, the incubation bottles were opened to the atmosphere allowing the headspace to equilibrate with atmospheric conditions. At this time we weighed the bottles to determine if water was lost, and added water to reestablish the desired moisture content if needed. Three replicates were used for each treatment (n=3).

Potential Nitrification Rate

The potential nitrification rates were determined for unburned and charcoal-amended JP and LP soils using branch material that corresponded to the soil type (e.g., JP soil amended with JP branch charcoal). Nitrification potential was measured using a shaken soil slurry method (Belser and Mays, 1980, modified by Hart *et al.,* 1994). We mixed 15 g of soil in a 250 mL Erlenmeyer flask with 100 mL of deionized water, 1.5 mM NH_4^+, and 1 mM PO_4^{-3} solution to ensure that nitrifiers were not limited by NH_4^+ and P availability. The slurries were shaken in an orbital shaker at 180 rpm to maintain aerobic conditions for a 24 h period at room temperature (21^{o}C). Subsamples (10 mL) were taken at 2, 4, 22, and 24 h from each slurry, which included both soil and solution in order to keep the ratio of soil to solution constant. The subsamples were centrifuged at 2500 rpm, decanted, filtered using a 1.5 µm glass fiber filter and analyzed for NO_3^- concentration using a Lachat Autoanalyzer. The nitrification rate was determined by obtaining the slope of the regression line between the NO_3^- concentration and time. The slope was subsequently corrected for temperature, water content, and mass of soil in the sample. Potential nitrification was measured at the beginning of the incubation and after 4 and 8 weeks. During this period samples were incubated at 25^{o}C and maintained for water content as described for the microbial respiration. Three replicates were used for each treatment (n=3).

Polyphenol Adsorption

To determine the effects of charcoal on polyphenol adsorption we extracted charcoal-amended soils with deionized water. Ten g of soil and 30 mL of deionized water were added to 50 mL centrifuge tubes and were shaken for 24 h using an overhead shaker. The mixture was then centrifuged at 2500 rpm, decanted, and filtered using a 0.45 µm nylon fiber filter. Next, 25 mL of each sample was mixed with 0.5 mL of tannin-lignin reagent and 5 mL of carbonate reagent in rapid succession and allowed to react for 30 min to enable color development (Rice *et al.,* 2012). Subsamples were analyzed using a Shimadzu spectrophotometer (Shimadzu, Columbia, MD) at 760 nm wavelength in quartz cuvettes. Three replicates were used for each treatment (n=3).

Soil and Charcoal Characterization

To measure total C and total N, all soil types and charcoal types were ground for 48 h using a Mavco vial rotator. Total C and N were measured using a Leco Truspec CN analyzer. All analyses were performed using three replicates (n=3). Soil pH was determined using a 1:2 soil to water (g g^{-1}) ratio for all combinations of soil treatment, charcoal type, and charcoal amendment level. Five g of unburned soil or soil/charcoal mixtures were combined with 10 mL deionized water in centrifuge tubes and mixtures were shaken and allowed to set for 1 h. Prior to the pH measurement the soil slurry was stirred and the pH was measured after the pH had stabilized. All measurements were performed with three replicates (n=3). Cation exchange capacity (CEC) was measured using the ammonium saturation method. The CEC was measured at the Waters Agricultural Laboratories Inc., Georgia, USA. Only a single measurement of CEC was recorded for each treatment (n=1). Surface area of charcoal was quantified nitrogen adsorption isotherms at 77 K using a Nova 2000 Surface Area Analyzer (Quantachrome Corp., Boynton Beach, FL, USA at the USDA-ARS Southern Regional Research Center by Isabel Lima. Specific surface areas, which included micropores >2 nm, were taken from adsorption isotherms using the Brunner-Emmett-Teller (BET) equation. All measurements were performed in duplicate (n=2).

Charcoal amounts in the burned field soil were quantified using the method of Mooney and Tinner (2011). Briefly, 8 mL of 6% hydrogen peroxide (H_2O_2) was mixed with 1 mL of soil without agitating the mixture to reduce charcoal disintegration. Mixtures reacted for 24 h to dissolve/bleach organic material. Subsequently, samples were rinsed with water on a 100 µm sieve to remove H_2O_2, but retain particles greater than 100 µm. The remaining soil was placed in a petri dish and any remaining water was allowed to evaporate. Next, photographs were taken of the soil in the petri dish and edited in Adobe Photoshop to increase brightness and contrast to clearly delineate the darker colors of charcoal particles compared to the rest of the soil in the sample. The images were then uploaded into Scion Imaging software to calculate the surface area of charcoal in a sample by outlining darker colored particles. Charcoal surface area was then converted to volume using the equation: $V \approx \Sigma A_i^{(3/2)}$, where A_i is the surface area of an individual particle of charcoal. Mass of charcoal in a sample was then estimated assuming a charcoal density range from 240 to 481 kg m^{-3} (engineeringtoolbox.com). We calibrated the method by adding known amounts of charcoal to charcoal-free soil samples.

Data Analysis

Microbial respiration was expressed as average CO_2 production rate per g of soil, soil C, or total C (soil C plus charcoal C). Respiration and potential nitrification data were analyzed using an Analysis of Co-variance. Statistical analysis for C respiration was performed using charcoal type and soil type as discrete independent variables and time and amendment level as continuous independent variables. Potential nitrification rate data expressed as mg N kg^{-1} day^{-1} were analyzed using soil type as discrete independent variables, and charcoal amendment level and time as continuous independent variables. Outliers were removed if the value exceeded the standard deviation by 2.5 times.

Carbon respiration and the potential nitrification rate in soils that experienced pile burning in the field were analyzed using soil type, burn treatment vs. control as discrete, and time as continuous independent variables. Polyphenol concentration data were analyzed using soil type and charcoal amendment level as discrete independent variables.

All statistical analyses were conducted with DataDesk V6.3. Probability levels ≤ 0.05 were considered to be significant. Following the ANCOVA analyses, we conducted post hoc tests to determine significant differences between treatments using a least significance difference (LSD) approach.

Results

Soil and Charcoal Characteristics

Unburned soils were slightly acidic and had an average pH of 6.08. The addition of charcoal increased pH of unburned soils. The highest pH was measured at the 5% addition level and was on average 0.59 units higher than unamended soils. Soil pH also tended to be higher in burned soils compared to unburned and amended soils, while the highest pH occurred in the most recent pile burns. Total C was significantly higher in LP control soil over JP control soil. Average charcoal content of soil collected from pile burns was 2.3% by mass of soil (Table 1) while charcoal content of unburned JP and LP soil was 0.03% and 0.02% by mass of soil, respectively (data not shown). The LP bole charcoal had a 30% lower surface area than other charcoal types, while total C and CEC were comparable between charcoal treatments (Table 2).

Table 1. Chemical properties of the soils used in the incubation study. Soil types have been categorized by dominant overstory vegetation type (JP or LP). Burned soils represent the eight year old pile burns. Three replicates were used for soil pH, total C, total N, and charcoal content. CEC was measured on one sample.

Soil Type	pH	Total C %	Total N %	CEC meq $100g^{-1}$	Charcoal %
JP Control	6.02±0.02	1.59±0.02	0.06±0.002	9.8	0
LP Control	6.13±0.01	2.24±0.21	0.08±0.006	11.2	0
Amended JP	6.66±0.04	-	-	-	5
Amended LP	6.68±0.03	-	-	-	5
JP Burned	7.61±0.01	2.05±0.04	0.04±0.004	10.9	2.08
LP Burned	7.45±0.03	1.84±0.04	0.06±0.005	11.2	2.52

Table 2. Chemical and physical properties of the charcoals used as amendments in the incubation study. Charcoal has been categorized by the dominant overstory vegetation type (JP or LP) and tree component (branch or bole). Three replicates were used for total C, two replicates for surface area and no replicates for CEC.

Charcoal Type	Total C %	CEC meq $100g^{-1}$	Surface Area m^2 g^{-1}
JP Branch	92.99±0.24	26.5	468.45±14.47
LP Branch	92.91±5.66	27.7	449.08±7.04
JP Bole	93.35±1.61	-	441.80±10.68
LP Bole	92.30±1.25	-	335.38±5.19

Carbon Mineralization

Respiration per g soil: When respiration was expressed as C respired per g soil, charcoal amendments caused a significant increase in C respiration compared to control soils at all levels of charcoal application (Table 3). On average, respiration rates in amended soils were 52% higher than in unamended soils. Respiration rates increased with increasing charcoal amendment until the 2.5% charcoal amendment level, after which respiration began to decline (Fig. 1). Average respiration rates were statistically similar at the 0.5, 1, and 2.5% charcoal amendment levels. The effect of charcoal amendment level varied by soil type. For instance, at the 2.5% amendment level, respiration was higher in LP soils (22.43 µg C day^{-1} g soil^{-1}) than in JP soils (13.04 µg C day^{-1} g soil^{-1}).

Post-hoc analysis showed that the stimulatory effect of charcoal on respiration did not vary by charcoal type (Fig. 2). The effect of charcoal type varied by soil type, however (Table 3). For instance, the addition of LP branch charcoal had a larger stimulatory effect on LP than on JP soil. Respiration rates in amended JP soils were 12.11 µg C day^{-1} g soil^{-1} (25% increase over the JP control soil) compared to 20.08 µg C day^{-1} g soil^{-1} when LP branch material was amended to LP soil (a 63% increase over the LP control soil).

Soil type significantly affected microbial respiration. Respiration rates were 44% higher in LP than in JP soils (Table 3; Fig. 3) when averaged across all amendment levels and charcoal types. The change in C respiration rate over time also differed significantly between soil types. Throughout the incubation, the decrease in respiration rates was slower in JP soils than in LP soils.

Table 3. ANOVA table of C respiration rate expressed as µg C day^{-1} g soil^{-1}, µg C day^{-1} g soil C^{-1}, µg C day^{-1} g total C^{-1}) and cumulative C respiration expressed as µg C g soil^{-1} for amended and unamended soil treatments. P-values < 0.05 are given in bold. P-values between 0.05 and 0.10 are listed in italics.

	C Respiration Rate									Cumul. C Respiration		
	µg C day^{-1} g soil^{-1}			µg C day^{-1} g soil C^{-1}			µg C day^{-1} g total C^{-1}			µg C g soil^{-1}		
Source	df	F	P	df	F	P	df	F	P	df	F	P
Charcoal Type (CT)	**4**	**10.470**	**<0.001**	**4**	**9.182**	**<0.001**	**4**	**2.780**	**0.026**	3	0.633	0.595
Time (T)	**1**	**227.720**	**<0.001**	**1**	**231.660**	**<0.001**	**1**	**431.490**	**<0.001**	**1**	**141.000**	**<0.001**
Soil Type (ST)	**1**	**208.620**	**<0.001**	**1**	**38.428**	**<0.001**	**1**	**97.914**	**<0.001**	**1**	**116.680**	**<0.001**
Charcoal Amendment Level (CA)	**1**	**13.377**	**<0.001**	**1**	**12.785**	**<0.001**	**1**	**661.910**	**<0.001**	**4**	**10.697**	**<0.001**
CT * T	4	0.848	0.495	4	1.016	0.398	**4**	**4.151**	**0.002**	3	0.147	0.931
CT * ST	**4**	**8.505**	**<0.001**	**4**	**6.910**	**<0.001**	**4**	**14.696**	**<0.001**	*3*	*2.218*	*0.088*
T * ST	**1**	**45.133**	**<0.001**	**1**	**20.478**	**<0.001**	**1**	**39.798**	**<0.001**	*1*	*3.890*	*0.050*
CT * CA	3	2.031	0.108	*3*	*2.478*	*0.060*	3	1.120	0.340	9	1.099	0.366
T * CA	*1*	*2.826*	*0.093*	1	2.516	0.113	**1**	**55.008**	**<0.001**	4	1.168	0.327
ST * CA	**1**	**5.008**	**0.025**	*1*	*3.105*	*0.078*	**1**	**16.779**	**<0.001**	**4**	**2.951**	**0.022**
CT * T * ST	*4*	*2.096*	*0.079*	**4**	**2.459**	**0.044**	**4**	**3.991**	**0.003**	3	0.068	0.977
CT * T * CA	3	1.361	0.253	3	1.623	0.182	3	0.637	0.591	9	0.108	1.000
CT * ST * CA	**3**	**7.047**	**<0.001**	**3**	**8.280**	**<0.001**	**3**	**6.576**	**<0.001**	**9**	**3.929**	**<0.001**
T * ST * CA	1	1.785	0.182	1	1.312	0.252	**1**	**9.485**	**0.002**	4	0.242	0.914
CT * T * ST * CA	3	0.697	0.554	3	0.856	0.464	3	1.025	0.381	9	0.432	0.916

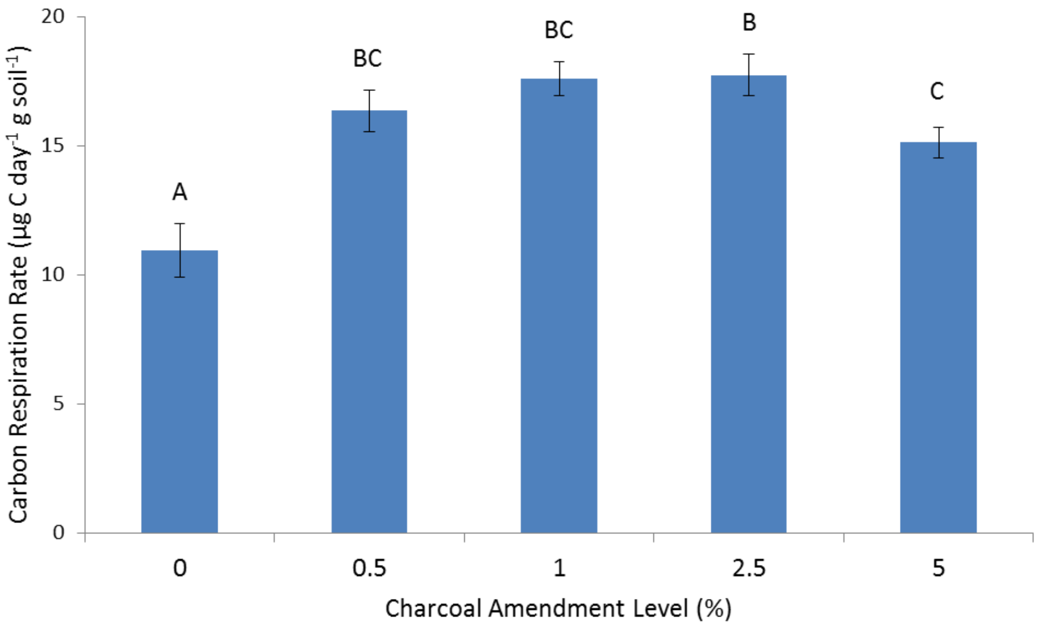

Figure 1. Carbon respiration rate expressed as µg C respired day^{-1} g soil^{-1} as a function of the charcoal amendment level (% charcoal, by mass) averaged across all soil types and charcoal types. Error bars represent standard error of the mean C respiration. Capital letters indicate significant differences between amendment levels ($P<0.05$).

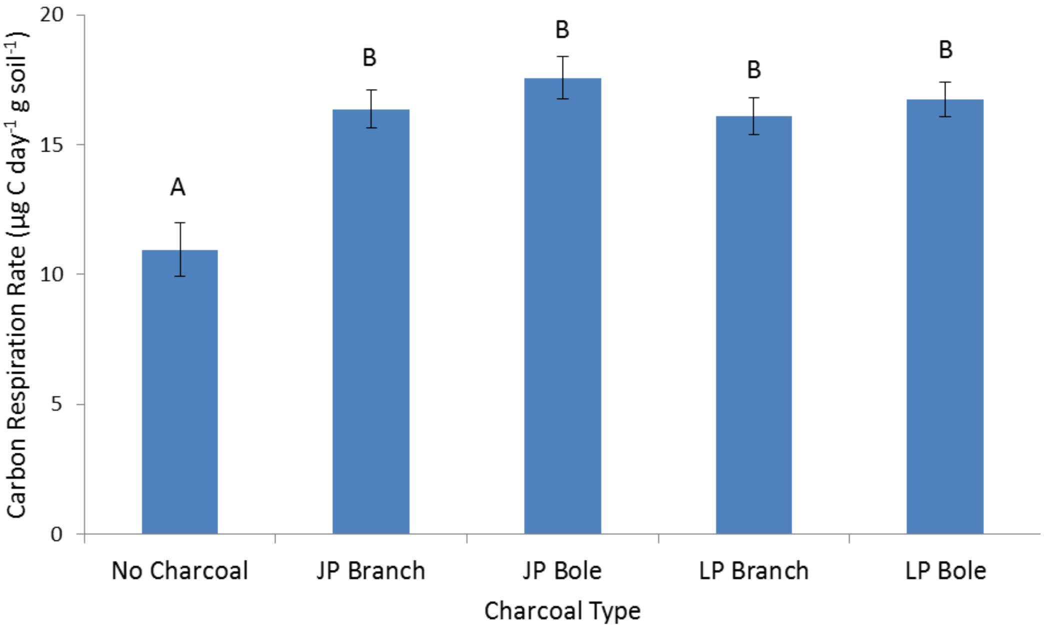

Figure 2. Carbon respiration rate expressed as µg C respired day^{-1} g soil^{-1} as a function of the type of charcoal (Jeffrey pine (JP) branch, Jeffrey pine bole, lodgepole pine (LP) branch, lodgepole pine bole, and no charcoal amendment) averaged across all soil types and amendment levels. Error bars represent standard error of the mean C respiration. Capital letters indicate significant differences between charcoal types ($P<0.05$).

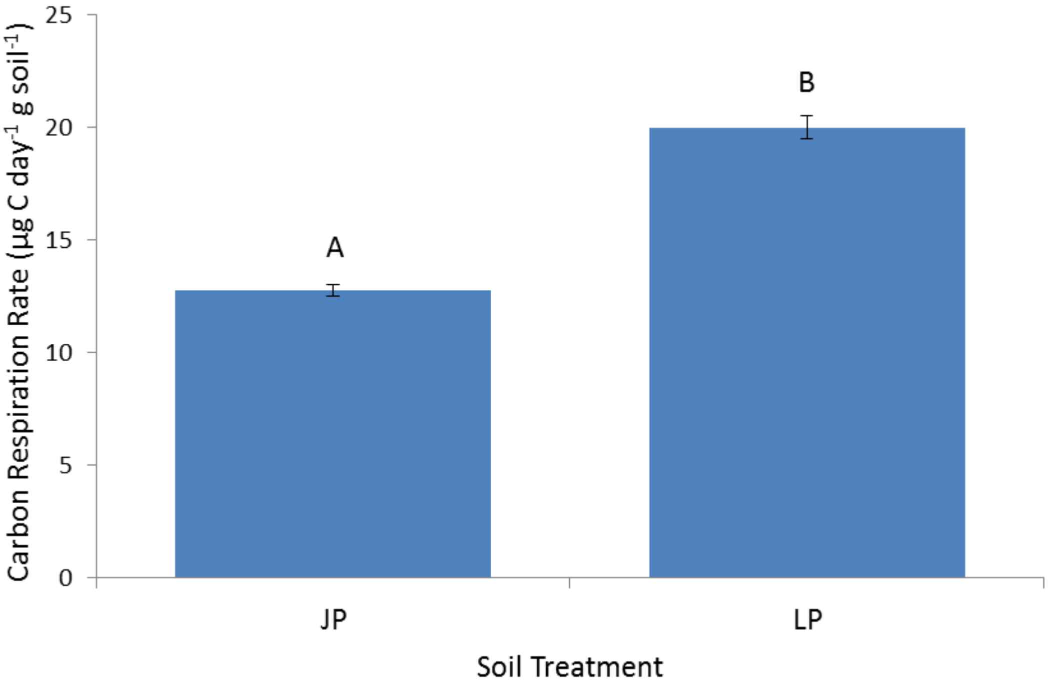

Figure 3. Carbon respiration rate expressed as µg C respired day^{-1} g soil^{-1} as a function of the soil treatment (Jeffrey pine (JP) soil treatments and lodgepole pine (LP) soil treatments) averaged across all charcoal types and charcoal amendment levels. Error bars represent standard error of the mean C respiration. Capital letters indicate significant differences between soil types ($P<0.05$).

Respiration per g soil C: Potentially, differences in respiration rates between LP and JP soils could be due to differing amounts of C in these soils. By expressing respiration rates per gram of soil C, we can account for differences in quantity and instead assess quality of organic matter. Even when accounting for differences in soil C between LP and JP soils, respiration rates were 10.5% higher in LP soil than JP soil, indicating that the quality of organic matter varied between the soil types.

Trends in respiration as a function of charcoal amendment level when expressed as C respired per g soil C were comparable to those when respiration was expressed as C respired per g soil (Fig. 4). Overall, respiration rates increased by 49.7% in the amended soils compared to unamended soils (Table 3; Fig. 4). Respiration rates were similar for the 0.5, 1, and 2.5% charcoal amendment levels and were higher than the 5% amendment level. Respiration rates for the 1% charcoal amendment level treatment were 58.5% higher compared to the control soils.

Post-hoc tests showed that charcoal type did not affect the stimulatory response of charcoal when averaged across all charcoal application levels and soil types (Fig. 5) however, charcoal type stimulated respiration in the two soils to different degrees. The addition of LP branch charcoal to JP soil produced an average respiration of 761.94 µg C day^{-1} g soil C^{-1} whereas LP branch charcoal amended to LP soil resulted in an average respiration of 894.90 µg C day^{-1} g soil C^{-1}, equivalent to a 16.1% difference in respiration between soil treatments.

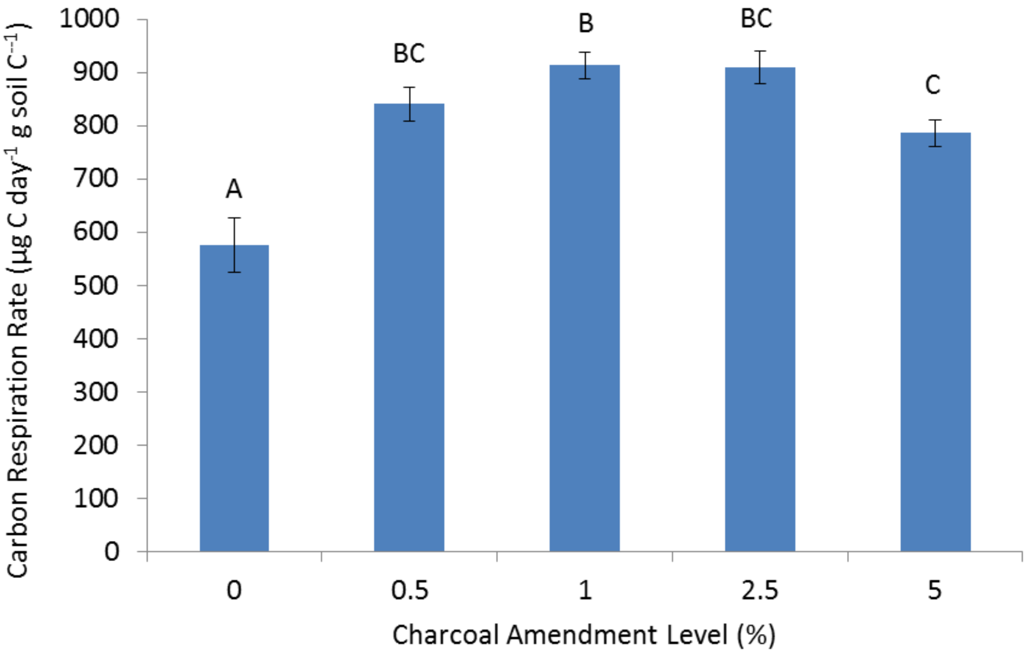

Figure 4. Carbon respiration rate expressed as µg C respired day^{-1} g soil C^{-1} as a function of the charcoal amendment level (% charcoal, by mass) averaged across all soil types and charcoal types. Error bars represent standard error of the mean C respiration. Capital letters indicate significant differences between charcoal amendment levels (P<0.05).

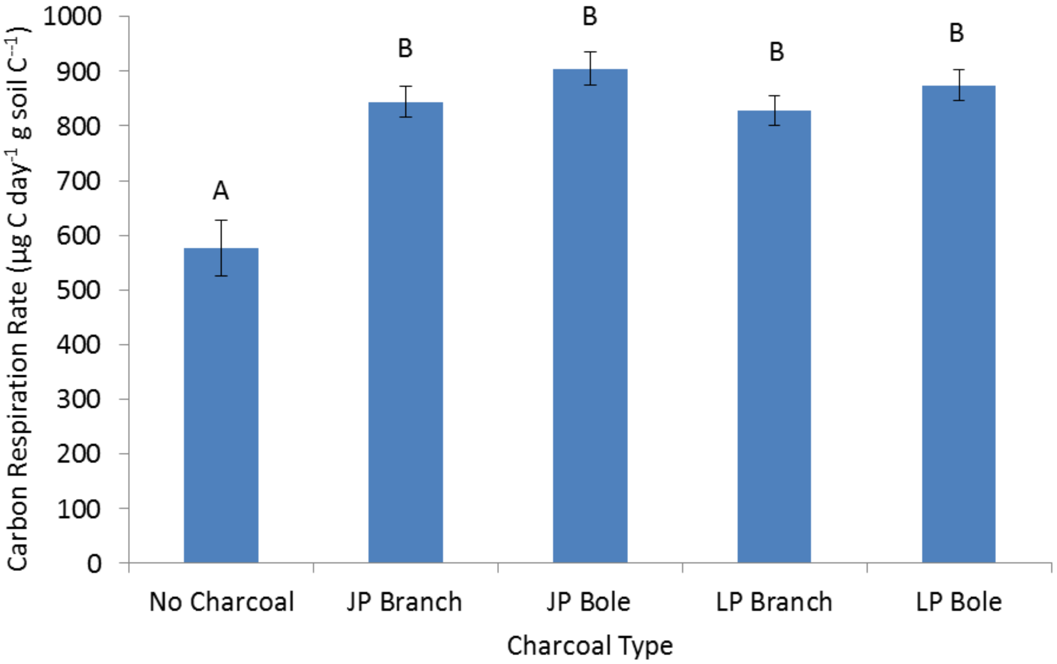

Figure 5. Carbon respiration rate expressed as µg C respired day^{-1} g soil C^{-1} as a function of the type of charcoal (Jeffrey pine (JP) branch, Jeffrey pine bole, lodgepole pine (LP) branch, lodgepole pine bole, and no charcoal amendment) averaged across all soil types and charcoal amendment levels. Error bars represent standard error of the mean C respiration. Capital letters indicate significant differences between charcoal amendment levels (P<0.05).

Respiration per g total C: When C respiration rate was expressed per gram of total C, which includes both soil C and charcoal C (µg C day^{-1} g total C^{-1}), the highest respiration rates were observed in the 0.5% amendment level treatments which exceeded respiration in the control treatments by 17.2% (Table 3; Fig. 6). Respiration was statistically similar between the 0, 0.5, and 1% charcoal addition levels, but declined with increasing charcoal amendment level. Respiration rates were lowest in the 5% charcoal amendment treatments and were approximately 99% lower than the rates observed at the 0.5% treatment. The effect of charcoal amendment level depended on the soil type. Respiration rates tended to be greater in the LP soils than in JP soils, but the differences in rates varied across amendment levels. For example, the greatest difference in respiration between the soil types occurred at the 2.5% amendment level where respiration was 38.3% higher in LP soils than in JP soil. At the 0% amendment level respiration rates in JP soils were only 9.8% greater than in LP soils. Respiration rates declined fastest in the control soils, whereas rate of decline in respiration rates decreased with increasing charcoal application.

Post-hoc tests did not show significant overall effects of charcoal type on respiration (Fig. 7) but the effect of charcoal type on respiration rates varied by soil type. For example, the respiration rate in LP soils amended with LP branch charcoal was 21.9% greater than JP soils amended with LP branch material. We also observed similar differences between soil types when amended with JP branch or LP bole material. Respiration rates in JP branch amended soils were 23.2% higher in LP than in JP soils, while rates in LP bole amended soils were 19.8% higher in LP than in JP soils. Respiration rates declined faster in soils amended with bole-derived charcoal (5.8 µg C day^{-1} g total C^{-1}) compared to charcoal derived from branches (4.4 µg C day^{-1} g total C^{-1}).

Similar to when respiration rates were expressed per g of soil or soil C, the LP soil respiration rates exceeded the JP respiration rates. Respiration rates in the LP soils were 22.4% higher than in the JP soils and respiration rates declined more rapidly in LP soils than in JP soils.

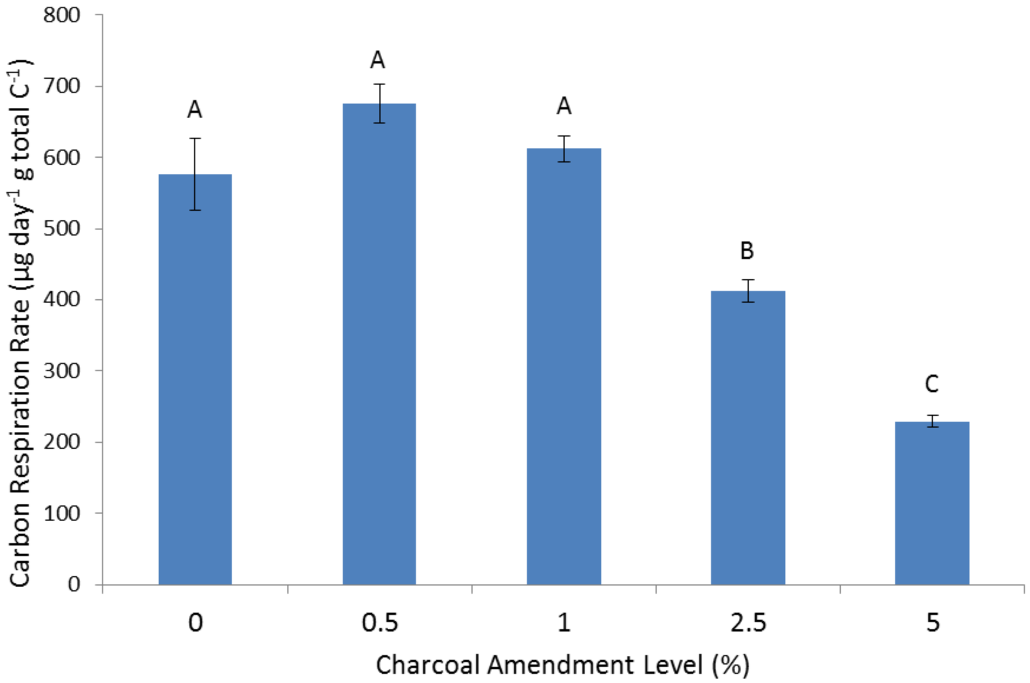

Figure 6. Carbon respiration rate expressed as µg C respired day^{-1} g total C^{-1} as a function of the charcoal amendment level (% charcoal, by mass) averaged across all soil types and charcoal types. Error bars represent standard error of the mean C respiration. Capital letters indicate significant differences between charcoal amendment levels ($P<0.05$).

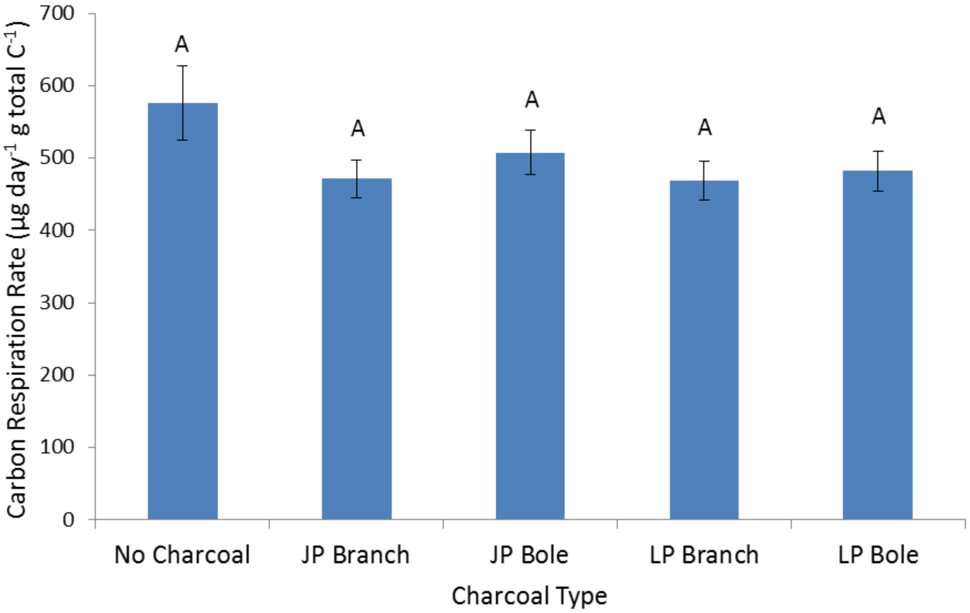

Figure 7. Carbon respiration rate expressed as µg C respired day^{-1} g total C^{-1} as a function of the type of charcoal (Jeffrey pine (JP) branch, Jeffrey pine bole, lodgepole pine (LP) branch, lodgepole pine bole, and no charcoal amendment) averaged across all soil types and charcoal application levels. Error bars represent standard error of the mean C respiration. Capital letters indicate significant differences between charcoal types ($P<0.05$).

Naturally burned soil: In general, both burning and soil type significantly affected C respiration rate regardless of how the respiration rates were expressed (Table 4). Soil type did however not significantly affect respiration when respiration was expressed per g of total C. Respiration rates were greater in JP control soils than in JP soils three to four years after exposure to a pile burn (Fig. 8) and on average rates were 42% lower in three to four year old burned soil than in unburned soils. However, respiration rates in soils from piles that were burned more than six years prior to the incubation were similar to unburned soil. Additionally, respiration rates were lower in eight year old burned JP soil compared to eight year old burned LP soil (Fig. 8). The burning x soil type interaction was significant in all cases except when respiration was expressed as µg C day^{-1} g soil^{-1} (Table 4).

Similar to the amended soils, respiration rates in LP soils decreased more rapidly than in JP soils when respiration was expressed as µg C day^{-1} g soil^{-1}, but this was not the case when other respiration units were used (Table 4).

Table 4. ANOVA table of C respiration expressed as µg C day^{-1} g $soil^{-1}$, µg C day^{-1} g soil C^{-1}, and µg C day^{-1} g total C^{-1} for unburned and naturally burned soil treatments. P-values < 0.05 are given in bold while P-values between 0.05 and 0.10 are listed in italics.

		µg C day^{-1} g $soil^{-1}$		µg C day^{-1} g soil C^{-1}		µg C day^{-1} g total C^{-1}	
Source	df	F	P	F	P	F	P
Soil Type (ST)	1	**50.924**	**<0.001**	**13.024**	**<0.001**	1.204	0.274
Burned/Unburned (BR?)	1	**35.294**	**<0.001**	**55.085**	**<0.001**	**516.22**	**<0.001**
Time (T)	1	**249.640**	**<0.001**	**272.02**	**<0.001**	**289.52**	**<0.001**
ST * BR?	1	0.237	0.627	**24.579**	**<0.001**	**8.540**	**0.004**
ST * T	1	**7.434**	**0.007**	2.157	0.143	0.478	0.490
BR? * T	1	1.860	0.174	*3.479*	*0.063*	**61.212**	**<0.001**
ST * BR? * T	1	0.119	0.730	1.708	0.193	0.223	0.637

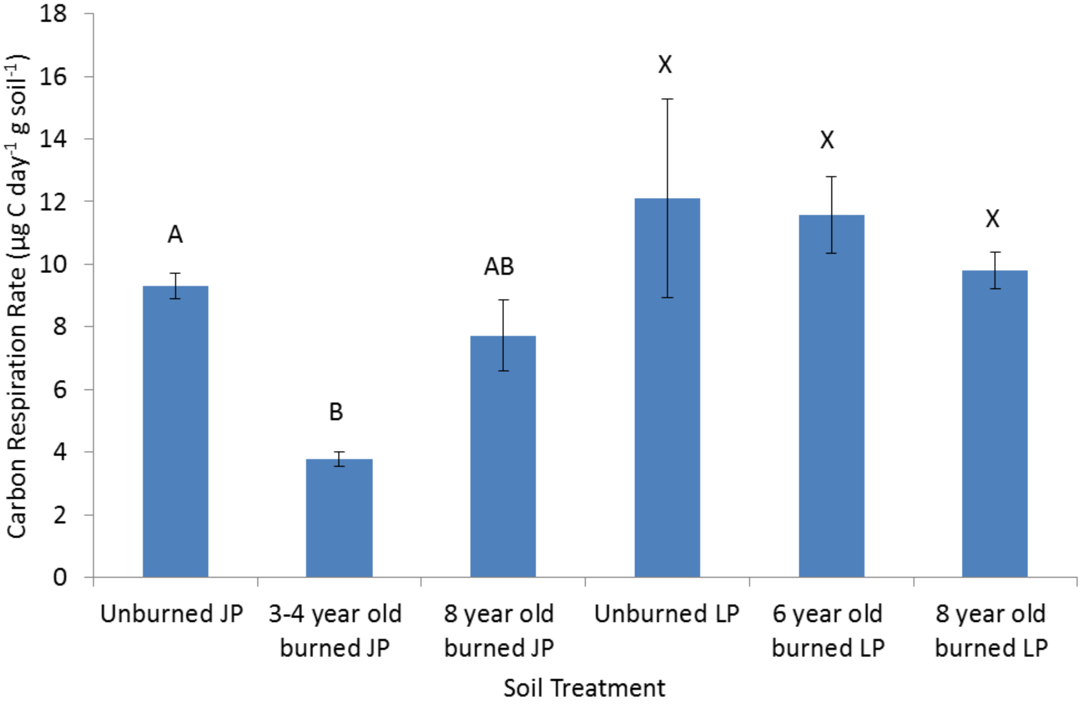

Figure 8. Mean C respiration rate expressed as µg C day^{-1} g $soil^{-1}$ as a function of soil treatment and time since burned soil experienced a pile burn. Error bars represent standard error of the mean C respiration. Capital letters denote significant differences within each soil type (JP or LP; P<0.05).

Potential Nitrification Rate

The potential nitrification rate significantly increased with time for all amendment levels. At the start of the study (day 0), we did not observe a significant difference in potential nitrification between the control and the 0.5, 1, and 2.5% amendment levels, but the control, 0.5, and 1% treatments significantly exceeded the potential nitrification rate of the 5% charcoal treatments (Table 5; Fig. 9). At day 30, nitrification potentials were similar for the 0.5, 1, and 2.5% charcoal amendment treatments and exceeded rates for the unamended soils and soils having a 5% amendment. At day 64, samples with 1, 2.5, and 5% charcoal amendment treatments exceeded the control treatment in potential nitrification rate by 87.8%.

Nitrification potential was significantly higher in LP soils than in JP soils (Table 5; Fig. 10). When potential nitrification rate was averaged over the entire incubation and across all charcoal addition levels nitrification rates in LP soils were 19.1% higher than in JP soils. By day 64, respiration rates in LP soils were 26% higher than in JP soils.

Table 5. ANOVA table of potential nitrification rate (mg N kg^{-1} day^{-1}) for amended and unamended soil treatments. Treatments and interactions with P-values < 0.05 are given in bold. Treatments and interactions with P-values between 0.05 and 0.10 are listed in italics.

Source	df	F	P
Soil Type (ST)	**1**	**16.493**	**<0.001**
Charcoal Amendment Level (CA)	1	1.964	0.166
Time (T)	**2**	**22.506**	**<0.001**
ST * CA	*1*	*3.037*	*0.086*
ST * T	**2**	**4.134**	**0.020**
CA * T	**2**	**10.604**	**<0.001**
ST * CA * T	**2**	**5.354**	**0.007**

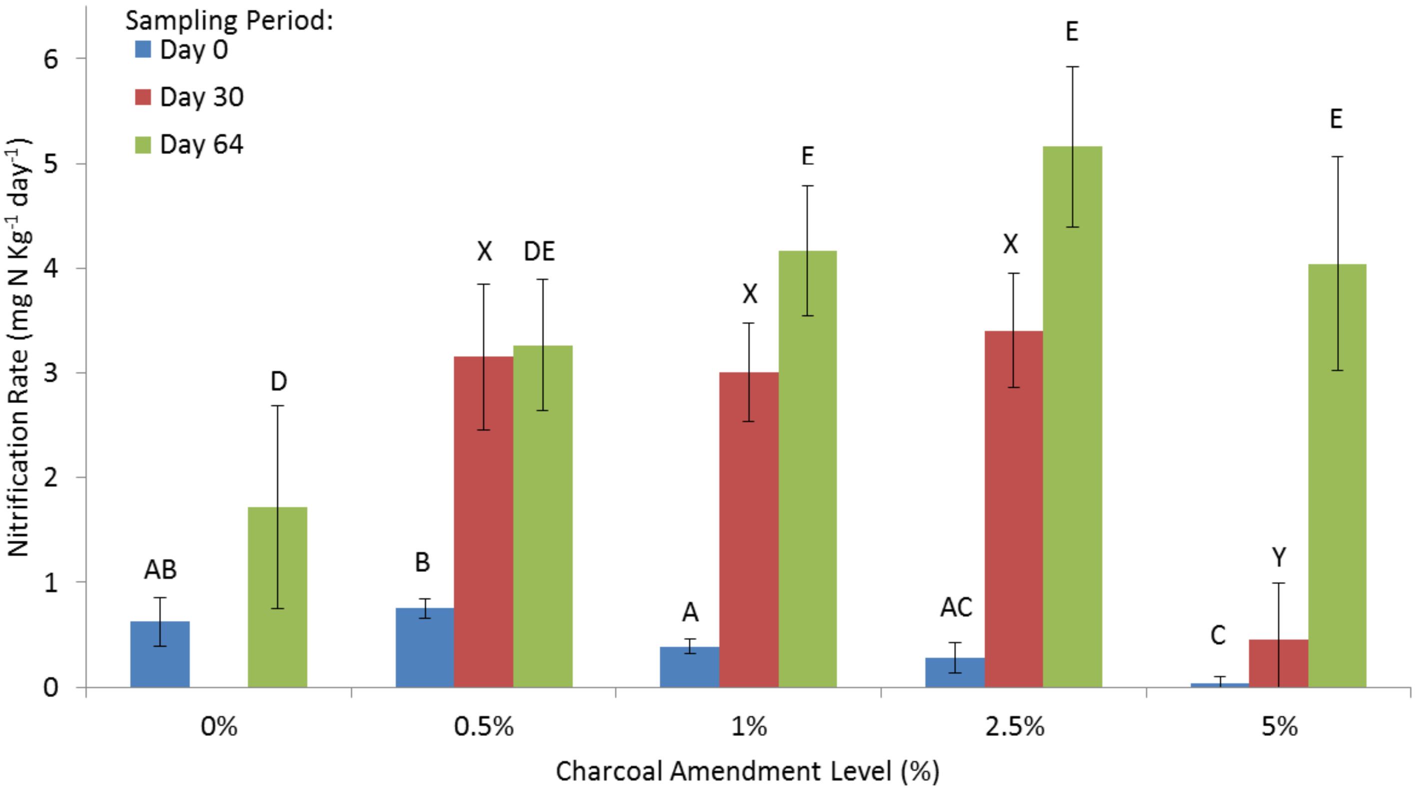

Figure 9. Potential nitrification rate (mg N kg^{-1} day^{-1}) as a function of the charcoal amendment level (% charcoal, by mass) averaged for both Jeffrey pine (JP) and lodgepole pine (LP) soil treatments across three time intervals (0, 30, and 64 days). Error bars represent standard error of the mean potential nitrification rate. Capital letters denote significant differences within each time interval (P<0.05).

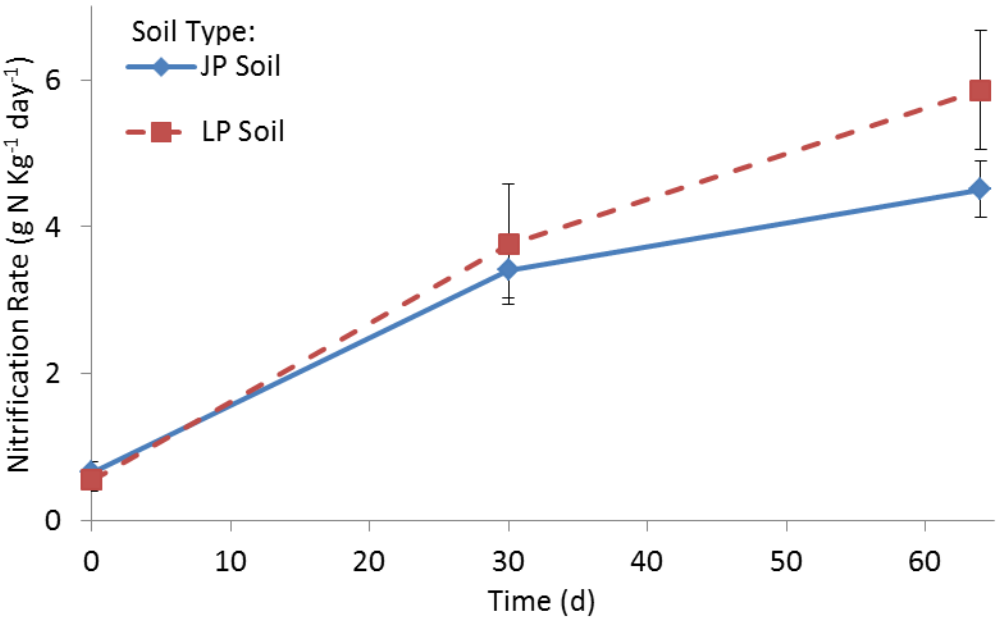

Figure 10. Mean potential nitrification rate (g N kg^{-1} day^{-1}) as a function of time for both Jeffrey pine (JP) and lodgepole pine (LP) soil treatments across three time intervals (0, 30, and 64 days). Error bars represent standard error of the mean potential nitrification rate.

Polyphenol Adsorption

Polyphenol concentrations in the soil extracts were inversely related to charcoal amendment level (Table 6); as charcoal concentration increased, polyphenol concentrations in solution decreased in both JP and LP soils (Fig. 11). The polyphenol concentration in JP soils decreased linearly with increasing charcoal amendment level (R^2=0.94). The decrease in polyphenol concentrations in LP soils showed a more curvi-linear pattern, but a linear regression was still significant. On average, charcoal amended soils experienced a 34.5% reduction in extractable polyphenolic compounds compared to the control soils.

The polyphenol concentrations were significantly greater in extracts of amended and unamended LP than JP soils (Fig. 12). Unburned soils had greater concentrations of polyphenolic substances than soils that had been subjected to prescribed burning, but this effect was only significant for LP soils (data not shown).

Table 6. ANOVA table of polyphenol concentration for amended, unamended, and naturally burned soil treatments. P-values < 0.05 are given in bold.

	Polyphenol Concentration (mg L^{-1})		
Source	df	F	P
Soil Type (ST)	**1**	**5.493**	**0.027**
Charcoal Amendment Level (CA)	**1**	**62.233**	**<0.001**
ST * CA	1	2.812	0.106

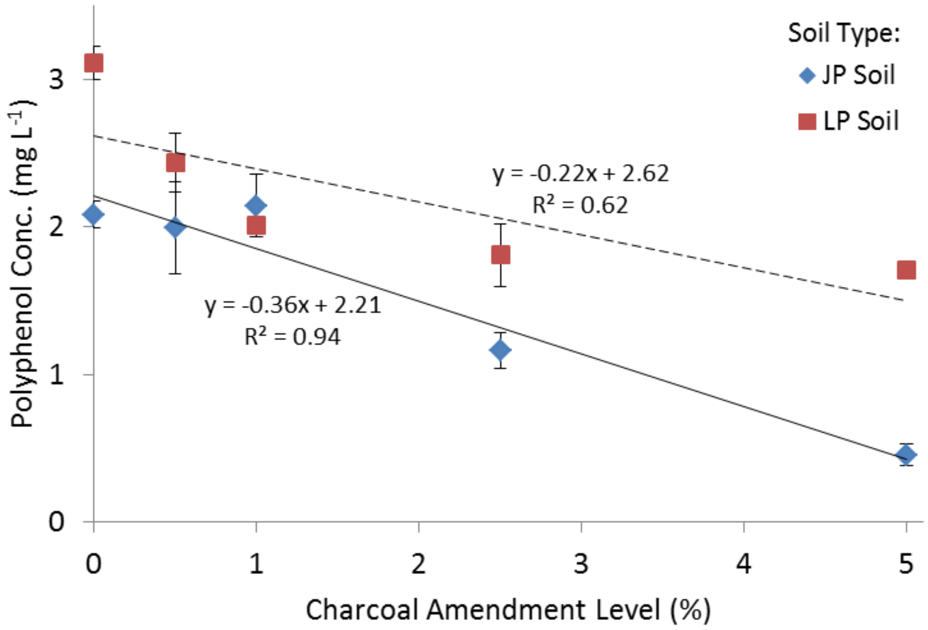

Figure 11. Polyphenol concentration (mg L^{-1}) in soil extracts as a function of charcoal amendment level (% by mass) in Jeffrey pine (JP) and lodgepole pine (LP) soils averaged across all charcoal amendment levels and charcoal types. Error bars represent standard error of the mean polyphenol concentration.

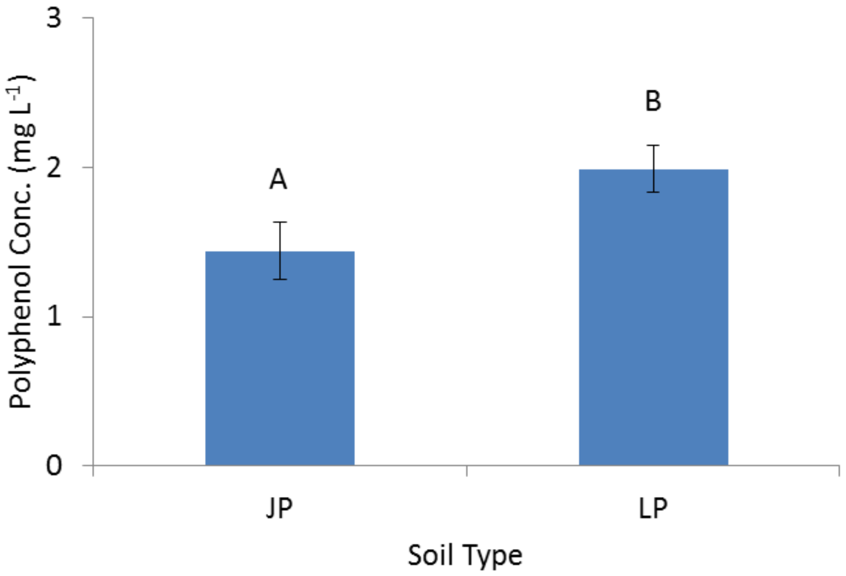

Figure 12. Polyphenol concentration (mg L^{-1}) in soil extracts as a function of soil type (JP and LP soil) averaged across all charcoal amendment levels and charcoal types. Error bars represent standard error of the mean polyphenol concentration. Capital letters denote significant differences between soil types (P<0.05).

Discussion and Conclusion

The lab incubation study showed that the addition of charcoal to unburned soils significantly stimulated microbial respiration at levels of charcoal up to 2.5%, supporting our first hypothesis. However, further increases in amounts of charcoal caused respiration rates to decline. Our results are consistent with other studies showing that microbial activity increases with the addition of charcoal/biochar in boreal and temperate systems (Wardle *et al.,* 1998; Pietikäinen *et al.,* 2000; Kolb *et al.,* 2009; Smith *et al.,* 2010). However, in contrast to our study most other studies show that respiration rates continue to increase with increasing amendment level, even when studies used similar charcoal application levels as ours (e.g., Kolb *et al.,* 2009). Temporal patterns in respiration indicated that the presence of charcoal increased the availability of labile C, since not only were average respiration rates higher, declines in rates were much slower when charcoal was added to the soil.

Several mechanisms may explain the stimulation of respiration by the addition of charcoal. First, charcoal could have a priming effect causing increased decomposition of native organic matter. This priming could be due to charcoal providing a refuge for microbes, thereby avoiding predation by larger soil fauna (Zackrisson *et al.,* 1996). The decrease in respiration at the highest level of charcoal addition could be due to a physical separation between the microbes associated with the charcoal and the soil. If we assume mineral particle density to be 2.65 g cm^{-3} and the charcoal particle density to be 0.21 g cm^{-3}, charcoal would occupy approximately 42% of the solid soil volume at the 5% application level. This could potentially decrease accessibility of the substrate due to the increased distance between microorganisms and the native organic matter.

Second, charcoal may have been able to adsorb labile C compounds that can subsequently be used as a substrate by microorganisms (Zackrisson *et al.,* 1996; Wardle *et al.,* 1998; Pietikäinen *et al.,* 2000; DeLuca *et al.,* 2002; Berglund *et al.,* 2004). Our polyphenol data indicated that indeed charcoal can adsorb organic compounds. However, polyphenol sorption increased with increasing amount of charcoal while respiration did not continue to increase. Potentially, at the 2.5% amendment level, all labile C present in the soil was adsorbed and additional amounts of charcoal did not result in additional sorption of labile C.

The decomposition of charcoal itself may have contributed to the increase in microbial respiration (e.g., Gundale and DeLuca, 2007; Smith *et al.,* 2010). If this were the case, we would have expected to see an increase in respiration with an increase in charcoal application when respiration was expressed per g total C. Instead, respiration per g of total C decreased with increasing charcoal amendment level indicating that, even if charcoal itself decomposed, it had a lower quality of C than the native organic matter in the soil and was most likely not the main reason that overall respiration rates increased.

Finally, charcoal may have affected microbial respiration by influencing other soil parameters. For instance, in our study charcoal caused an increase in pH similar to other studies (Pietikäinen *et al.,* 2000; Appendix A). Again, if microbial activity was stimulated by increases in pH alone, we should have observed a positive correlation between respiration rate and charcoal amendment level across all amendment levels. The observed pattern in respiration as a function of amendment level suggests that the protection of microbes by charcoal and/or the adsorption of organic compounds may be the most likely mechanisms explaining our observations. Still, our study did not allow for the exact assessment of each of these proposed mechanisms and further study is needed to confirm the exact mechanism responsible for the observed patterns.

Overall, all charcoal types equally stimulated microbial activity in the amended soils (Table 3). However, we cannot necessarily accept or reject our second hypothesis because differences in the chemical and physical properties between the different charcoal types used in this study were relatively small. For example, differences in cation exchange capacity were less than 5% between JP and LP branch charcoal. The only exception was LP bole charcoal having a 30% smaller surface area than the three other charcoal types (Table 3). Tracheid diameter of the wood used to generate charcoal, which has been related to the surface area of charcoal, has been reported to increase the capacity of charcoal to adsorb phenolic compounds (Gundale and DeLuca, 2006). Our findings suggest that, despite the difference in the surface area of LP bole charcoal, charcoal source (JP vs. LP, bole vs. branch) did not differ enough in physical or chemical properties to differentially stimulate microbial respiration. Our results are similar to those of Pietikäinen *et al.* (2000) who compared microbial activity in soils amended with two different charcoal types originating from *Empetrum nigrum* twigs and forest humus material to activated C and pumice and found that both charcoal types stimulated basal respiration similarly.

Microbial activity significantly varied by soil type (Table 3) with respiration rates being consistently higher in LP soils compared to JP soils (Fig. 3) when respiration was expressed as CO_2 production per g of soil C. This indicates that organic matter was more easily decomposable in LP than JP soils. However, over the duration of the incubation, respiration rates in LP soils declined faster than in JP soils suggesting that labile C was used more rapidly in LP than in JP soils. The LP sites are located in a meadow-forest interface and contained herbaceous vegetation that may have produced litter that was more easily decomposed than the pine needle litter present at the JP sites. The interaction between charcoal amendment level and soil type (Table 3) also showed that charcoal had a larger stimulatory effect in LP than in JP soils, further indicating that the presence of charcoal stimulated decomposition of native organic matter rather than decomposition of charcoal itself.

During our 64 day incubation, potential nitrification rates were equally stimulated across all charcoal amendment levels, which leads to the partial acceptance of our third hypothesis. By the eighth week of the incubation, the potential nitrification rates at the 1, 2.5, and 5% charcoal amendment level were higher than those in the unburned soils (Fig. 9), consistent with other studies that found that mineralization/ nitrification rates increase in the presence of charcoal (DeLuca *et al.,* 2002; 2006; Berglund *et al.,* 2004; Kolb *et al.,* 2009; Ball *et al.,* 2010). Potentially, charcoal could stimulate adsorption of nitrification-inhibiting substances, such as polyphenols, present in the soil solution onto the surface of charcoal. When in solution, these substances can either immobilize N through complexation or cause enzymatic inhibition of nitrification, but when removed from solution by the adsorption to charcoal, stimulation of nitrification can occur (Pietikäinen *et al.,* 2000; DeLuca *et al.,* 2006; Ball *et al.,* 2010). Extractable polyphenol concentrations significantly decreased with increasing charcoal application for both soil types (Table 6), which is in agreement with previous studies (Zackrisson *et al.,* 1996; Wardle *et al.,* 1998; Berglund *et al.,* 2004; Deluca *et al.*, 2006; Kolb *et al.,* 2009). The reduction of polyphenolic substances in solution with increasing charcoal amendment (Fig. 11) can be attributed to the sorption of these substances to the surfaces of the amended charcoal particles (Zackrisson *et al.,* 1996; DeLuca *et al.,* 2002; Gundale and DeLuca, 2007). Second, the increase in soil pH with increased charcoal application can also provide a more suitable environment for the nitrifying community which, through the oxidation of NH_4^+, creates more acidic conditions. A third mechanism responsible for increased potential nitrification rates at higher levels of charcoal application could be related to the physical protection that charcoal provides for nitrifying microorganisms within the pore spaces (Zackrisson *et al*., 1996) as discussed above. However, by the eighth week of the incubation, all levels of charcoal application equally stimulated nitrification indicating that other factors besides the three mentioned above may have contributed to the observed patterns, such as limitations in NH_4^+ supply.

Similar to the respiration data, potential nitrification rates were higher in LP than in JP soils (Table 5) indicating a higher capacity of LP soils to support nitrifying microorganisms. The higher nitrification rate between soil types was not due to lower concentrations of potentially inhibitory compounds, since polyphenol content was higher in LP than in JP control soils. Also, the difference in pH between the soil types (0.1 unit) was likely not enough to cause differences in nitrification rates (Table 1). The higher potential nitrification rates may be related to the presence of more easily decomposable organic matter, as indicated by the respiration data, potentially causing increased rates of N mineralization and thus increased NH_4^+ availability.

When comparing unamended soils with naturally burned soils, we observed that soils exposed to a pile burn 3-4 yr ago had lower respiration rates than unburned control soils, in contrast with hypothesis 4 (Table 4). Pile burns that were ignited between 6 and 8 years ago had respiration rates similar to unburned control soil. This pattern suggests that pile burned sites may have lowered microbial populations 3-4 yr after soils were exposed to a pile burn. Our data are consistent with the findings from Choromanska and DeLuca (2001) who recorded slow microbial recovery corresponding to reductions in available C and depressed respiration rates 2 yr after a fire. However, 6-8 yr following pile burn, microbial populations may have recovered in burned sites. In this study, we also observed significant differences between the soil types, specifically a greater difference in respiration rates between 2-3 yr old burned and unburned JP soils compared to LP soils (Fig. 8), indicating that more available C may have been present in LP soils compared to JP soils. The results for the natural burn appear to be in contrast with the amended soils where charcoal additions resulted in greater microbial activity. This opposing trend may be due to a variety of reasons. First, pile burns cause heat transfer into soils, and depending on the fire intensity this heating can greatly reduce microbial populations (Knicker, 2007). It is possible that after a prescribed pile burn, microbial populations are slow to recolonize burned soils. Second, fire causes combustion of native organic matter, thereby reducing available C in the burn scars. Depending on the recovery of the vegetation, this may result in long-term decreases in C availability and thus microbial activity. The piles sampled in our study had very little vegetation development even eight years after burning, so organic matter levels most likely had been reduced substantially compared to native soils. The similarities in total C content between control and burned soils may have been caused by formation of charcoal in the soils. Since our amended treatments used unburned soil as the substrate for charcoal addition, microbial populations and nutrient availability prior to amendments were largely unaltered. This is in contrast with burned soils, which were subject to nutrient and microbial population losses caused by pile burning. The addition of charcoal in unburned soil stimulated microbial activity beyond that of the controls, possibly because the habitat became more favorable for microbes with charcoal additions in combination with available C and nutrients present within soil that did not experience heating losses. Conversely, burned soils contained charcoal and ash through fire deposition, but since losses of native organic C as well as microbial populations occurred, the positive response from microbial activity seen in charcoal amended soils was absent.

In conclusion, both microbial activity and potential nitrification rates were stimulated by the addition of charcoal in incubated soils, particularly at the 2.5% amendment level, suggesting that similar mechanisms were responsible for increasing the activity of heterotrophic and autotrophic organisms. These mechanisms may include the sorptive capacity of charcoal as well as the protection of microbes against predation due to the porosity of charcoal. The presence of charcoal increased microbial activity, the potential rate of nitrification, adsorption of allelopathic substances, and soil pH. The source of charcoal had a limited effect on microbial processes mainly because properties of charcoal originating from different tree species and tree parts were modest. Soil type was important however, and the effects of charcoal were largest in soils under lodgepole pine compared to Jeffrey pine species, potentially due to differences in the quality of organic matter present in lodgepole pine soils. The effects of charcoal additions alone were different from those observed in actual pile burns, suggesting that the positive impacts of the presence of charcoal were more than offset by direct effects of heating on soil properties.

The results from our study suggest that charcoal by itself can impact soil microbial processes. Charcoal generated in pile burns could stimulate microbial activity in forest soils. While presence of charcoal may aid in restoration of burned areas if charcoal were added as an amendment to unburned soils, it could also increase decomposition of native organic matter. This may temporarily increase N availability, but can cause losses of native organic matter and N by stimulating nitrification. Even though charcoal may aid in restoration of microbial populations, the respiration data from burned soil indicate that the recovery of microbial populations in soils exposed to a pile burn may take up to six years. However, more studies are needed to understand the mechanisms by which charcoal stimulates microbial activity in soil exposed to a pile burn and to better assess implications for restoring soils and vegetation that have been exposed to pile burns.

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Appendix A: Soil pH response to charcoal addition study using a 1:2 soil to water (g g^{-1}) ratio for all combinations of soil treatment, charcoal type, and charcoal amendment level (0, 0.5, 1, 2.5, and 5% charcoal by mass).

Treatment	pH 1	pH 2	pH 3	SD
JP Control	5.99	6.03	6.04	0.026
JP 0.5% JP Branch	6.18	6.2	6.14	0.031
JP 1% JP Branch	6.23	6.21	6.2	0.015
JP 2.5% JP Branch	6.41	6.38	6.47	0.046
JP 5% JP Branch	6.55	6.57	6.59	0.020
JP 0.5% LP Branch	6.16	6.13	6.21	0.040
JP 1% LP Branch	6.18	6.18	6.2	0.012
JP 2.5% LP Branch	6.41	6.4	6.41	0.006
JP 5% LP Branch	6.5	6.54	6.5	0.023
JP 0.5% JP Bole	6.17	6.2	6.18	0.015
JP 1% JP Bole	6.34	6.3	6.3	0.023
JP 2.5% JP Bole	6.52	6.52	6.56	0.023
JP 5% JP Bole	6.77	6.79	6.75	0.020
JP 0.5% LP Bole	6.28	6.23	6.15	0.066
JP 1% LP Bole	6.33	6.29	6.32	0.021
JP 2.5% LP Bole	6.54	6.51	6.55	0.021
JP 5% LP Bole	6.79	6.79	6.76	0.017
LP 0.5% JP Branch	6.15	6.21	6.27	0.060
LP 1% JP Branch	6.31	6.29	6.33	0.020
LP 2.5% JP Branch	6.47	6.49	6.47	0.012
LP 5% JP Branch	6.7	6.69	6.72	0.015
LP Control	6.16	6.11	6.13	0.025
LP 0.5% LP Branch	6.2	6.17	6.16	0.021
LP 1% LP Branch	6.29	6.25	6.24	0.026
LP 2.5% LP Branch	6.34	6.38	6.42	0.040
LP 5% LP Branch	6.5	6.53	6.56	0.030
LP 0.5% JP Bole	6.22	6.24	6.28	0.031
LP 1% JP Bole	6.36	6.24	6.35	0.067
LP 2.5% JP Bole	6.5	6.42	6.49	0.044
LP 5% JP Bole	6.7	6.7	6.76	0.035
LP 0.5% LP Bole	6.29	6.2	6.21	0.049
LP 1% LP Bole	6.3	6.3	6.28	0.012
LP 2.5% LP Bole	6.47	6.48	6.45	0.015
LP 5% LP Bole	6.75	6.77	6.72	0.025
JP 3-4 YO Burn	8.75	8.73	8.73	0.012
JP 8 YO Burn	7.6	7.63	7.61	0.015
LP 6 YO Burn	7.34	7.33	7.35	0.010
LP 8 YO Burn	7.51	7.42	7.41	0.055

Appendix B: Corrected polyphenol concentration at varying charcoal amendment levels (0, 0.5, 1, 2.5, and 5% charcoal by mass) and in pile burned soils.

Treatment	Average Corrected Polyphenol (mg/L)	SD
JP Unburned	2.085	0.159
JP 0.5%	1.993	0.540
JP 1%	2.146	0.374
JP 2.5%	1.163	0.212
JP 5%	0.454	0.128
LP Unburned	3.114	0.196
LP 0.5%	2.436	0.342
LP 1%	2.010	0.000
LP 2.5%	1.811	0.369
LP 5%	1.707	0.032
JP Natural Burn 8YO	1.845	0.088
LP Natural Burn 8YO	2.055	0.077

CHAPTER 3

The Effects of Pile Burning on Nutrient Stocks in Eastern Sierra Nevadan Forested Soils

Zachary Carter, Robert Blank, Paul Verburg

Introduction

Fire affects numerous attributes of North American semi-arid forests including composition, health and productivity, prevention of non-native species encroachment, and landscape heterogeneity. Fire suppression has been the dominant form of forest management for over a century and has contributed to increases in fuel loads that may lead to more severe, stand-replacing wildfires. It is estimated that severe wildfires threaten more than 72 million ha of federal land in the United States (Moghaddas and Stephens, 2007). To reduce the severity of future wildfires, fuel loads can be reduced using mechanical and/or fire-based treatments. Pile burning is a commonly used technique to dispose of woody materials following mechanical treatments. While pile burning is often preferred over broadcast burning because of the ease of containment and reduced concerns about excessive smoke production compared to broadcast burning, the high temperatures achieved may cause local detrimental impacts on soils beneath the piles that can affect plant available nutrients, including nitrogen (N), phosphorus (P), and potassium (K) species (Certini, 2005; Kolb *et al*., 2009; Busse *et al.,* 2013; Hubbert *et al.,* 2015).

Nitrogen is often the most growth-limiting nutrient in coniferous forests, followed by P, and fire can play an important role in the availability of these nutrients (DeBano and Klopatek, 1988; Choromanska and DeLuca 2001; Prieto-Fernández *et al.,* 2004; Certini, 2005). The majority of soil N is present in an organic form and is contained in the O horizon and upper mineral horizon (Choromanska and DeLuca, 2001). The concentration and speciation of N in soil following a fire is a function of the type and amount of organic material oxidized by fire, degree of heating, soil depth, water content, and leaching potential (Prieto-Fernandez *et al.,* 1998; Certini, 2005). Fire typically decreases the size of the organic N pool because of the low temperature required to volatilize N (200^{o}C; Certini, 2005; Murphy *et al.,* 2006). However, inorganic forms of N including ammonium (NH_4^+) and nitrate (NO_3^-) typically increase following a fire. Pyrolysis of organic matter produces NH_4^+, through the chemical mineralization of organic material, the deposition of ash, and the lysis of soil microorganisms (Prieto-Fernandez *et al.,* 2004). Following an increase in NH_4^+, NO_3^- availability can increase in soils through the stimulation of nitrification. Following this initial pulse in inorganic N, elevated levels of inorganic N can be sustained through mineralization of N and the subsequent formation of NO_3^- through nitrification (Canfield *et al.,* 2010).

The persistence of N pulses following fire is dependent upon climatic conditions, N speciation, fire intensity and duration, and the deposition of ash and charcoal. The duration of elevated NH_4^+ availability in soils exposed to a fire is variable in the literature and has been reported to last for one to five years before returning to pre-fire levels (Choromanska and DeLuca, 2001; Mackenzie *et al.,* 2004). Increases in NO_3^- following a prescribed fire have been reported to last for up to five years before returning to pre-fire levels (Covington *et al.,* 1991).

The volatilization temperature of P is greater than N (>774^{o}C) resulting in relatively small atmospheric losses (Richter *et al*., 1982; Cade-Menun *et al.,* 2000; Murphy *et al.,* 2006). Inorganic orthophosphate (ortho-P) can increase in availability following a fire due to the mineralization of organic P and increases in pH, and typically increases with increasing burn intensity and depth of heating (Cade-Menun *et al*., 2000). Heating of soil at temperatures greater than 450^{o}C can cause a marked increase in soil pH due to denaturing of organic acids and the input of bases into soils (Certini, 2005). Biological P availability is highly dependent on pH, so depending on the pH change following a pile burn, P availability can change; at pH levels below 6.5, P can sorb to sesquioxide surfaces, while at higher pH levels P may precipitate as insoluble Ca-phosphates. (Romanya *et al.,* 1994; Cade-Menun *et al.,* 2000; Certini, 2005; Johnson *et al.,* 2011). The duration of elevated P levels can vary between 45 days to ten years following a fire (DeBano and Klopatek, 1988; Cade-Menun *et al.,* 2000). Still, P availability is determined by biological and geochemical processes, both of which are affected by fire, making prediction of the long-term patterns in P availability challenging.

Similar to P, only a small portion of K in burned organic matter is released to the atmosphere because of the relatively high volatilization temperature of K (>760^{o}C) (Richter *et al.,* 1982; Murphy *et al.,* 2006). Base cations, such as Ca^{2+}, Mg^{2+}, and K^{+}, are deposited in ash on the forest floor after burning (Certini, 2005) and K becomes biologically available once ash is assimilated in soils (Johnson *et al.,* 2011). Since K is highly soluble, K may be lost from the soil due to leaching (Richter *et al.,* 1982). The persistence of increased K levels following a fire is relatively short and has been recorded to last between three to six months, after which elevated concentrations decreased to background levels (Adams and Boyle, 1980; Kutiel and Inbar, 1993).

The effects of fire on N, P, and K depend on duration and intensity of a fire. Burn conditions within pile burns can vary due to the distribution of fuels within a pile. For instance, Jiménez Esquilín *et al*. (2007) found that soil in the center of a pile burn were exposed to greater temperatures (300^{o}C) over a longer period of time than soil at the edge of a pile burn (175^{o}C), but soil temperatures may be up to 600^{o}C in the center of burn piles depending on fuel loads (Busse *et al.,* 2013). Consequently, the effects of a pile burn on short- and long-term nutrient availability are likely to vary as a function of position within a pile burn.

Our study follows up on research conducted by Johnson *et al.* (2011), who studied nutrient dynamics of pile burns located in the Whittell forest of Little Valley, Nevada. Burn scars in Johnson *et al.* (2011) ranged in age between less than three months to two years. Johnson *et al.* (2011) found that soil pH increased following pile burning in response to ash incorporation into burned soils. Using ion exchange resins they also found that NO_3^- was the dominant inorganic N species, and that NO_3^- concentrations were five times greater in young burn scars located in meadows compared to young upland burn scars. Orthophosphate concentrations were greatest in the center of young meadow pile burns and old upland pile burns but lowest in the center of young upland pile burns, which was attributed to P precipitating as Ca-phosphate or binding to sesquioxide surfaces. Finally, K^+ was highest in the center of pile burns located in meadows, but no trends existed with regard to position in upland pile burns regardless of age.

The objective of this research is to understand the long-term effects of pile burning on N, P, and K availability in forest soils. We attempted to observe the long-term effects of pile burning on soil nutrients by sampling pile burns similar to the piles sampled by Johnson *et al*. (2011) that were 3-4, 6, and 8 years old. We also sampled at varying distances away from the pile burn centers to observe if the position within a burn pile affects nutrient availability, given that heating temperature typically decreases with increasing distance from the center of the pile (Jiménez Esquilín *et al.,* 2007; Busse *et al.,* 2013). In our study, we tested the following hypotheses:

H1: The age of the pile burn will significantly affect the concentration of N, P, and K. We expect that the concentration of each nutrient species will decrease with increasing pile burn age. Decreased N and K availability may be caused by leaching because of the high solubility of both N and K, while decreased P availability may be caused by precipitation as insoluble Ca-phosphates.

H2: The position within the pile burn will significantly affect the concentration of N, P, and K. We expect that concentrations of each nutrient species will decrease with increasing distance from the center of the pile burn because of the higher burn temperatures in the center of the pile burn compared to the edge of a pile burn. Higher burn temperatures will increase N, P, and K concentrations in the center of a pile burn through greater chemical mineralization of organic material, ash deposition, and soil pH in the center of a pile burn.

Methods

Site Description

The study area is located in Little Valley, Nevada, approximately 30 km south of Reno, Nevada. Little Valley is part of the Carson Range and elevation ranges from 1900 to 2500 m. The overstory vegetation is dominated by Jeffrey pine (*Pinus jeffreyi*) and lodgepole pine (*P. contorta*), while the understory is dominated by bitterbrush (*Purshia tridentata*) and manzanita (*Arctostaphylos patula*). The climate is semi-arid characterized by a dry and warm summer and a cold wet winter. The mean annual precipitation is 87.5 cm and the mean annual air temperature is 5^{o}C. Approximately half of the precipitation in Little Valley occurs as snow, with snowmelt being the dominant hydrologic event (Johnson *et al.,* 2011; http://environment.unr.edu/whittell/ climate/index.html).

Burn piles were selected based on the dominant overstory vegetation and pile burn age (referenced from the Whittell Forest Fuel Reduction and Ecosystem Enhancement Plan). The dominant overstory vegetation closest to the pile burns consisted of either Jeffrey pine (referred to as JP) or lodgepole pine (referred to as LP) tree species. Burn piles with a JP overstory were located on areas having a 10-15% slope with a sparse understory consisting of manzanita and bitterbrush. Soils belonged to the Marla series (loamy sand, mixed, frigid Aquic Dystroxerepts; USDA, 2014). Burn piles with a LP overstory were located in a meadow with LP encroaching. These areas had slopes of less than 5% and soils belonged to the Marla series (loamy sand, mixed, frigid Aquic Dystroxerepts) and Toiyabe series (Mixed, frigid, shallow Typic Xeropsamments; USDA, 2014). A total of three piles per vegetation type/age were selected. Pile burns were separated into three age classes representing the time since a pile burn was ignited: (1) “old” JP and LP ignited in late May 2008; (2) “intermediate” LP ignited in February 2010; (3) “young” JP estimated to have been ignited autumn of 2012 (S. VanderWall, personal communication). Three pile burns were selected for each vegetation type/age combination for a total of 12 pile burns. Pile burn scars were on average 1.8-2.5 m in diameter and vegetation was absent even in the older burn scars.

Nutrient Availability

Nutrient availability was monitored using Plant Root Simulators™ (PRS; Western Ag Innovations Inc., Saskatoon, Saskatchewan, Canada). These probes consist of either a positively- or negatively-charged ion exchange resin. In each burn pile, we established three transects of four sampling points. One positively- and negatively-charged probe was inserted into the mineral soil, 2-5 cm apart to a depth of 10 cm at four points along each transect (center [position 1], midway [position 2], edge of burn pile [position 3], and outside of the burn pile [position 4]; Fig. 1; Bengston *et al.*, 2007; Switzer *et al.,* 2012). During the fall season, probes were installed on 12 August 2015 and retrieved on 22 October 2015, so the probes were in the soil for a total of nine weeks. During the winter/spring season, probes were installed on 6 December 2015 and retrieved on 13 May 2016 for a total incubation period of 23 weeks. Following each monitoring period, the PRS probes were cleaned and sent to Western Ag Innovations for nutrient analysis. Probes were extracted with 17.5 mL of 0.5 N HCl in zip lock bags for 1 hour followed by NH_4^+-N and NO_3^--N analysis using a Technicon Autoanalyzer II (TIC 1977), while the remaining nutrients were analyzed using inductively coupled plasma (ICP) emission spectroscopy (Perkin Elmer Optima 3000-DV ICP). Ammonium was at or below detection limits for most resin stakes during each incubation period, so NH_4^+ data will not be reported. Nutrient availability units were reported as mg m^{-2} burial $period^{-1}$, where the burial periods for the fall and winter/spring incubations are nine and 23 weeks respectively.

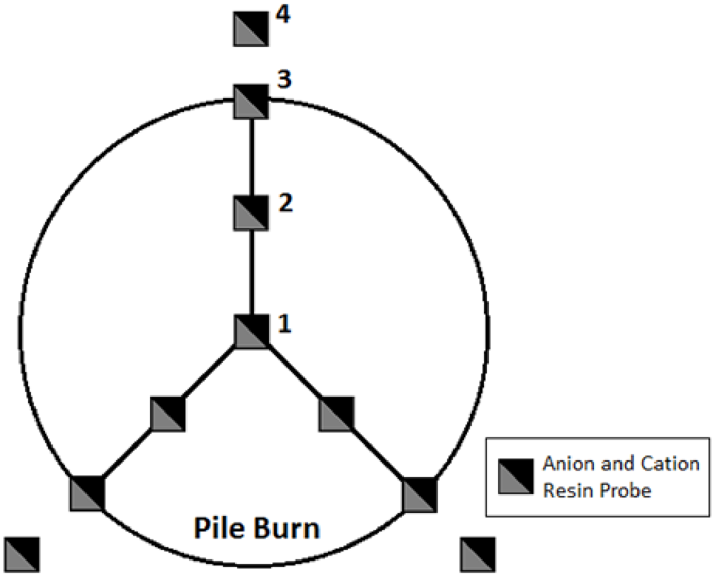

Figure 1. Sampling design for in-situ ionic resin installation. The numbers indicate the different sampling locations used in this study. Samples were combined for each location.

Soil and Climatological Measurements

Precipitation, temperature, and snow depth were recorded at a nearby SNOTEL site (site number 1242; wcc.sc.egov.usda.gov). During the fall incubation, total precipitation was 4.8 cm and the mean air temperature was 14°C. During the winter/spring incubation total precipitation was 68 cm and mean air temperature was 2.9°C and maximum snow depth was 73.7 cm. During the winter/spring incubation PRS probes were removed 43 d after the winter snow pack snow had entirely melted, during which 9 cm of precipitation occurred.

Soil Characteristics

To measure soil C and N concentrations, soil from the center of pile burns and unburned control soil was ground for 48 hours using a Mavco vial rotator. Total C and N concentrations were measured using a Leco Truspec CN. Soil pH was measured using a 1:2 dilution of soil to water (g g^{-1}). Five grams of soil and 10 mL of deionized water were added to centrifuge tubes, shaken and allowed to settle for an hour. The soil slurry was stirred with an electrode and the measurement was taken after the pH had stabilized. All C, N, and pH measurements were performed with three replicates (n=3). Cation exchange capacity (CEC) analyses were performed using the ammonium saturation method at the Waters Agricultural Laboratories, Inc., Georgia, USA. Only a single measurement of CEC was recorded for each treatment (n=1).

Data Analysis

All statistical analyses were conducted with DataDesk V6.3 using an analysis of covariance (ANCOVA) with relative pile burn age, position within the burn pile, and vegetation type as independent variables. Age was treated as a continuous variable, while location and vegetation were considered discrete variables. Statistical analysis of nutrient availability was not compared between the two installation periods because of the differences in the duration of the incubation periods. Nutrient concentration was averaged for each position within each pile and the pile was our experimental unit resulting in an n=3 for our statistical analyses. The NO_3^--N, P, and K concentrations were log transformed to ensure a normal distribution of the data. Probability levels of ≤ 0.05 were assumed to be significant. Post hoc tests to determine significant differences between treatments were conducted using a least significance difference (LSD) approach.

Results

Soil Characteristics

Unburned soils were slightly acidic and had an average pH of 6.08. Soil pH tended to be higher in burned than in unburned soils. The highest pH occurred in the most recent pile burns. Total C was higher in LP than in JP control soils (Table 1).

Table 1. Chemical properties of the soils in burn scars sampled in the PRS incubation study. Soils are categorized by dominant overstory vegetation type (JP or LP). Burned soils have been further categorized by relative pile burn age ('younger'; 3-4 years old, 'intermediate'; 6 years old, and 'older'; 8 years old). Three replicates were used for soil pH, total C, and total N. CEC was measured on one sample.

Veg. Type	pH	Total C %	Total N %	CEC meq $100g^{-1}$
JP Control	6.02±0.02	1.59±0.02	0.06±0.002	9.8
LP Control	6.13±0.01	2.24±0.21	0.08±0.006	11.2
JP Older Burn	7.61±0.01	2.05±0.04	0.04±0.004	10.9
LP Older Burn	7.45±0.03	1.84±0.04	0.06±0.005	11.2
JP Younger Burn	8.74±0.01	2.15±0.04	0.08±0.001	-
LP Intermediate Burn	7.34±0.01	2.12±0.03	0.07±0.003	-

Fall Season

During the fall season, burn age was the only significant factor affecting NO_3^-, ortho-P, and K^+ adsorbed to PRS probes (Table 2). Ammonium was at or below detection limits for most resin probes during the fall installation period and is not reported in the results (N is reported as NO_3^-- N in the figures). Nitrate in the younger and intermediate age burns was significantly greater than in the older burns with NO_3^- being more than twice as high in the intermediate compared to the oldest burn (Fig. 2a). Both PRS-P and K significantly decreased with burn age. Potassium levels in younger burns were nearly four-times higher than in intermediate age burns. Potassium levels were 45% higher in the intermediate than in older pile burn ages. Phosphorus tended to decrease linearly with increasing age and ln(P) decreased by on average a 44% between each increase in burn age.

Overall, location within the piles did not affect N, P, and K but we observed a significant interaction between burn age and position within each pile for K during the fall season (Table 2). Potassium in the center position of younger burns was on average 1760 mg m^{-2} burial $period^{-1}$ and decreased with increasing distance from the center of the pile burn. An opposite trend in K occurred however in intermediate and older burns. Potassium in the center position averaged 160 mg m^{-2} burial $period^{-1}$ and increased with increasing distance from the center of the burn pile, while K in soils outside of the burn scars averaged 345 mg m^{-2} burial $period^{-1}$.

Table 2. ANOVA table of the log-transformed NO_3^--N, K^+, and ortho-P during the fall season. Treatments and interactions with P-values < 0.05 are given in bold.

		Ln (NO_3^--N)		Ln (K^+)		Ln (P)	
Source	df	F	P	F	P	F	P
Veg. Type	1	0.226	0.638	1.688	0.203	0.819	0.372
Burn Pile Age	**2**	**5.261**	**0.011**	**43.847**	**<0.001**	**12.461**	**<0.001**
Burn Pile Position	3	0.177	0.911	0.167	0.918	2.140	0.115
Veg. Type * Age	-	-	-	-	-	-	-
Veg. Type * Position	3	0.138	0.937	0.245	0.864	0.797	0.505
Age * Position	6	0.436	0.850	**3.030**	**0.018**	0.889	0.514

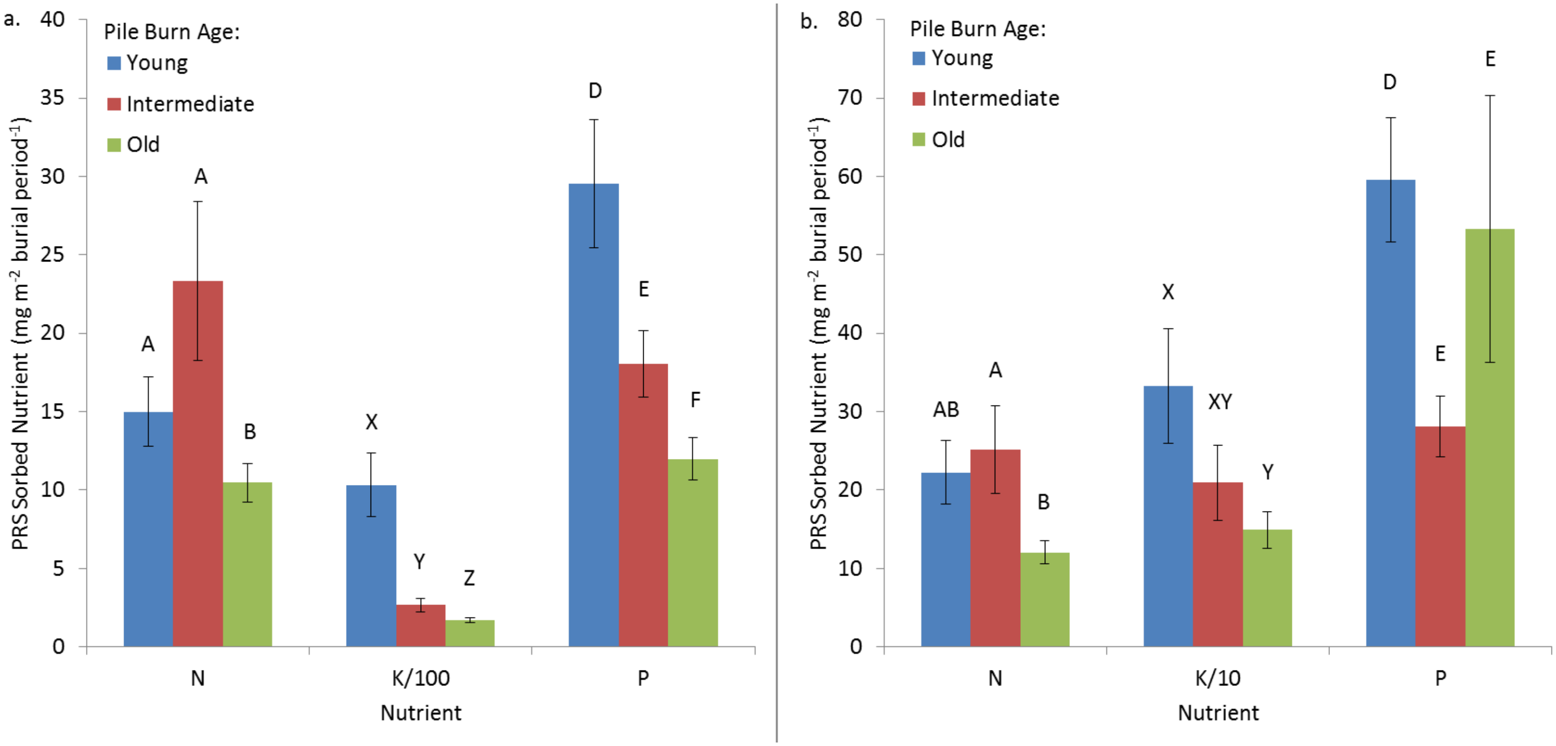

Figure 2. (a) PRS-sorbed NO_3^--N, K^+, and ortho-P for the fall season and **(b)** for the winter/spring season. Values for K^+ have been divided by 100 during the fall season and 10 during the winter/spring season. Error bars represent standard error of the mean using untransformed data. Capital letters indicate significant differences between pile burn ages, specific for each nutrient species (P<0.05).

Winter/Spring Season

During the winter/spring season the age of a pile burn significantly affected P and tended to be significant for NO_3^--N and K (Table 3). Sorbed NO_3^- showed similar trends during the winter/spring season as during the fall season, where the highest levels occurred in the intermediate age burns. The NO_3^- levels in the intermediate age burns were significantly greater than in the older burns, but NO_3^- in the younger burns was not significantly different from the intermediate and older burns (Fig. 2b).

Winter/spring trends in PRS-K as a function of burn age were also similar to trends observed in the fall season. The highest K levels were found in the most recent pile burns and decreased with increasing, decreasing by about 40% between each age class (Fig. 2b).

Temporal trends in P during the winter/spring season were different from the fall season. The highest levels of PRS-P occurred in the most recent burns, followed by the older burns and intermediate age burns. Phosphorous values in the younger and older burn ages appear to have similar levels of nutrient availability (Fig. 2b), however statistical analyses, including post-hoc tests, were conducted with the log transformed data, causing P levels in the younger pile burn to be significantly greater than in intermediate and older pile burns even though untransformed data do not appear to support these trends (Fig. 2b).

During the winter/spring season, burn pile position significantly affected PRS sorbed K and P (Table 3). Potassium levels increased exponentially with increasing distance from the center of the burn pile and K outside the pile burn was more than 3.5 times greater than in the center of the pile burn (Fig. 3). Phosphorus levels decreased exponentially with increasing distance from the burn center with P in the center of the burn being more than three times higher than P found outside the burn scar (Fig. 3). Vegetation type also significantly affected K during the winter/spring season (Table 3) with levels being 55% higher in the JP soils than in the LP soils.

Table 3. ANOVA table of the log-transformed NO_3^--N, K^+, and ortho-P during the winter and spring season Treatments and interactions with P-values < 0.05 are given in bold. Treatments and interactions with P-values between 0.05 and 0.10 are listed in italics.

		Ln (NO_3^--N)		Ln (K^+)		Ln (P)	
Source	df	F	P	F	P	F	P
Veg. Type	1	1.652	0.208	**7.276**	**0.011**	1.923	0.175
Burn Pile Age	*2*	*2.916*	*0.069*	*2.735*	*0.080*	**4.491**	**0.019**
Burn Pile Position	3	0.347	0.791	**5.651**	**0.003**	**2.897**	**0.050**
Veg. Type * Age	-	-	-	-	-	-	-
Veg. Type * Position	3	0.169	0.916	0.963	0.422	1.018	0.398
Age * Position	6	1.239	0.313	1.540	0.197	1.036	0.420

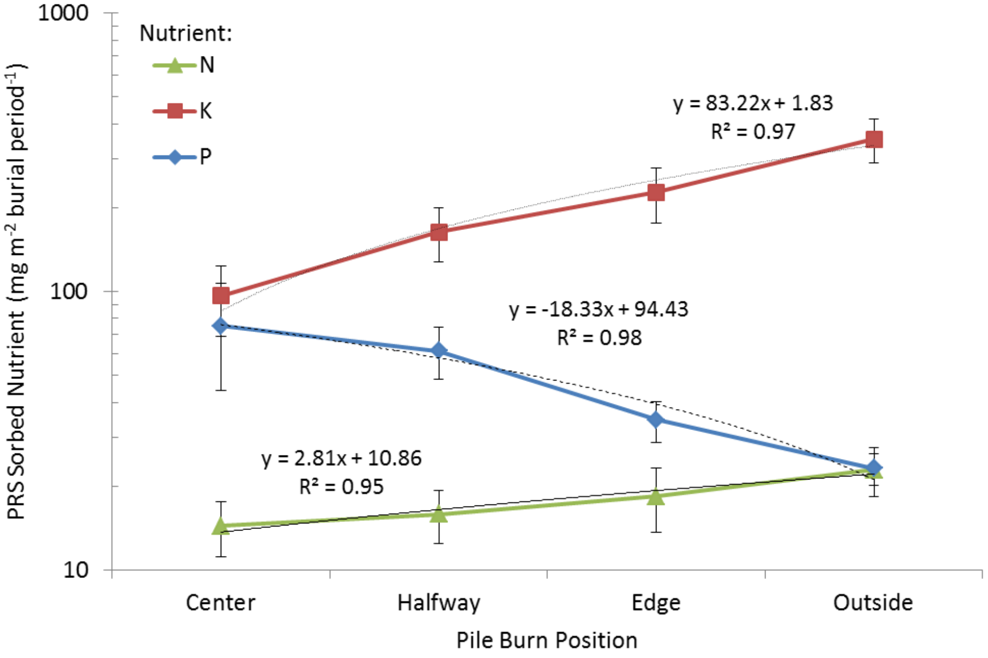

Figure 3. Winter/spring season PRS-sorbed NO_3^--N, K^+, and ortho-P as a function of position within a pile burn. Error bars represent standard error of the mean. Regressions were carried out on log-transformed data.

Discussion and Conclusion

Trends in PRS-sorbed nutrients as a function of pile burn age were similar for both incubation periods, but absolute N and P levels tended to increase from the fall to the winter/spring seasons, most likely as a result of a longer incubation period. Potassium decreased between the two seasons, which may have been due to leaching during snowmelt in combination with lower mineralization rates of K compared to N and P under snowpack (Williams *et al.,* 1996).

In support of our first hypothesis, nutrient availability for N, P, and K tended to decrease with increasing pile burn age. Nitrate was the dominant form of N sorbed to PRS probes. The predominance of NO_3^--N is similar to the findings in Johnson *et al.*

(2011). The greater NO_3^--N presence relative to NH_4^+-N may be partly attributed to NO_3^- moving onto resins by mass flow, whereas NH_4^+ is more strongly adsorbed to clay particles and moves onto resins through diffusion (DeLuca *et al.,* 2002). Nitrate levels were highest in burns that were between three and six years old, but declined in eight year old pile burns. Our results are similar to Covington and Sackett (1986) who found that fire-induced increases in mineral N declined after four to five years following prescribed burns in Ponderosa pine forests. The initial pulse of NH_4^+ created by fire potentially increased the activity of nitrifiers leading to increased NO_3^- that was subsequently leached from the soil. It was unlikely that the decrease in NO_3^- was due to uptake by the vegetation since no vegetation was present in any of the burn scars.

Similar to NO_3^-, older pile burns had significantly lower levels of K compared to younger pile burns, which may be due to the incorporation of base cations into soils from ash in younger burns followed by leaching to deeper horizons. Previous studies suggest that increases in base cation concentrations are the result of fire-induced mineralization of organic matter (Certini, 2005). Increased K concentrations have been observed to be relatively short-lived and return to background levels in three to six months following a fire as a result of cation leaching or retention onto secondary silicate minerals (Adams and Boyle, 1980; Kutiel and Inbar, 1993). The significantly greater PRS-K found in JP soils compared to LP soils may be related to the 52% higher initial K levels found in unburned JP soils and may be due to differences in litter quality between vegetation occupying JP and LP areas.

Similar to N and K, P levels decreased with increasing time, but intermediate and older pile burns had similar P levels during the winter/spring measurement period. Phosphorus availability in burned soils has been documented to be more persistent than N and K, which can be attributed to geochemical processes in addition to biological processes controlling P availability. Initially, soil pH increased following the fire potentially due to ash incorporation (Table 1), thereby affecting ortho-P availability in addition to the conversion of organic P to ortho-P upon combustion of organic matter (Certini, 2005). Over time, ortho-P availability may have decreased as it precipitated as Ca-phosphates (Certini, 2005). Potentially P could be lost due to erosion of P contained in ash (DeBano and Klopatek, 1988) or assimilation by undamaged roots under pile burns. While these last two mechanisms may occur, we found little evidence of surface and wind erosion. In addition, burn scars were devoid of vegetation so it is unlikely that P uptake by plants in the surface soil was important in reducing surface soil P levels.

Overall, position within the pile burn did not significantly affect NO_3^- availability, but K levels significantly increased with increasing distance from the pile burn center during the winter/spring season, rejecting our second hypothesis for both N and K. In addition, N and K levels were lower, albeit not significantly for N, in the center of the burn scar compared to the unburned soils. Burn severity is likely to be higher in the center of a pile burn and may cause increased NH_4^+-N and K levels compared to the edges of piles (Jiménez Esquilín *et al.,* 2007; Busse *et al.,* 2013). Our research shows that three to eight years after burning, N and K in the center of a pile burn are lower than outside of the pile burn indicating continued losses of N and K, which may be caused by leaching (N and K) or fixation (K) onto secondary silicate mineral surfaces (Adams and Boyle, 1980). The decrease in N and K levels below those observed in unburned soils further suggest that N and K losses were not compensated for by new supplies through mineralization. This may be due to the absence of litter inputs and/or larger amounts of soil organic matter combusted in the center of the pile limiting N and K mineralization. Johnson *et al.* (2011) found pile burning had no effect on PRS-N. Johnson *et al.* (2011) also found that pile burns that took place less than one year prior to sampling increased PRS-K in the center of burns located in meadows, but two years after pile burning, K levels in the center of burns were similar to unburned soil. Our results suggest that N and K levels continue to decrease to levels below that of unburned soils.

In contrast to N and K, during the winter/spring period P availability decreased with increasing distance from the pile burn center and overall was higher than in the unburned soil, in support of our second hypothesis. Our data are consistent with the results from Johnson *et al.* (2011) who found decreasing ortho-P levels with increasing distance from the center in two year old upland burns and <1 year old meadow burns. Also in contrast to N and K, P levels inside the burn scars remained consistently higher than in the unburned areas, again pointing at different mechanisms controlling P availability compared to N and K. Increased P availability in the center of a pile burn can be attributed to greater temperatures reached in the center, resulting in increased P mineralization and soil pH compared to areas further removed from the center

(Romanya *et al.,* 1994; Cade-Menun *et al.,* 2000; Certini 2005). Retention of P following the initial increase may have been due to sorption of P to mineral surfaces and/or precipitation of Ca-phosphates.

In conclusion, prescribed pile burning has the ability to alter the availability of N, P, and K in soils. Previous studies have shown that the period of enhanced availability for each of these nutrients is variable, ranging from a few months in the case of K (Adams and Boyle, 1980; Kutiel and Inbar, 1993) to ten years as in the case of P (Cade-Menun *et al.,* 2000). The data from our study is consistent with other studies showing that nutrient availability tends to decline with increasing age of a pile burn. Our research indicates that N levels were highest in intermediate aged burns and K levels were highest in younger burns, but overall, both N and K concentrations decreased over time. In addition, while initially N and K levels may be highest in the areas that were subjected to the highest burn temperature (e.g. Johnson *et al.,* 2011), in the long term, these also appear to be the areas from which most N and K is lost, resulting in levels that are even below unburned, background levels. The observed patterns may be due to a combination of high leaching and absence of resupplying available N and K through mineralization. Phosphorus levels were highest in younger burns and remained elevated beyond unburned controls particularly in the center of burn scars that were exposed to the highest temperatures. Our study indicates that P availability increases in pile burns up to eight years following a fire which can potentially enhance biological availability over a sustained period of time. Still, our results indicate that particularly N and K availability decreases in surface soils exposed to a pile burn and may limit regrowth of vegetation and restoration of burn scars for years after a pile burn. It is unclear if our results have implications for permanent nutrient losses as our research focused on surface soils. Future research should assess the movement of nutrients deeper into the soil profile as well as monitoring any nutrient enrichment of neighboring vegetation that would allow for better insight into the fate of a fire-generated nutrient pulse in surface soils.

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Chapter 4: General Conclusions

Our laboratory studies showed that the addition of charcoal in unburned soils significantly stimulated microbial activity. Our research also showed that the stimulatory effect of charcoal on microbial respiration was greatest at an intermediate charcoal application level of 2.5% compared to other levels of charcoal application (0.5, 1, and 5% charcoal by mass of soil). This level is comparable to charcoal concentrations found in pile burn scars at the field site. Our results differ from previous work since there appeared to be an optimum amendment level, whereas other studies showed that microbial activity typically continues to increase with increasing charcoal application when comparable application rates were used (Kolb *et al.,* 2009). Several mechanisms could explain our results. First, charcoal may cause a priming effect on soil leading to an increase in the decomposition of native organic matter. The priming effect may be caused by the porous structure of charcoal protecting soil microorganisms against larger predatory soil fauna (Zackrisson *et al.,* 1996; Pietikäinen *et al.,* 2000; Gundale and DeLuca, 2006), thereby increasing the rate of organic matter decomposition. The decline in respiration at the highest amendment level may be due to the large volume occupied by the charcoal (almost 50%), physically separating the microorganisms from native soil organic matter. Second, the increase in respiration may have been caused by the adsorption of labile organic compounds to the charcoal surfaces that can be used as a C source for microorganisms. However, this mechanism does not explain the decrease in respiration at higher application levels. Our polyphenol sorption data indicated that adsorption continues to increase with increasing charcoal application, whereas microbial respiration declines at the highest charcoal application levels. Potentially, all labile soil C was adsorbed at the 2.5% amendment level, so increased amounts of charcoal would not result in additional labile C sorption. Again, the decline in respiration may have been caused by physical separation between the substrate and microorganisms. Still, our research did not allow for assessment of the exact mechanisms responsible for the observed patterns, so more studies are needed specifically focusing on these processes.

Both tree species (Jeffrey pine and lodgepole pine) as well as tree component (branch and bole) used as the source for charcoal equally stimulated microbial activity in amended soils which may be explained by the relatively similar physical and chemical properties of the different charcoal types used in this study. Even though the adsorption of organic compounds to charcoal surfaces may be regulated by the surface area of charcoal particles (Gundale and DeLuca, 2006), the 30% less surface area of LP bole-derived charcoal did not appear to be enough to differentially stimulate microbial respiration compared to the other charcoal types. This indicates that perhaps other properties, aside from surface area and porosity, regulate the responses of soil microorganisms to charcoal additions.

Microbial activity significantly varied as a function of vegetation type where microbial respiration was higher in lodgepole pine than in Jeffrey pine soil when respiration was expressed per unit organic matter, indicating that lodgepole pine soils contained more labile C. The understory of lodgepole pine sites was dominated by herbaceous vegetation while little understory vegetation was present at the Jeffrey pine sites. Potentially, the litter from the herbaceous vegetation was more easily decomposable causing labile C pools to be larger in lodgepole than in Jeffrey pine soils.

Soils that were burned three years prior to sampling had lower microbial respiration rates than unburned soils, while respiration rates in soils that were burned between six and eight years ago were similar to unburned soil. We initially hypothesized that microbial activity would be greater in burned soils than unburned soils because of a more favorable habitat for soil microorganisms following pile burning due to the presence of charcoal, yet that was not the case. Initially, heat transferred into soils during a pile burn may have reduced microbial populations (Knicker, 2007) and it may have taken up to eight years for microbial populations to recover. In addition, a substantial amount of the native organic matter may have been lost during the burn, further depressing microbial recovery. In contrast, in the amended soils both microbial populations and native organic matter were still present, indicating that in natural burns the negative impacts of fire outweigh the potentially positive direct impacts of charcoal on microbial activity.

Similar to microbial respiration, charcoal amendments also significantly increased potential nitrification rates. Nitrification rates increased during the incubation and after eight weeks potential nitrification was highest at the 1, 2.5, and 5% charcoal amendment levels. The addition of charcoal could stimulate the nitrifying community in several ways. First, charcoal can adsorb organic compounds including nitrification-inhibiting and N-complexing organic compounds, such as polyphenols, thereby increasing the concentration of freely available NH_4^+. Second, soil pH increased with increasing levels of charcoal application, thus creating a more favorable habitat for nitrifying microorganisms. Finally, charcoal may provide pore spaces that protect nitrifying microorganisms from predation, similar to heterotrophic organisms, which may allow for increases of potential nitrification rates at higher charcoal application levels. However, further studies are needed to elucidate the exact mechanism(s) responsible for the observed patterns.

Our field study showed that nutrient availability following pile burning differed between nutrient species. In all piles inorganic N and K levels were lower in burned than in unburned soils and levels decreased with increasing burn age. The initial fire-generated N and K pulse can increase the short-term availability of both nutrients but the lack of vegetation inside the burn scars, and thus the supply or fresh organic matter, may have caused the system to be depleted of N and K as these nutrients continued to be mineralized and ultimately leached from the surface soils. The N and K concentrations increased with increasing distance from the center of the pile, most likely due to the increased combustion of organic matter and higher N and K losses associated with higher temperatures and subsequent volatilization/leaching.

Orthophosphate also tended to decrease with increasing pile burn age similar to N and K but in contrast to N and K, ortho-P levels were higher in burned than unburned soil. Changes in soil pH due to denaturing of organic acids and deposition of ash and charcoal (Certini, 2005; Johnson *et al.*, 2011) may have increased the availability of P. Availability of P usually decreases over time when P binds to Al-, Fe-, and Mn-oxides at low pH or precipitates as Ca-phosphates at high pH (Certini, 2005). Still, soil pH inside the burn area decreased with increasing age to a level where P would actually be expected to become more available. Potentially, P is increasingly being immobilized by microbial biomass as microbial communities rebound from the effects of a pile burn. Overall, our research indicates that long-term losses of N and K can occur in pile burns, but available P levels can remain elevated within burned soils eight years after a pile burn.

Implications

From the lab study we observed that the use of charcoal as a soil amendment positively influences heterotrophic and autotrophic (nitrifier) soil microbial activity. However, the positive effects of charcoal additions in the lab incubation study differed from field observations, suggesting that overall pile burning negatively affects microbial processes in soil exposed to a pile burn for up to eight years following a burn. While the presence of charcoal in forest soils may stimulate microbial activity, other effects of pile burning, such as the loss of microbial biomass through heat transfer and loss of native organic matter, appear to outweigh the positive effects of charcoal on the microbial community, and it may take years for soils exposed to a pile burn to recover to pre-burn conditions.

While previous studies have shown that pile burning can increase the short-term availability of nutrients such as N, P, and K, our research further indicated that sustained losses in N and K can occur in pile burns greater than three years old. Pile burning may locally benefit vegetation surrounding burned areas by increasing available nutrients generated either directly or indirectly by fire (Covington and Sackett, 1992; Johnson *et al.,* 2011). However, restoration of burn scars may be challenging due to a sustained depletion of nutrients such as N and K relative to unburned areas. In addition, concentrating pile burns in forested ecosystems could potentially have deleterious impacts on water quality, particularly if N and K leach from the soil causing eutrophication of water bodies. Consequently, frequent pile burning or having a high density of pile burns increases the potential for permanent nutrient losses and localized long-term reductions in soil fertility.

Future Research

While our studies showed that charcoal positively affected microbial activity, the exact mechanisms responsible for the observed patterns remain unclear. The use of isotopically labeled C compounds could potentially aid in understanding if the stimulation of microbial respiration is due to priming of native organic matter, sorption of labile compounds, or direct decomposition of charcoal itself. Similar isotopic studies could be conducted using labeled N compounds to further help explain why nitrification increases in the presence of charcoal. Future studies should also focus on determining what microbial communities are stimulated by the addition of charcoal and how microbial populations change over time in charcoal amended soils. This may help assess how different microbial communities respond to the presence of charcoal and what these responses mean for C and N cycling in soils. Since burn temperature can affect physico-chemical properties of charcoal, lab studies could also focus on the microbial response to charcoal created at a variety of temperatures. Future field research should focus on the movement of nutrients deeper into the soil profile following pile burns, as well as monitoring any nutrient patterns of neighboring vegetation, allowing for better insight into the fate of a fire generated nutrient pulse in the surface soil. In addition, characterization of the microbial communities would help determine if patterns observed in lab studies follow those observed in the field.

Chapter 4 References

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