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# Impacts of mountain pine beetle outbreak on biogeochemical cycling in a high elevation whitebark pine ecosystem

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# IMPACTS OF MOUNTAIN PINE BEETLE (*DENDROCTONUS PONDEROSAE*) OUTBREAK ON BIOGEOCHEMICAL CYCLING IN A HIGH ELEVATION WHITEBARK PINE (*PINUS ALBICAULIS*) ECOSYSTEM

By

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ABSTRACT

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Ecological disturbances can significantly impact biogeochemical cycles in terrestrial ecosystems, but the effects of the current widespread mountain pine beetle outbreak on ecosystem processes like carbon (C) and nitrogen (N) cycling are poorly understood. This is especially true in high elevation whitebark pine (WbP) (*Pinus albicaulis*) ecosystems of western North America. WbP has been described as a keystone species, providing a critical food source and regulating hydrologic regimes. However, widespread WbP mortality caused by the mountain pine beetle drives structural and physiological changes in WbP forests, which could result in shifts in pools and fluxes of C and N within these ecosystems. To assess the biogeochemical consequences of the mountain pine beetle outbreak on whitebark pine ecosystems, I measured above and belowground nitrogen and carbon pools and fluxes around trees at three different times since beetle attack, including unattacked trees. Litterfall inputs under beetle-attacked WbP trees were more than ten times higher than those under unattacked trees. In response, soil $NH_4^+$ concentrations in the organic horizons increased from 15 $\mu$g N/g soil under unattacked trees to 33 $\mu$g N/g soil under attacked trees. However, there were not significant differences in ammonium ($NH_4^+$) concentrations in the mineral soil horizons. Overall, soil nitrate ($NO_3^-$) concentrations were low and highly variable, but generally increased following beetle attack. Additionally, there was no change in microbial biomass N in the soil between attacked and unattacked trees, implying that changes in N cycling in response to the initial stages of WbP attack were subtle. Soil $CO_2$ efflux rates were generally higher under unattacked trees, but overall, the similarities were more apparent than the differences. My results indicate that while beetle attack drives a large pulse of C and N canopy to the forest floor after beetle attack, changes in litterfall quality and quantity do not have immediate and profound effects on soil biogeochemical cycling. However, continuous observation of these important ecosystems will be crucial to determining the long-term biogeochemical effects of the mountain pine beetle outbreak.

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# Introduction

## Whitebark pine ecology

Whitebark pine (*Pinus albicaulis*; hereafter referred to as WbP) is a coniferous tree species found throughout subalpine regions of western North America (Figure 1). WbP distribution is divided into western and eastern ranges, extending from northern British Columbia to southeastern California in the west, and from the northern Rockies of British Columbia to the mountain ranges of Nevada in the east (McCaughey and Schmidt 2001). Depending on latitude, WbP can be found at elevations ranging from 900-3660m (Arno and Hoff 1990). The species can tolerate harsh conditions including extremely cold temperatures, poorly-developed soils, exposure to wind and steep slopes (Arno and Hoff 1990). Throughout the distribution of WbP, January temperatures range from an average minimum of -14°C to an average maximum of -5° C. Summer temperatures are cool, ranging from an average minimum July temperature of 4°C to an average maximum of 18° C. Precipitation in WbP ecosystems can range from 600 to 1600 mm/year, with over 85% falling as snow in the winter (Weaver 2001).

WbP occurs as a climax tree species at the upper ranges of its elevation (where conditions are often too harsh for most other species to survive) and as an early seral species at lower elevations (Tomback et al. 2001). Despite being restricted to subalpine regions, WbP can coexist with several community types ranging from grassland species in xeric sites to herbaceous and shrub species in more mesic sites. At its highest elevations, WbP often takes on a shrub-like "krummholz" form and associates with other low-lying alpine plants (Arno 2001).

Whitebark pine is the only North American member of the "stone pine" species group (subsection *Cembrae*, section *Strobes*, subgenus *Strobes*, genus *Pinus*, family Pinaceae), a group of pines with needles in bundles of 5 per fascicle and wingless seeds produced in indehiscent cones (Price 1998). The species is thought to have coevolved with the Clark's nutcracker (*Nucifraga columbiana)* to form a mutualism wherein the bird plays the role of primary seed disperser through the caching of WbP seeds across the landscape (Tomback 1982). The Clark's nutcracker will place anywhere from one to fifteen seeds in a single cache, producing the multi-stemmed, but genetically distinct boles characteristic of many WbP stands. Clark's nutcrackers preferentially cache seeds on steep, windswept, south-facing slopes that are typically rocky or gravelly (Tomback 2001).

Whitebark pine is a considered a keystone species because it plays multiple critical ecological roles in subalpine ecosystems. Its most well-known and extensively studied role is as an important food source for a large number of birds and mammals. Whitebark pine seeds weigh 175 mg on average, considerably more than any other co-occurring subalpine conifer species (Tomback and Linhart 1990). The seeds are rich in fat and nutrients, providing a high-energy food source for animals preparing to survive the harsh winters typical of WbP ecosystems (Lanner and Gilbert 1994). Seeds are ripe in middle to late August during which time a large variety of bird and rodent species feed on and cache them. Additionally, WbP seeds are a major food source for black bears (*Ursus americanus*) and grizzly bears (*U. arcos*), which obtain seeds by raiding the middens of red squirrels (*Tamiasciurus hudsonicus)*. Throughout WbP's range, the seeds serve as a primary food source for grizzlies in the fall, shortly before the bears enter

hibernation, and are considered critical for their winter survival and reproductive success (Mattson et al. 1992). Poor WbP cone production years in the Greater Yellowstone Ecosystem have co-occurred with a greater number of bear-human altercations as grizzlies move down the mountains towards roads and development in search of food (Mattson et al. 1992).

WbP plays multiple other critical ecological roles. For example, WbP strongly influences the radiation balance of subalpine ecosystems, and thus regulates the spring snowmelt runoff. Similarly, the root systems of WBP help to stabilize soil that would normally be carried downslope during periods of heavy rain and snowmelt, thus reducing soil erosion from steep mountain slopes (Farnes 1990). Because WbP can survive under conditions that are unsuitable for other trees, their canopy cover is particularly important in shading and regulating snowpack accumulation and subsequent spring and summer snowmelt at high elevations (Farnes 1990). In addition, WBP help to facilitate the upslope movement and establishment of less stress-tolerant tree species such as Engelmann spruce (*Picea engelmannii*) and subalpine fir (*Abies lasiocarpa)* by altering the abiotic environment to create microsites suitable for the germination of those species (Lanner 1980). For example, they provide pockets of higher moisture, shade and reduced wind exposure for newly establishing seedlings. Callaway (1998) found higher growth rates in subalpine fir seedlings clustered around WbP compared to those on their own.

Finally, though not used for timber or other commercial products, WbP is valued by humans for its aesthetic appeal as the quintessential subalpine conifer species, where its windswept, gnarled appearance symbolizes the harsh beauty of high elevation systems. For this reason and all of the above-mentioned ecological roles played by WbP,

the species has received an increasing amount of both research and media attention, and was recently named a candidate species for the Endangered Species List. This designation was deemed necessary because WbP is currently facing a number of threats to its continued existence and has already experienced significant declines in populations across its distribution (Tomback et al. 2001).

**Threats to Whitebark pine**

Across its entire range, WbP is experiencing threats on multiple fronts, ranging from historically natural beetle outbreaks, to infection by exotic pathogens, to fire suppression, as well as the interactions between these disturbances and climate change. First, white pine blister rust (*Cronartium ribicola*) is an exotic fungal pathogen introduced to North America from Asia around 1900 (McDonald and Hoff 2001). The fungus infects all North American white pines, including WbP. White pine blister rust undergoes a complicated life cycle involving five different spore types and two host types: white pines and woody shrubs in the genus *Ribes* (McDonald and Hoff 2001). Infected trees display characteristic "flagging" at the tips of their branches as well as cankers on their boles. Often, the upper, cone-bearing branches of the WbP are killed first, rendering the tree incapable of reproducing long before the 10-15 years it can take for them to die from the infection (McDonald and Hoff 2001). White pine blister rust has now spread to nearly the entire distribution of whitebark pine and stand level infection rates average 64% in recent surveys of Idaho, Montana, and the Greater Yellowstone Area (GYA) (Kegley et al. 2011). Fortunately, about 3-5% of WbP are genetically resistant to the fungus. Researchers and forest managers are currently isolating and

breeding blister-rust resistant WbP seedlings for outplanting back into the subalpine in an effort to slow mortality caused by the fungus (McDonald and Hoff 2001).

Another threat facing WbP is the active exclusion of fire from high elevation ecosystems that historically experienced fire return intervals ranging from fifty to four hundred years (Arno 1986**,** Romme 1982). Fires were either stand-replacing or mixed-severity in nature. Whitebark pine is better able to withstand mixed severity fires than subalpine fir and Engelmann spruce, meaning that occasional fires would eliminate competition and favor surviving WbP (Tomback et al. 2001). In the case of stand-replacing fires, whitebark pine has an advantage because the Clark's nutcracker disperses seeds in open, burned areas faster than wind can disperse the seeds of other conifers (Arno 1986). The lack of recurring fire in subalpine ecosystems has resulted in the encroachment and outcompetition of WbP by late seral conifer species (Tomback et al. 2001).

The current mountain pine beetle outbreak is unprecedented in terms of its extent, particularly in WbP ecosystems (Raffa et al. 2008). The mountain pine beetle (*Dendroctonus ponderosae*) is a native phloem-feeding insect that undergoes outbreaks in pine forests of the western U.S. and Canada every 30-40 years. Its most common tree hosts include lodgepole, ponderosa, and whitebark pine. The beetles preferentially attack mature large diameter trees. Mountain pine beetles deposit their eggs in the phloem of a host whitebark pine where the larvae develop and feed on the phloem for 1-3 years before emerging as adults and flying to a new host tree to begin the cycle again (Safranyik et al. 1999). As the larvae feed on the phloem, they effectively girdle the tree and kill it within two weeks of attack (Raffa et al. 2008).

The most recent previously recorded outbreak of any comparable magnitude happened in the 1930s under unusually warm and dry conditions and affected large areas of the GYA whitebark pine population (Logan et al. 2010). The current outbreak continues with a 320-fold increase in the number of mountain pine beetle-infested whitebark pine in the GYA occurring between 1999 and 2007 (Logan et al. 2009). Recent satellite evidence has revealed that 79% of whitebark pine ecosystems in the GYA have some level of canopy mortality most likely due to MPB infestation (Goetz et al. 2009). Climate change has contributed to this increased mortality with warming temperatures allowing more beetles to survive the winter and enabling them to complete a life cycle in one year instead of three (Bentz et al. 1991**,** Bentz and Schen-Langenheim 2006).

**Ecological Disturbances and Biogeochemistry**

An ecological disturbance is any discrete event that disrupts ecosystem structure and causes shifts in resources, substrate availability and/or the physical environment (White and Pickett 1985). Ecosystems experience a wide variety of disturbances, ranging from natural events such as insect outbreaks and wildfires to anthropogenic activities like clearcuts and land-use change. Each disturbance leaves its own unique signature on an ecosystem in terms of the extent and type of mortality affecting the dominant vegetation, which can alter several ecosystem characteristics including species composition, age structure, productivity and biogeochemical cycling (e.g. Swank et al. 1981, Bardgett et al. 1998, Ostertag et al. 2003, Reynolds et al. 2000, Frost and Hunter 2004, Lovett et al. 2008, White and Pickett 1985). Previous research has uncovered a wide variety of

biogeochemical responses to disturbance, responses that hinge upon characteristics of both the ecosystem and disturbance itself (Vitousek et al. 1979, Allen 1985, Hicke et al. 2011).

Some of the most important early work investigating the biogeochemical effects of disturbance took place in clear-cut watersheds at the Hubbard Brook Experimental Forest, where the potential for long-term consequences in terms of nutrient transformations and losses after disturbances was first recognized (Bormann et al. 1969). Subsequent clear-cut research in a variety of ecosystems complicated the picture by revealing a wide range of potential nutrient responses to disturbance, with some sites showing large, long-term impacts on the system and others virtually none (Vitousek et al. 1979, Allen 1985). Trenching experiments intended to mimic disturbance-induced mortality attempted to pick apart the mechanisms behind why ecosystems reacted to disturbance on such a wide biogeochemical spectrum (Vitousek et al. 1982). It was determined that ecosystem characteristics such as climate, vegetation, soil type, etc. played large roles in regulating the magnitude of biogeochemical responses to disturbance (Vitousek et al. 1982).

Regardless of the disturbance or ecosystem in question, the mortality of dominant vegetation produces some predictable structural and physiological changes in a disturbed ecosystem. Following disturbance, important elements such as carbon (C) and nitrogen (N) that were previously locked up in living biomass often arrive as a large pulse on the forest floor and become available for decomposition and immobilization by microbes (Reynolds et al. 2000, Chapin et al. 2002). Simultaneously, if the disturbance significantly reduces plant biomass, plant nutrient demand and nutrient uptake from the

soil are also reduced (Clow et al. 2011), and the pulse of biomass combined with the decrease in plant nutrient uptake contribute to an increase in C and N pools on the forest floor (Reynolds et al. 2000). The magnitude and duration of the increase depends on how various components of the ecosystem respond. For example, in the absence of an increase in plant uptake by early successional species and/or immobilization of N by microbes, excess inorganic N can be nitrified and ultimately converted to $NO_3^-$, an anion that is easily leached from the soil (Eshlemann et al. 1998, Aber et al. 2002, Riscassi and Scanlon 2009). Disturbances can also indirectly influence C and N cycling through their effect on abiotic characteristics. Changes in canopy structure following disturbance frequently alter microclimate conditions in the soil that can cause changes in soil temperature, light conditions, and moisture levels, all of which may influence C and N cycling processes (Jenkins et al. 1999, Morehouse et al. 2008, Lovett et al. 2008).

The above-mentioned structural and physiological shifts occur following most disturbances. However, the spatial and temporal characteristics of the disturbance, as well as ecosystem traits such as climate, vegetation type and soil qualities will determine the extent to which these shifts produce large or small changes in nutrient cycling (Schowalter et al. 1991, Vitousek et al. 1979). Bark beetle outbreaks like the current MPB infestation impart their own unique signature on an ecosystem. In the initial stages of an outbreak, the beetles tend to attack older and weaker host trees in patches across a landscape; however, if the outbreak is severe enough, the entire population of mature host trees may eventually be affected, leaving only seedlings and smaller trees alive (Safranyik and Carroll 2006). Once a tree has been successfully attacked, its nutrient supply is cut off and the tree dies within a year (Bentz et al. 2010, Raffa et al. 2008).

For approximately five years following attack, infested stands undergo a number of shifts that could influence biogeochemical cycling in the affected ecosystem**.** Shortly after beetle attack, nutrient and water uptake by host trees stops, potentially altering soil moisture and soil nutrient pools (Huber 2005, Clow et al. 2011, Griffin et al. 2011). Within two years of attack, needles on the tree typically turn red and begin falling to the ground, signifying the "red" stage of beetle infestation. In host tree species where litterfall nutrient content has been analyzed, attacked tree litterfall has higher N content than normally senescing litterfall, because the attacked trees do not resorb nutrients from their needles before they fall (Morehouse et al. 2008**,** Riscassi and Scanlon 2009**,** Griffin et al. 2011). Five years after attack, trees have typically lost all their needles to the forest floor and reach the "gray" stage. The large, relatively rapid pulse of needlefall to the ground provides a substantial pool of C and N for the ecosystem to process (Chapin et al. 2002). Once trees have reached the gray stage, reduced canopy cover allows more sunlight to reach the forest floor, with the potential for effects on soil temperature and moisture. All of the above characteristics of mountain pine beetle attack have the ability to impact belowground internal C and N cycling, as well as above and belowground C and N fluxes (Clow et al. 2011, Hicke et al. 2011). The likely increase in inputs of C and N to the system, at least in the short term, leads to two primary possibilities: they will be absorbed by the ecosystem through mechanisms that include increases in biomass and microbial immobilization, or they may be lost from the system through leaching and soil $CO_2$ efflux (Clow et al. 2011, Hicke et al. 2011).

The current MPB outbreak has just recently begun to receive research attention in terms of its impacts on nutrient cycling (Morehouse et al. 2008, Griffin et al. 2011, Huber

2005). ***To date, however, there have been no studies published describing nutrient cycling impacts of the outbreak in WbP ecosystems, despite the fact that potential biogeochemical shifts accompanying this disturbance may play a critical role in regulating the future of WbP ecosystems.*** There have also been no studies published describing the biogeochemical characteristics of WbP ecosystems in general, meaning that baseline data is needed to assess the effects of mountain pine beetle attack on these systems. In this case, consulting previous research on other disturbances and ecosystems becomes necessary to formulating hypotheses about how C and N cycling in WbP ecosystems might change following the current mountain pine beetle outbreak.

Although only a few studies have been conducted in ecosystems after bark beetle attack, much work has been done on the aftermath of defoliating insect outbreaks (e.g. Eshleman 1998, Orwig et al. 2008, Swank et al. 1981, Stadler et al. 2005, Riscassi and Scanlon 2009, Gandhi 2010). These disturbances are the most similar to bark beetles in terms of spatial and temporal mortality, and can be used to help fill in gaps that currently exist in our understanding of bark beetle impacts on biogeochemistry. The overarching objectives of this research project were to determine how the current mountain pine beetle outbreak affects abiotic characteristics and biogeochemical cycling in whitebark pine ecosystems in the short term. More specifically, I explored the short-term responses of two critical element cycles, nitrogen and carbon, following mountain pine beetle attack in these systems.

**Carbon**

Subalpine forests – including those dominated by whitebark pine – currently represent a substantial sink for atmospheric $CO_2$ (Dixon et al. 1994). The unprecedented

MPB-induced tree mortality has the potential to disrupt the C storage capacity of these ecosystems (Busby and Canham 2011, Clark et al. 2010, Forrester et al. 2003, Nuckolls et al. 2009). Other forest disturbances such as fire, land-use change, and defoliating insect outbreak have been shown to drive net transfers of C to the atmosphere (Crutzen and Andreae 1990**,** Dixon et al. 1994, Dymond et al. 2010). MPB outbreaks are more complex in their impact on the C cycle in that they do not always cause complete mortality across a landscape due to their host specificity and preference for larger diameter trees (Pfeifer et al. 2011).

Immediately following beetle attack, there is often a sharp decrease in gross primary productivity (GPP) due to mortality of the dominant tree species (Schafer et al. 2010, Morehouse et al. 2008, Nuckolls et al. 2009). In the short term, an MPB outbreak produces an initial pulse of C in the form litterfall to the forest floor (le Mellec et al. 2009, le Mellec and Michalzik 2008, Morehouse et al. 2008). Over longer time scales, the more recalcitrant standing dead woody boles and branches begin to decompose and slowly release a second C pulse to the ground (Harmon et al. 1986, Busse et al. 1994). In addition to and because of these changes in C inputs, C fluxes leaving the system can also shift following beetle attack (Brown et al. 2010, Clark et al. 2010). Autotrophic root respiration decreases with the death of the dominant vegetation, as might heterotrophic respiration coincident with the termination of labile C exudates from tree root systems (Hogberg et al. 2001, Bhupinderpal-Singh et al. 2003)**.** Conversely, the increase in C inputs to the ground may result in an increase in heterotrophic respiration through increased biomass available for decomposition (Hogberg et al. 2001, Morehouse et al. 2008). Rates of regeneration, changes in species composition, and shifts in understory

productivity all play a role in how ecosystem C storage may shift (McCambridge et al. 1982, Stone and Wolfe 1996). The overall effect on the C cycle, and ultimate sink/source designation of the ecosystem, depends on the magnitude of changes in the above-mentioned fluxes into and out of the ecosystem.

Studies across multiple ecosystems and disturbances have investigated C cycling impacts at large and small scales, and can aid in predicting what may happen in WBP ecosystems after MPB attack. Several studies have measured decreases in overall productivity immediately following a variety of insect outbreaks, including MPB (Morehouse 2008, Lovett et al. 2010, Nuckolls et al. 2009, Pfeifer et al. 2011**,** Schafer et al. 2010). Inputs of C to the forest floor in the form of litterfall or insect frass often increase after insect outbreaks (e.g., le Mellec and Michalzik 2008), although some systems do not see this shift after infestation (Nuckolls et al. 2009). Ecosystem respiration responses to insect outbreak differ greatly. Decreases in ecosystem respiration of varying magnitude are the most common response and have been observed in multiple systems and outbreaks (Heliasz et al. 2011**,** Nuckolls et al. 2009, Amiro et al. 2010, Pfeifer et al. 2011). Outbreak severity, type of insect, and forest type were hypothesized to account for the variation in magnitude of ecosystem respiration changes observed across studies (Hicke et al. 2011). For example, higher tree mortality may lead to sharper declines in autotrophic root respiration compared to a less severe outbreak (Pfeifer et al. 2011). In general, large-scale studies and modeling analyses have determined that insect outbreaks result in the transition of an ecosystem to a weaker C sink or a C source in the short-term. For example, a recent study determined that

Canada's western pine forests have shifted from a C sink to a source as a result of widespread MPB outbreaks (Kurz et al. 2008).

**Nitrogen**

Research suggests that N availability frequently limits net primary productivity (NPP) in temperate ecosystems (Gutschik 1981, Vitousek and Howarth 1991, Bormann and Likens 1994), and N losses following disturbance could delay forest regrowth (Vitousek et al. 1982). In addition, N losses often end up in streams leaving the ecosystem and can affect the water quality of aquatic systems downstream from the disturbance (Eshleman et al. 1998, Jenkins et al. 1999, Riscassi and Scanlon 2009). In MPB-infested stands, trees drop their needles before N resorption occurs and halt N uptake from the soil, meaning that there is a pulse of litter with low C:N ratios and less removal of available N from the soil by plant uptake (Morehouse et al. 2008, Clow et al. 2011). Belowground, soil microbial processes such as N mineralization and nitrification may increase in order to accommodate the increase in organic N. Through the process of nitrification, microbes convert available soil N into a more mobile form, (*e.g.,* nitrate [$NO_3^-$]) which can be easily leached from ecosystems into groundwater and streams if it is not taken up by microbes or other plants (Vitousek et al. 1979, Aber 2002). Understory plants or regenerating seedlings may take up the available N before it is leached from the ecosystem, and can exhibit increases in biomass or foliar N as a result (Lovett et al. 2010, Griffin et al. 2011).

Several studies have documented changes in the N cycle following insect outbreaks. Defoliating insects such as the gypsy moth and hemlock woolly adelgid produce many short-term effects including a pulse of N to the ground as frass and

unconsumed foliage (Lovett et al. 2002**,** Kosola et al. 2004, le Mellec and Michalzik 2008). Much of the N is immobilized by microbes (Lovett and Ruesink 1995), incorporated into organic matter (Christenson et al. 2002**,** Orwig et al. 2008), or taken up by surviving plants (Frost and Hunter 2004). In some cases of severe outbreaks, large increases in N losses from the ecosystem were measured in streamwater (Webb et al. 1995, Eshleman et al. 1998**,** Houle et al. 2009). After bark beetle attack, Morehouse et al. (2008) observed higher amounts of available N as well as higher rates of N mineralization in soil under infested ponderosa pine (*Pinus ponderosa*) stands compared to uninfested stands. In a 17-year chronosequence study following a bark beetle (*Ips typographus*) attack on a Norway spruce forest in Germany, Huber (2005) found the highest soil N pools in the first year following the attack, with the total soil N remaining higher than pre-attack levels for 7 years. Griffin et al. (2011) observed increases in N mineralization and nitrification under MPB- attacked lodgepole pine stands compared to unattacked stands.

**Abiotic characteristics**

Abiotic factors also play important roles in controlling various C and N cycling processes such as decomposition and N mineralization. Insect outbreaks often alter the abiotic characteristics of an ecosystem, thereby indirectly affecting C and N cycling. For example, increases in soil moisture and temperature as a result of canopy structure changes caused by disturbance can increase heterotrophic respiration rates and in turn soil $CO_2$ efflux (Concilio et al. 2005). Another abiotic variable, photosynthetically active radiation (PAR), may change after disturbance and subsequently affect understory

productivity as well as soil temperature and moisture (McCambridge et al. 1982, Stone and Wolfe 1996, Orwig et al. 2008).

After MPB attack, transpiration decreases, resulting in the potential for increased soil moisture (Morehouse et al. 2008, Clow et al. 2011). The loss of canopy needles allows more sunlight penetration to the forest floor, potentially increasing soil temperature and evaporation (Orwig and Foster 1998). Alternatively, increased litterfall after the outbreak may serve to insulate the soil, reducing evaporation (Byers 1984). Defoliating insect outbreaks have been shown to result in increased soil temperature and moisture (Jenkins et al. 1999, Lovett et al. 2002). Morehouse et al. (2008) observed higher soil temperature and moisture in MPB attacked pine stands compared to unattacked stands. They also measured higher solar radiation reaching the forest floor after attack. These changes have the potential to influence C and N processes beyond the direct biogeochemical effects induced by the MPB outbreak.

**Objectives**

The overall objective of this work was to assess the short-term impacts of the current MPB outbreak on WPB ecosystem processes. Within that context, I was specifically interested in assessing the effects of beetle attack on soil abiotic factors, (i.e., soil moisture and pH) and on C and N cycling. Thus, I took measurements under WbP at three different stages of beetle attack in the Pioneer Mountains of southwestern Montana over a 3-month period in 2010. In effect, I took advantage of the "natural experiment" that has been initiated by the ongoing beetle outbreak to investigate the effects of tree mortality as a result of beetle attack on a number of belowground ecosystem properties

and processes, and how those processes change through the early stages of the disturbance.

Given the widespread WbP mortality that is occurring in my study area and WbP ecosystems all over western North America, I predicted that there would be measurable shifts in abiotic characteristics, C, and N cycling with time since beetle infestation, and I designed my study to address the following hypotheses. First, I hypothesized that soil moisture would increase under beetle-attacked trees due to reduced transpiration following mortality. Next, I predicted that litterfall mass would be significantly higher under red stage trees compared to green and gray stage trees because of increased needle fall following tree mortality, that the C:N of the litterfall would be lower under infested trees due to the lack of nutrient resorption after beetle attack (Morehouse et al. 2008, Clow et al. 2011), and that soil inorganic N would be higher under red and gray stage trees compared to uninfested trees. An increase in available soil inorganic N could follow a number of different pathways, including microbial immobilization, uptake by understory plants, and conversion to $NO_3^-$ with the potential for loss from the ecosystem through leaching (Huber 2005, Webb et al. 1995, Eshleman et al. 1998**,** Houle et al. 2009). Thus, I hypothesized that I would observe changes in one or all of these pathways under infested trees. Finally, I predicted that the reduction in autotrophic root respiration and microbial respiration of labile root carbon exudates after beetle attack would be larger than any increase in heterotrophic respiration that may occur with increased microbial substrate availability from litter inputs and decomposition of root biomass (Hogberg et al. 2001, Bhupinderpal-Singh et al. 2003), and thus net soil respiration would be lower under attacked trees than under green trees.

# Materials & Methods

## Study Site

This study was conducted at Vipond Park (45.6974258° N, -112.9105898° W) in the Pioneer Mountains in the Beaverhead-Deerlodge National Forest of southwestern Montana, USA (Figure 2). Average temperatures in the region range from -9°C in January to 13°C in July (SNOTEL site 656, 2530 meters above sea level (m.a.s.l.), 1979-2009 average). Mean annual precipitation is ~770 mm falling mostly as snow (SNOTEL site 656, 2530 m.a.s.l., 1979-2009 average). Snow covers the ground for about 8 months out of the year, leaving a relatively short window of time for conducting field work. The study site sits 2500m above sea level. Soils in the area consist of Typic calcicryepts (inceptisols) and Eutic haplocryalfs (alfisols) derived from limestone colluvium parent materials (USDA Natural Resources Conservation Service, Web Soil Survey). The site is an open canopy forest with whitebark pine (*Pinus albicaulis)* as the dominant canopy tree species co-occurring with occasional lodgepole (*Pinus contorta)* and limber pine (*P. flexilis)*. The understory is sparse, consisting primarily of perennial grasses and forbs. The current mountain pine beetle outbreak at Vipond Park was first observed in 2005 and has now progressed to the point where over 70% of the whitebark pine (WbP) are at some stage of beetle infestation.

## Sampling Design

To assess the influence of WbP mortality resulting from beetle infestation on C and N cycling, I established ten 4X4 $m^2$ plots around individual "focal trees" (Zinke 1962). This sampling design allowed for the isolation of tree level effects of beetle infestation at a site that is heterogeneous in terms of both canopy cover and beetle attack.

Three common stages of beetle infestation were investigated in this study: 1) ***green***, uninfested whitebark pine; 2) ***red***, recently (within 2 years) infested whitebark pine with needles that have turned red but not fallen; and 3) ***gray***, whitebark pine infested more than two years ago with complete loss of needles. Ten whitebark pine individuals at each of the three infestation stages (30 total) were used as focal trees. In addition to beetle infestation stage, focal trees were chosen based on a 15 cm minimum DBH cutoff and were located in patches of trees at the same infestation stage.

Soil samples were collected monthly from July 2010 to October 2010 at four different distances from the bole to the crown drip line of the focal tree in each of four cardinal directions (90° apart) starting 0.5m from the base of the tree and moving outward in 0.5m increments (Figure 3). Soil organic horizon samples were taken to a depth of ~5cm at each sampling point. Mineral soil samples were collected directly below the organic horizon cores to 15 cm depth. In cases where rocks obstructed the mineral cores, samples were taken adjacent to the organic sampling point after removing the organic material from the mineral soil surface. During each sampling event, soil samples were composited by tree and depth interval, placed in coolers and transported back to the laboratory at the University of Montana for analysis. Within 48 h, soil samples were sieved (4mm) and subsampled for physical and chemical analyses including gravimetric moisture, inorganic N and microbial biomass analysis (methods below), and the remaining soil air-dried for pH and total C and N. Soil pH was determined at 2:1 (water: soil) using a Beckman Instruments 265 pH meter (Fullerton, CA).

## Foliage and Litter Quantity and Quality

*Relative litterfall and foliage inputs*

One litter trap was placed 0.5 m from the bole of each focal tree in July 2010. Litter was collected after one month to obtain an index of relative litter mass among beetle infestation stages and to perform litter nutrient analyses. Following collection, the litter was dried at 70 °C for 48 hours and subsequently weighed. Oven-dried subsamples from each litter trap were ground with a Wiley Mill (20-mesh screen), weighed into tin capsules (~4 mg each), and combusted on a CHNS-O elemental analyzer (CE Instruments EA 1110, Thermo Fisher, USA) for total litter C and N (Environmental Biogeochemistry Laboratory, University of Montana).

Canopy foliage was collected in July 2010 from green and red stage trees. Samples were taken approximately 2.5 m from the ground at various points around each tree. Foliage was analyzed for total C and N using the above protocols. Standing litter was sampled from below each focal tree in July 2010. A 0.25 $m^2$ sampling quadrat was placed 0.5 m away from each tree and all intact standing litter in the quadrat was collected down to the organic horizon, dried at 70 °C for 48 hours and weighed. Subsamples were ground, weighed into tin capsules (4 mg), and combusted on a CHNS-O elemental analyzer (CE Instruments EA 1110, Thermo Fisher, USA) for total standing litter C and N (Environmental Biogeochemistry Laboratory, University of Montana).

## Understory Vegetation and Foliar N

Understory vegetation under each focal tree was characterized in July 2011. Two 0.25 $m^2$ quadrats were placed 0.5 m from the bole of each tree oriented toward the north

and south. Percent ground cover in each quadrat was visually estimated according to plant functional group (grass, forb, sedge, shrub, seedling). Foliar samples from two common understory species, *Viola praemorsa* (Canary violet) and *Oxytropis sericea* (Silky locoweed*)*, were collected from the above quadrats and composited by tree and species. Samples were oven-dried at 70°C for 48 hours, ground, weighed into tin capsules (4mg), and combusted on a Carlo Erba elemental analyzer for total C and N (Environmental Geochemistry Laboratory, University of Montana).

**Soil N and C Pools**

Within 24 hours of collection, soil inorganic N (ammonium [$NH_4^+$] and nitrate [$NO_3^-$]) was extracted from the September 2010 soil samples. Ten grams of each soil sample were extracted in 30ml 2M KCl for 18 hours, vacuum filtered through 11 μm Whatman (Grade 1) filter paper, and stored at 4°C prior to analysis. Extracts were analyzed colorimetrically for inorganic N ($NH_4^+$ and $NO_3^-$) using a Synergy 2 Microplate Reader (BioTek, USA) after Weatherburn (1967) and Doane and Horwath (2003), respectively.

Microbial biomass C and N were analyzed using the chloroform fumigation-extraction method (Brookes et al. 1985). Ten grams of fumigated and unfumigated samples from the October 2010 collection (organic and mineral samples composited) were extracted with 0.5 M $K_2SO_4$ for one hour and vacuum-filtered through 11 μm Whatman (Grade 1) filter paper. Organic C and N in extracts were analyzed using a Shimadzu TOC-V CPN/TNM-1 analyzer (Shimadzu, Inc, Kyoto, Japan). Microbial biomass C was determined as the difference between extractable organic C in fumigated

and unfumigated samples using a proportionality constant (K*c*) of 0.45 (Vance *et al*. 1987). Microbial biomass N was determined using a correction factor (K*n*) of 0.54 (Brookes *et al*. 1985). Finally, composited organic and mineral soil samples from the October 2010 soil collection were ground, weighed into tin capsules (7mg), and combusted on a CHNS-O elemental analyzer (CE Instruments EA 1110, Thermo Fisher, USA) for total soil C and N determination (Environmental Geochemistry Laboratory, University of Montana).

**Soil N and C fluxes**

*N mineralization/nitrification*

I conducted a 28-day lab aerobic incubation of soil samples collected in September 2010 (organic and mineral horizons kept separate) to measure net N mineralization rates. Ten grams of field moist, sieved soil were mixed and weighed into plastic vials, covered with perforated plastic wrap, and incubated in the dark at room temperature (22 °C) for 28 days. Vials were reweighed weekly and water added to maintain field moisture of each sample. After 28 days, samples were extracted with 30 ml 2M KCL (18 hours) vacuum filtered, and analyzed for inorganic N ($NH_4^+$ and $NO_3^-$) colorimetrically after Weatherburn (1967) and Doane and Horwath (2003), respectively. Net N mineralization values were calculated by subtracting the pre-incubation September 2010 inorganic N values from final incubated values. Net N nitrification values were obtained in the same way, using only nitrate concentrations.

*Soil N fluxes*

Soil N fluxes (to 15 cm) were assessed using ion-exchange resin capsules (Unibest, Inc, Bozeman, MT, USA). Capsules were deployed two times, first from July 2010 to October 2010, and again from October 2010 to July 2011, following snowmelt. Resin capsules were inserted under each focal tree to 10-15 cm depth by carefully creating a slit in the soil with a hand trowel, inserting the capsule, and carefully removing the blade to minimize disturbance. Once the capsules were collected from the field, resin-exchanged inorganic N ($NH_4^+$ and $NO_3^-$) was determined following extraction in 2 M KCl and colorimetric analysis as mentioned in the above sections.

*Soil CO2 fluxes*

To quantify soil respiration, I installed one 4-inch PVC collar (80 $cm^2$) 0.5 m from each focal tree in July 2010. $CO_2$ fluxes were measured every two weeks using a Li-Cor vented, closed soil chamber system; this combination allowed continuous flow-through measurements with the ability to monitor and control environmental variables within the chamber (*e.g.,* Scott-Denton et al. 2003, Buchmann 2000). Any herbaceous vegetation, including roots, was removed from the PVC collar area prior to taking measurements. Soil respiration was measured with a LI-COR 6400 with soil flux chamber (LI-COR, Lincoln, Nebraska, USA) under each tree approximately every two weeks from July-October 2010. Soil temperature near each collar was also measured with every respiration measurement.

**Litter Incubation Experiment**

To complement the field-based analyses and in an attempt to isolate the effects of differences in litter quality (i.e., from attacked "red" trees vs. unattacked "green" trees) on soil C mineralization rates, I conducted a laboratory incubation experiment. Ten grams of soil from the October 2010 sampling period (organic and mineral composited) were weighed into 50ml glass tubes. Three replicates of each sample were prepared for a total of 90 tubes. Subsamples of litter from the littertraps under the "green" and "red" trees were dried and ground using a Wiley Mill (40mm). There were three litter addition treatments in total: green needles (0.5g), red needles (0.5g), and no needles. Every sample received all three treatments, one for each of the three replicates. Once litter was added, the samples were mixed to incorporate the litter into the soil. Additional samples included each litter and soil type alone along with empty tubes to act as blanks, for a total of 102 tubes. Each mixture was adjusted to ~55% of water-holding capacity (WHC) on day 0 and maintained at that level throughout the experiment. Water was added on a weekly basis to keep tubes at their starting weight. Tubes were incubated in the dark in a closed plastic cooler and left uncapped and loosely covered with aluminum foil at 22°C. Moist paper towels were placed in the cooler to minimize water loss.

Prior to sampling for C mineralization, tubes were sealed with airtight, rubber-septa plastic caps and incubated for four hours. Using a plastic syringe, headspace was mixed, 10 mL samples were taken, and carbon mineralization rates were calculated as carbon dioxide production per hour using a thermal conductivity detector in a Shimadzu gas chromatograph (Shimadzu Inc, Kyoto, Japan). Carbon mineralization rates were measured eight times over the course of the 37-day incubation.

### Statistical Analyses

All statistical analyses were conducted using SPSS (IBM SPSS Statistics, Version 19). Analysis of variance tests (ANOVA) were used to determine whether there were significant differences between the three beetle infestation stages in terms of litterfall, standing litter mass, soil C and N pools and fluxes, and understory vegetation cover and nutrient content. Repeated measures ANOVA was used to test for the effects of both time and beetle infestation stage on soil moisture and soil $CO_2$ efflux. Repeated measures ANOVA was also used to detect the effects of time and treatment on the litter addition incubation. For post-hoc analyses, Tukey's HSD test was used to determine differences between stages when ANOVAs were significant ($\alpha = 0.05$). Linear regression was used to assess relationships between various N and C cycling metrics across all stages of beetle infestation.

## Results

### Soil Abiotic Characteristics

Soil pH at the site did not vary significantly with beetle infestation stage (Table 1), but soil moisture did vary significantly between trees at different infestation stages. Soil moisture under both the red and gray stage trees was significantly higher than soil moisture soil under the green stage trees ($P=0.026$, $P=0.039$). The repeated measures ANOVA showed that sampling time was a significant factor ($P<0.001$) in explaining differences in soil moisture over the course of the growing season (Fig. 4). There was not

a significant time × infestation stage interaction; however, infestation stage alone explained a significant proportion of the variation in soil moisture among treatments.

**Litterfall: Relative rates and litter chemistry**

In July 2010, litterfall inputs were an order of magnitude higher under the red stage trees (278.38 ± 44.08 g/m$^2$) than rates under either green stage or gray trees (11 ± 5 g/m$^2$ and 77 ± 21 g/m$^2$ respectively) ($P<0.001$ for both). However, episodic litterfall inputs did not vary significantly between the gray and green stages (Fig. 5). Total N concentrations were significantly higher in gray and red stage litterfall (1.45 ±0.05% and 1.16 ±0.07% respectively) compared to green stage litterfall (1.06 ±0.04%) ($P=0.001$, $P<0.001$, respectively). This was reflected in the significantly higher standing litter N pools under the red and gray stages compared to the green stage ($P<0.001$ for both) (Fig. 6). Litterfall C:N ratios varied between stages, with the red and gray stages having significantly lower C:N ratios than the green stage needles ($P=0.046$, $P<0.001$ respectively) (Fig 7). Foliar C:N ratios varied as well, with the green stage trees having approximately double the foliar C:N (109.88 (2.35)) of the red stage trees (55.66 (2.91)) ($P<0.001$). There was no significant difference in standing litter mass among infestation stages, although the red and silver stages tended to be higher than the green stage ($P=0.203$, $P=0.467$, respectively) (Table 1). Similarly, standing litter biomass % N and C:N did not vary among infestation stages.

Understory ground cover did not significantly vary across the three stages of beetle infestation, although grasses and forbs displayed an increasing trend moving from the green to red and gray stages (Fig. 8). Neither of the two understory forb species

(canary violet and silky locoweed) exhibited significantly different C:N values across the three stages of beetle infestation (Table 1).

**Soil N and C Pools**

Soil inorganic N concentrations ($NH_4^+ + NO_3^-$) were higher ($p<0.001$) in the organic horizon compared to the mineral horizon for all stages. However, across infestation stages, the organic horizon under red stage trees had higher extractable inorganic N concentrations than the organic horizon of either the green or gray stage trees ($p=0.005$, $p=0.071$) (Fig 9). In the mineral horizon, extractable inorganic N did not vary across infestation stages (Fig. 9), and soil $NO_3^-$ concentrations in both organic and mineral horizons were very low (i.e., near detection limits) and highly variable. There were no differences in $NO_3^-$ levels across infestation stages in either soil horizon (Table 1), and there were no significant differences in either microbial biomass C or N concentrations or total soil C and N concentrations across infestation stages in either the organic or mineral soil horizons (Table 1). However, soil microbial biomass C:N was significantly lower under the red stage trees compared to the gray stage trees ($P = 0.012$; Table 1).

**Soil N and C cycling**

Net N mineralization rates were higher in both organic and mineral soils under gray trees, followed by red and then green trees (Table 1), but the differences among stages were not significant. Similarly, net nitrification rates were very low and highly variable in both soil horizons and across all stages (Table 1). Soil inorganic N fluxes as measured with resin capsules did not vary across stages of beetle infestation (Table 1).

$CO_2$ fluxes varied through the 2010 growing season, and a repeated measures ANOVA analysis showed that sampling time was the only significant factor ($P<0.001$) in explaining temporal differences in soil $CO_2$ efflux. Soil $CO_2$ efflux tended to be higher under green stage trees compared to the other red and gray stages, however infestation stage was not a significant factor over the entire sampling period (Fig. 10). There was also no significant effect of a time × infestation interaction on soil $CO_2$ efflux throughout the growing season.

**Litter Addition Incubation**

The experimental addition of green and red stage litter types did not have an effect on laboratory soil $CO_2$ mineralization rates**.** Repeated measures ANOVA analysis showed that time was a significant factor ($p<0.001$) in explaining differences in $CO_2$ mineralization during the litter addition incubation. The four litter/soil treatments were not significant throughout the incubation. Figure 11 illustrates that for a portion of the incubation, however, samples that received red stage needles tended to have higher $CO_2$ mineralization rates than those that received green stage needles. There was no significant interaction between time and treatment during the incubation.

## Discussion

Shifts in nutrient cycling and abiotic factors have been observed following insect outbreaks in many forested ecosystems around the world. In some ecosystems the changes are relatively subtle and short-lived (Griffin et al. 2011, Swank et al.1981), while others experience significant, long-term impacts (Huber 2005, Riscassi and Scanlon 2009), at times accompanied by shifts in species composition (Orwig and Foster 1998)

and deterioration of water quality (Riscassi and Scanlon 2009). The current MPB outbreak has the potential to affect ecosystem processes in critically important high elevation WbP systems. Here, I investigated the short-term impacts of the current MPB outbreak on two features of WbP ecosystems: abiotic factors and soil C and N cycling. To assess possible short-term shifts in these variables, I measured soil abiotic characteristics and pools and fluxes of C and N under WbP at different stages of beetle attack. I hypothesized that inputs of C and N would increase following WbP mortality and that this would in turn affect internal cycling and outputs, as well as abiotic factors. The results of the study varied in many ways from research conducted in other systems impacted by MPB outbreak.

Soil moisture varied with beetle infestation stage, with the red stage having the highest soil moisture. There are several possible explanations for this result, including an increase in litter depth under beetle-attacked trees and/or reduced transpiration following WbP mortality (Clow et al. 2011, Griffin et al. 2011, Griffiths et al. 2010, Jenkins et al. 1999). For example, deeper litter layers can insulate the soil and prevent moisture losses through evaporation. A litter addition experiment in Yellowstone National Park found higher soil moisture levels in its litter addition treatments compared to controls (Cullings et al. 2003). Solar radiation reaching the forest floor was not measured in this study, but increased radiation due to complete loss of canopy cover could promote evaporation, explaining why soil moisture under gray stage trees is lower than soil moisture under red stage trees. In ponderosa pine stands, Morehouse et al. (2008) measured significantly higher photosynthetically active radiation (PAR) reaching the forest floor under beetle-

killed trees compared to unattacked trees. Reduced canopy cover during the gray stage may partially counteract increases in soil moisture stemming from reduced transpiration.

Increased soil moisture can have important implications for a number of microbial processes, including N mineralization, respiration and decomposition (Concilio et al. 2005, Davidson and Janssens 2005)**.** Although I did not measure soil temperature, other studies have measured changes after insect infestation (Griffin 2011, Jenkins et al. 1999). Shifts in soil temperature and moisture most likely interact with and influence each other. For example, an increase in soil moisture may result in decreased soil temperatures despite increases in solar radiation reaching the forest floor.

Not surprisingly, episodic litterfall inputs were significantly higher under red stage trees compared to green and gray stage trees, reflecting the pulse of litterfall often observed soon after tree mortality following beetle attack. Litterfall nutrient chemistry also varied with infestation stage. Litterfall from gray stage trees had significantly lower C:N ratios than green stage trees, with the difference being primarily due to higher N concentrations in the needles falling from the infested trees. This result matches the findings of studies conducted in both MPB-attacked lodgepole and ponderosa pines (Morehouse et al. 2008, Griffin et al. 2011, Clow et al. 2011). Differences in litterfall nutrient content following MPB most likely reflect limited N resorption prior to senescence (Stone et al. 1999, Morehouse et al. 2008). However, while I expected these changes in litterfall quantity and quality to translate to subsequent shifts in C and N cycling as they reached the forest floor and became available for microbial processing and decomposition (Chapin et al. 2002, Riscassi and Scanlon 2009, Lovett et al. 2010), this was generally not the case. For example, increased litterfall mass did not translate

into differences in standing litter biomass under infested trees. Griffin (2011) observed similar patterns in MPB-attacked lodgepole pine stands, and attributed this to decomposition breaking down the gradual (3-4 year) influx of litter at a pace that did not allow buildup of a litter layer beyond that of a normal, uninfested stand of trees. Alternatively, the patchiness of WbP ecosystems in terms of canopy cover, as well as the movement of litter during snowmelt or wind or rain events, may contribute to the high variability of standing litter mass, thereby masking any shifts due to beetle infestation.

Once increased litterfall N inputs reach the forest floor, the N can have multiple possible fates, one of which is through uptake by surviving understory vegetation. This can manifest itself as an increase in productivity, or as an increase in foliar N concentration of understory plants after the disturbance (Stone and Wolfe 1996, Metzger et al. 2006). However, I observed no significant difference across infestation stage in the foliar N of two common understory species (*Viola praemorsa* and *Oxytropis sericea*). There was also no increase in understory ground cover under beetle-attacked trees. In lodgepole pine stands attacked by MPB, however, Griffin et al. (2011) observed higher foliar N concentrations in the understory sedge, *Carex geyerii*, in beetle-infested stands compared to uninfested stands, but did not measure higher sedge productivity in infested stands. Other studies of post-beetle stands have observed increases in understory growth in a number of forest types (Stone and Wolfe 1996, McMillen and Allen 2003, McCambridge et al. 1982). Increases in available N after disturbance may be responsible for some of this enhanced growth, but other abiotic factors such as increased light and moisture are also likely contributors, particularly in the longer term.

As hypothesized, the highest soil inorganic N values were observed in the organic horizon under red stage trees, and the differences in inorganic N concentrations observed were due to shifts in soil $NH_4^+$, not $NO_3^-$. The increase in soil inorganic N under red stage trees could be explained by one or a combination of a number of factors. For example, reduced plant uptake (Reynolds et al. 2000), higher microbial N mineralization (Swank et al. 1981, Jenkins et al. 1999, Morehouse et al. 2008), lower microbial immobilization of N, or leaching of N from the N-enriched litter layer (Riscassi and Scanlon 2009) could all contribute to the elevation in concentrations. Similar results have been found in other insect outbreak studies, with infested stands having significantly more extractable soil $NH_4^+$ than uninfested stands (Jenkins et al. 1999, Lovett et al. 2010, Clow et al. 2011). In beetle-infested vs. uninfested ponderosa pine stands, however, there were no significant differences measured in soil inorganic N pools (Morehouse et al. 2008).

In contrast to the differences in inorganic N in the organic (surface) soil horizons, inorganic N concentrations in the mineral soil horizons did not vary with infestation stage. This is most likely because sufficient time has not elapsed for the increased inputs of N to be transported into the lower mineral horizons. Leaching of inorganic N from the organic horizon and litter layers requires time and movement of water through the soil profile (Chapin et al. 2002). Alternatively, the increased inorganic N in the organic horizon may be immobilized by microbes or taken up by plants before it can move into the mineral horizon.

While I predicted that the relatively low C:N ratios in litter from beetle-infested trees would drive increases in soil N pools, not all forms of inorganic N shifted following

beetle attack. Soil $NO_3^-$ concentrations were consistently very low, highly variable and did not differ with beetle infestation stage. Low soil $NO_3^-$ levels are often measured in high elevation, N-limited ecosystems; any available N is rapidly taken up by plants or microbes (Chapin et al. 2002). Increases in soil $NO_3^-$ may also have been lost from the ecosystem quickly through leaching during rain events or snowmelt (Vitousek et al. 1979). In other disturbed systems, large increases in soil $NO_3^-$ have been observed but usually involved nearly 100% vegetation mortality or took place in systems with substantial N deposition (Bormann and Likens 1994, Aber et al. 2002). For example, Orwig (2008) measured soil $NO_3^-$ in hemlock stands that had been infested by the insect defoliator, hemlock woody adelgid, and observed significantly higher levels in attacked vs. unattacked stands. These forests were nearly pure hemlock and experienced almost complete mortality as the outbreak moved through the system. Many eastern forests also experience relatively high levels of atmospheric N deposition compared to western forests, which contributes to $NO_3^-$ levels in the soil (Aber et al. 2003). Soil texture and the timing of snowmelt or rain events also influences the magnitude of N losses from an ecosystem. For example, coarse, sandy soils are more prone to N losses after a disturbance, as are ecosystems with large rain events closely following a disturbance (Vitousek et al. 1982).

Microbial biomass C:N ratios were lower under red stage trees compared to gray stage trees, but surprisingly did not differ from green stage trees. Microbial immobilization of N is one pathway that N mobilized after a disturbance may take, particularly if N is limiting (Chapin et al. 2002). In such cases, the microbial biomass C:N reflects this with lower values, indicating uptake of N. Net nitrogen mineralization

rates did not vary across beetle infestation stage. This result is not consistent with other insect outbreak studies, which found significantly higher net N mineralization potential in soil from attacked stands (Morehouse et al. 2008, Jenkins et al. 1999, Lovett et al. 2010). My results appear to rule out increased microbial N mineralization as the factor behind the observed increase in soil inorganic N in the organic soil horizon. One possibility is that reduced plant uptake resulted in the increase in inorganic N rather than increased N mineralization. Alternatively, the timing of sampling may have missed an initial pulse of mineralization following tree mortality. High elevation WbP ecosystems most likely undergo the majority of their microbial processing of organic matter and decomposition during spring snowmelt, a time at which my study site is inaccessible.

Nitrogen fluxes through the soil profile as measured with resin capsules also did not vary with beetle infestation stage in our system. N losses are one of the most commonly measured biogeochemical variables in disturbance studies. Often measured in streams draining the disturbed ecosystem, increased $NO_3^-$ levels have been observed in a number of insect defoliation studies (Houle et al. 2009, Swank et al. 1981, Webb et al. 1995, Eshlemann et al. 1998). A spruce bark beetle outbreak in Germany resulted in elevated N concentrations 40 cm below the soil surface for five years following the outbreak (Huber 2005). The fact that no measurable N fluxes were observed in our WbP system is not entirely surprising considering the very low $NO_3^-$ levels measured in the soil profile. High losses of $NO_3^-$ like those observed in other disturbed ecosystems have the potential to negatively impact water quality downstream of the affected ecosystem.

In terms of C fluxes, there were no significant differences in soil $CO_2$ efflux between beetle infestation stages. The trend in my data, however, of higher soil

respiration under green stage trees, suggests that together autotrophic root respiration (of live trees) and microbial respiration of labile root C exudates are at least as high as any increase in heterotrophic respiration that may occur with increased microbial substrate availability from litter inputs and decomposition of root biomass. A similar study of beetle-killed lodgepole pines in Colorado measured significantly higher soil respiration under unattacked trees compared to attacked trees (N. Trahan, pers. communication). Whether or not these trends continue into the long-term, once decomposition of woody biomass begins, remains to be seen (Kurz et al. 2008, Hicke et al. 2011). In general, however, the decline in C fixation after tree mortality must also be considered when assessing the net C status of the ecosystem. In other words, similar rates of soil respiration in attacked and unattacked systems do not imply that overall C fluxes are the same. Instead, in the absence of significant C uptake, net C losses would almost certainly be higher in attacked stands than in unattacked stands in the short term (Kurz et al. 2008).

Overall, the results of my study suggest that from a biogeochemical perspective, WbP ecosystems experience only subtle changes in the years immediately following beetle attack. While C and N inputs to the ecosystem changed significantly following tree mortality, they were accompanied by only slight, if any, shifts in internal cycling and outputs of C and N from the ecosystem. In addition, the internal cycling characteristics that did change with beetle infestation stage varied in the upper, organic soil horizon, but these differences did not appear in the mineral soil horizon, and there was no evidence suggesting significant shifts in C and N outputs from the system. Overall, this study indicates that WbP ecosystems react differently to MPB attack compared to lower

elevation ecosystems, displaying either subtle or delayed responses in their biogeochemical cycling.

Considering the extent of WbP mortality at the site, the lack of many significant biogeochemical responses to the increased litter C and N inputs was unexpected. Looking at the characteristics of WbP ecosystems, however, provides some insight into potential lags in response time to the disturbance, which may allow regeneration to catch up before any long-term nutrient shifts occur. For example, the very short growing season and extreme climatic conditions that exist in WbP ecosystems most likely cause many microbial processes to progress more slowly than occurs in other ecosystems. In the case of a large-scale, high mortality disturbance, this may be a positive characteristic as far as ecosystem response to significant shifts in C and N cycling is concerned. Long-term monitoring is required to determine whether wholesale biogeochemical changes merely take longer to manifest themselves in WbP ecosystems, but so far the data look promising for the regeneration potential of these critically important ecosystems.

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## Table

*Table 1*. Site characteristics and soil N and C pools and fluxes under whitebark pine at three different stages of beetle infestation. Variables were analyzed using soil or litter samples from dates specified in the materials and methods section. Letters denote significant differences between stages. Error ranges are 1 S.E**.**

| **Variable** | **Infestation stage** | | |
|---|---|---|---|
| | **Green** | **Red** | **Gray** |
| **Soil pH** | 6.17 (0.22) a | 6.44(0.21) a | 6.30 (0.19) a |
| **Standing litter** | | | |
| Mass ($g/m^2$) | 1938.99 (261.79) a | 2994.08(451.98) a | 2655.96 (517.99) a |
| **Soil Inorganic N ($NH_4^+$ and $NO_3^-$) (μg N /g soil)** | | | |
| Organic horiz. | **14.96 (2.20) a** | **33.60 (4.98) b** | **21.19 (3.66) ab** |
| Mineral horiz. | 4.86 (0.94) a | 4.73 (0.93) a | 6.32 (1.23) a |
| **Soil $NO_3^-$ (μg N/ g soil)** | | | |
| Organic horiz. | 0 (0) a | 0.17 (0.10) a | 0.24 (0.13) a |
| Mineral horiz. | 0 (0) a | 0.06 (0.05) a | 0.32 (0.21) a |
| **Microbial Biomass** | | | |
| Carbon | 1.30 (0.09) a | 1.28 (0.02) a | 1.48 (0.19) a |
| Nitrogen | 0.15 (0.01) a | 0.17 (0.02) a | 0.16 (0.02) a |
| C:N | **8.53 (0.40) ab** | **7.65 (0.16) a** | **8.98 (0.30) b** |
| **Total Soil C:N** | 21.25 (1.24) a | 22.51 (1.01) a | 23.32 (1.35) a |
| **Foliage C:N** | **109.88 (2.35) a** | **55.66 (2.91) b** | N/A |
| **N Mineralization (μg N/g soil/day)** | | | |
| Organic horiz. | 1.51 (0.19) a | 1.53 (0.44) a | 1.65 (0.36) a |
| Mineral horiz. | 0.61 (0.21) a | 0.63 (0.19) a | 1.05 (0.33) a |
| **Resin capsule N flux (μg N/g soil)** | 0.46 (0.17) a | 1.13(0.49) a | 0.45 (0.22) a |
| **Understory C:N** | | | |
| Silky locoweed | 8.87 (0.28) a | 8.57 (0.17) a | 8.35 (0.22) a |
| Canary Violet | 11.14 (0.24) a | 11.87 (0.83) a | 11.27 (0.34) a |

## Figure Captions

Fig.1-Distribution of whitebark pine (*Pinus albicaulis*) in North America. (Adapted from Moscow Forest Sciences Laboratory, Moscow, ID).

Fig. 2 - Location of study site, Vipond Park in the Beaverhead-Deer Lodge National Forest, denoted by blue star (Google).

Fig. 3- Diagram of sampling design showing focal tree bole at the center and crown drip line as the outer circle. The green circles delineate different distances from the bole of the tree at which soil samples were collected. Blue arrows denote location of sampling sites at various distances with the intention of capturing soil variability under a given focal tree.

Fig. 4- Soil moisture of both the mineral and organic soil horizons under trees at three different stages of mountain pine beetle infestation in whitebark pine. Measurements were taken at three different time points throughout the 2010 growing season. Error bars denote one standard deviation.

Fig. 5 - Litterfall mass under trees at three different stages of mountain pine beetle infestation in whitebark pine. Stages with different letters are statistically different based on ANOVA and Tukey's HSD test. N= 10/stage, error bars denote one standard deviation.

Fig. 6- Litterfall N pool under trees at three different stages of mountain pine beetle infestation in whitebark pine. Stages with different letters are statistically different based on ANOVA and Tukey's HSD test. N= 10/stage, error bars denote one standard deviation.

Fig. 7- Litterfall C:N ratio under trees at three different stages of mountain pine beetle infestation in whitebark pine. Stages with different letters are statistically different based on ANOVA and Tukey's HSD test. N= 10/stage, error bars denote one standard deviation.

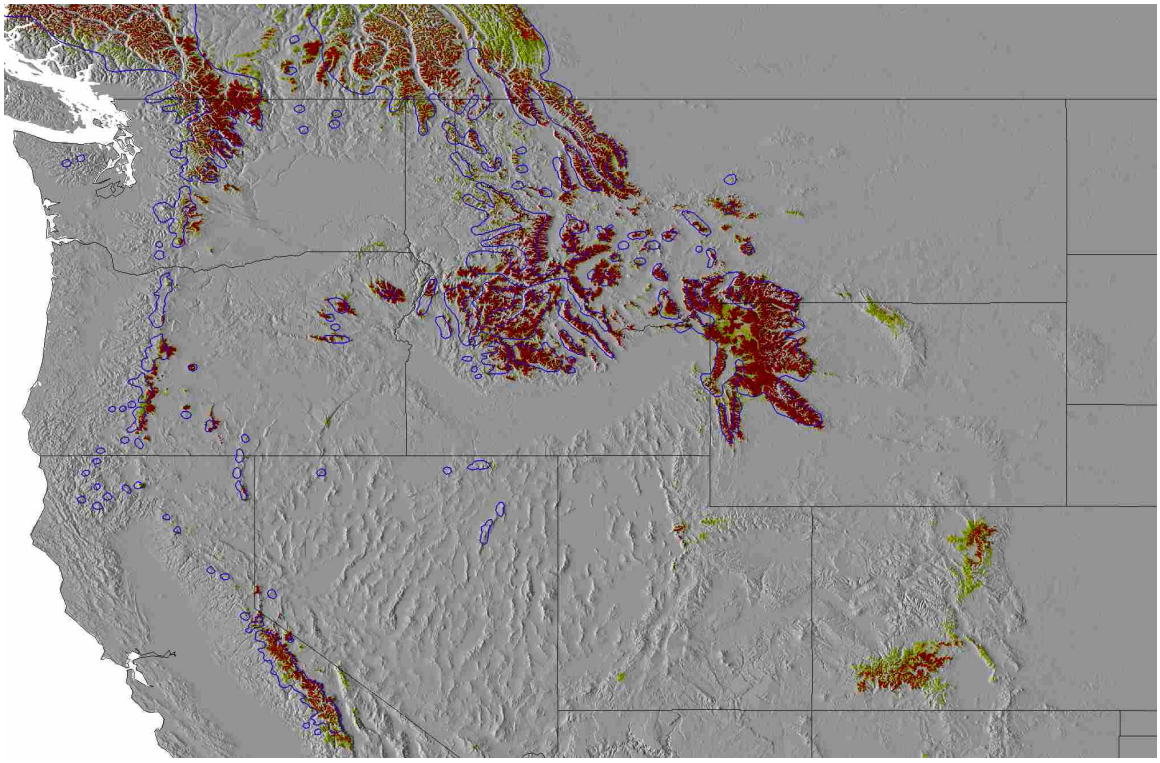
Fig. 8- Understory ground cover arranged by plant functional group under trees at three different stages of mountain pine beetle infestation in whitebark pine.

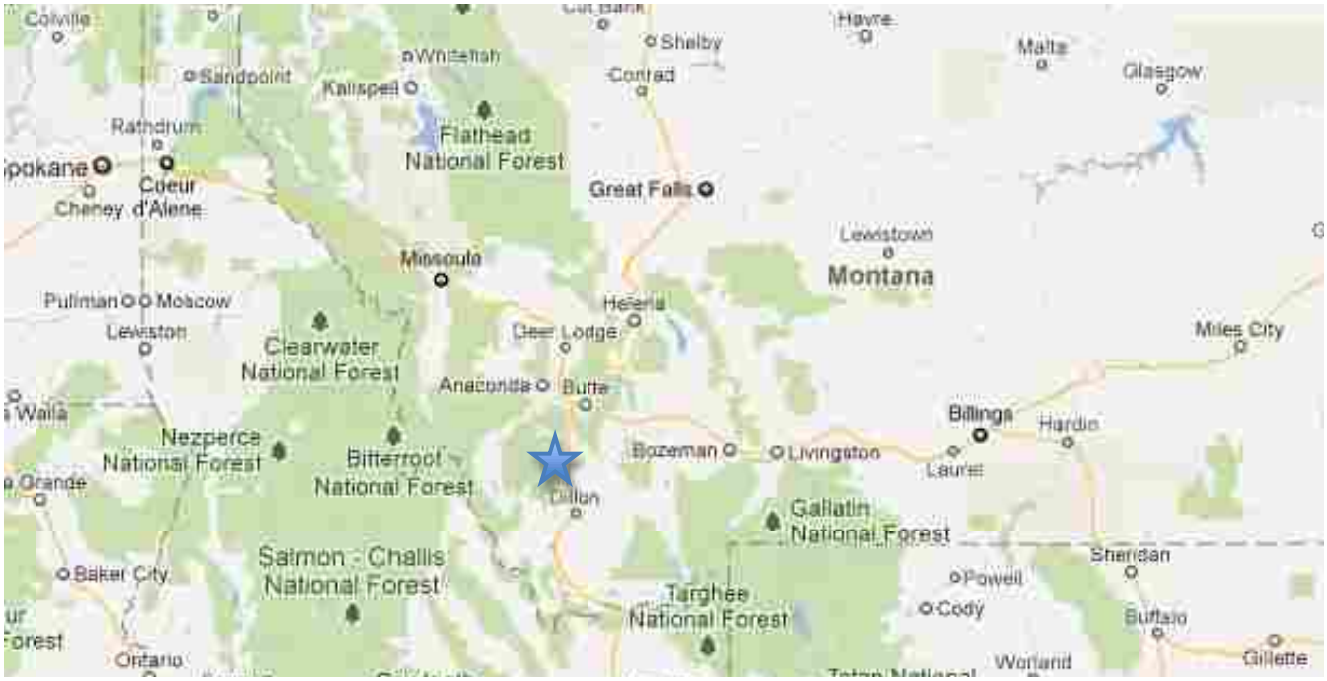
Fig. 9- Inorganic N ($NH_4^+$ and $NO_3^-$) pools in soils across three stages of beetle infestation in whitebark pine. The left side of the figure displays values from the upper organic soil horizon and right side the lower, mineral horizon. Stages with different letters are significantly different based on ANOVA and Tukey's HSD test (p=0.05) Error bars denote one standard deviation.

Fig. 10- Soil $CO_2$ efflux measurements throughout the growing season of 2010 under whitebark pine at three different stages of beetle infestation. Error bars denote one standard deviation.

Fig. 11- Soil respiration measured in a lab incubation under four different litter and soil combinations collected from whitebark pine at different stages of mountain pine beetle infestation. Hours represent time since 0.5 g litter addition to 10 g soil. In the key, the first color signifies the beetle infestation stage of the soil, and the second color the infestation stage of the litter. Error bars denote one standard deviation.

## Figures

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Pullman
Moscow
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Helena
Deer Lodge
Miles City
Clearwater
National Forest
Anaconda
Butte
Billings
Hardin
Nezperce
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Bitterroot
National Forest
Bozeman
Livingston
Laurel
Dillon
Gallatin
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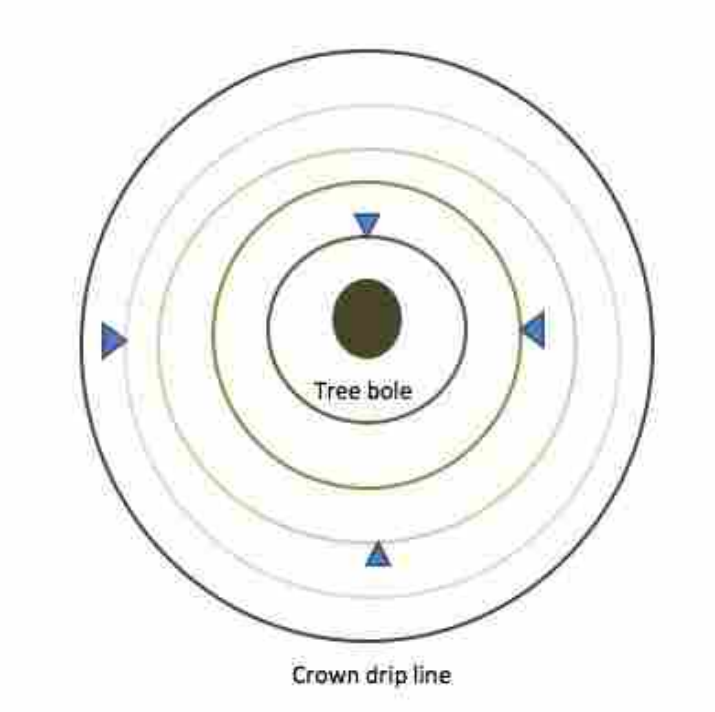
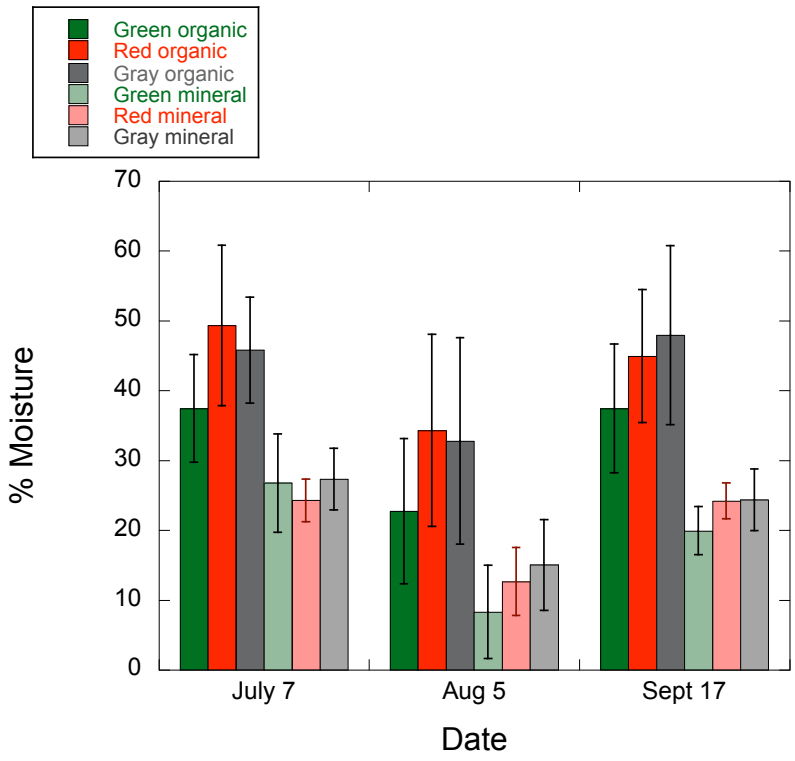

Tree bole
Crown drip line


Green organic
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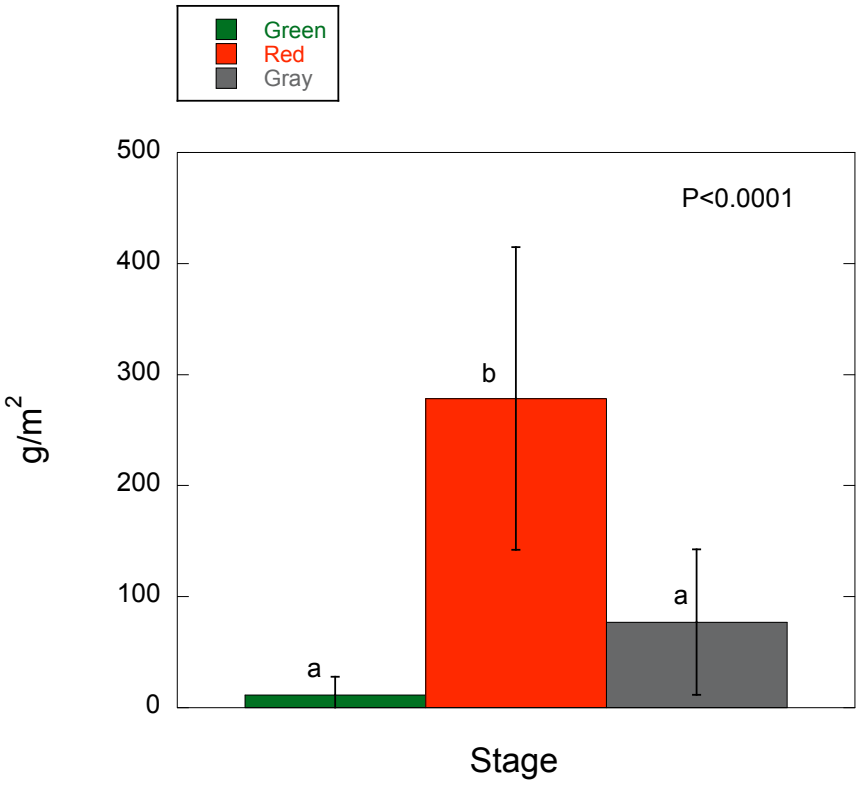
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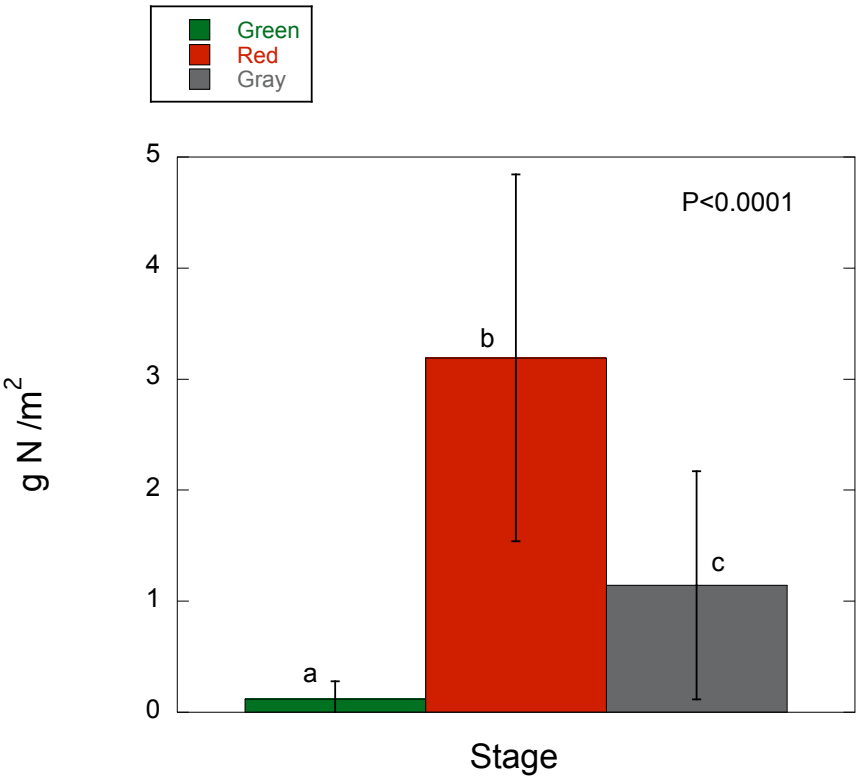
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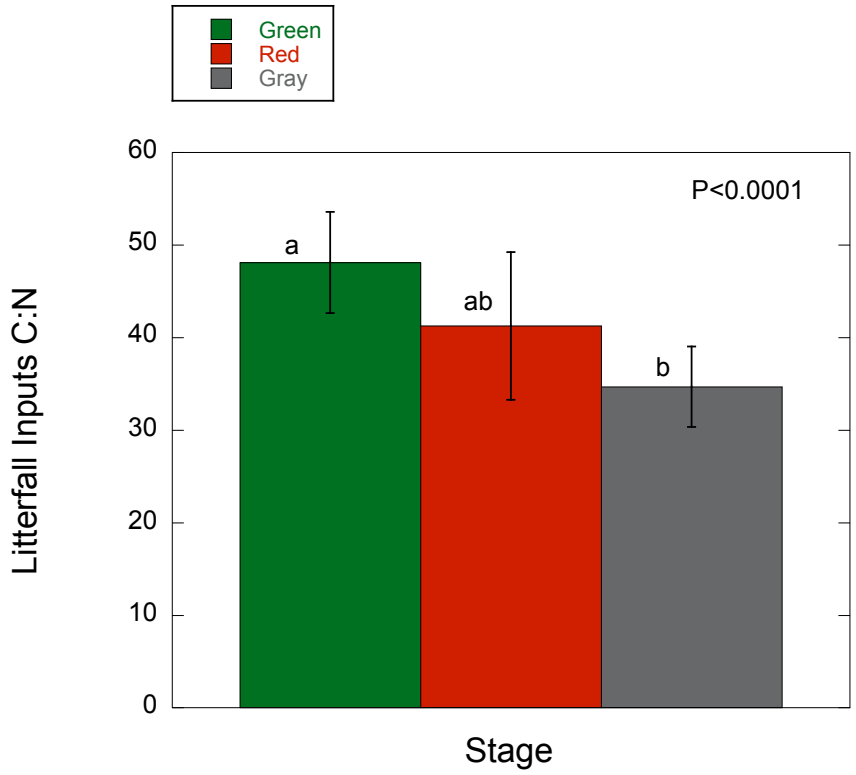
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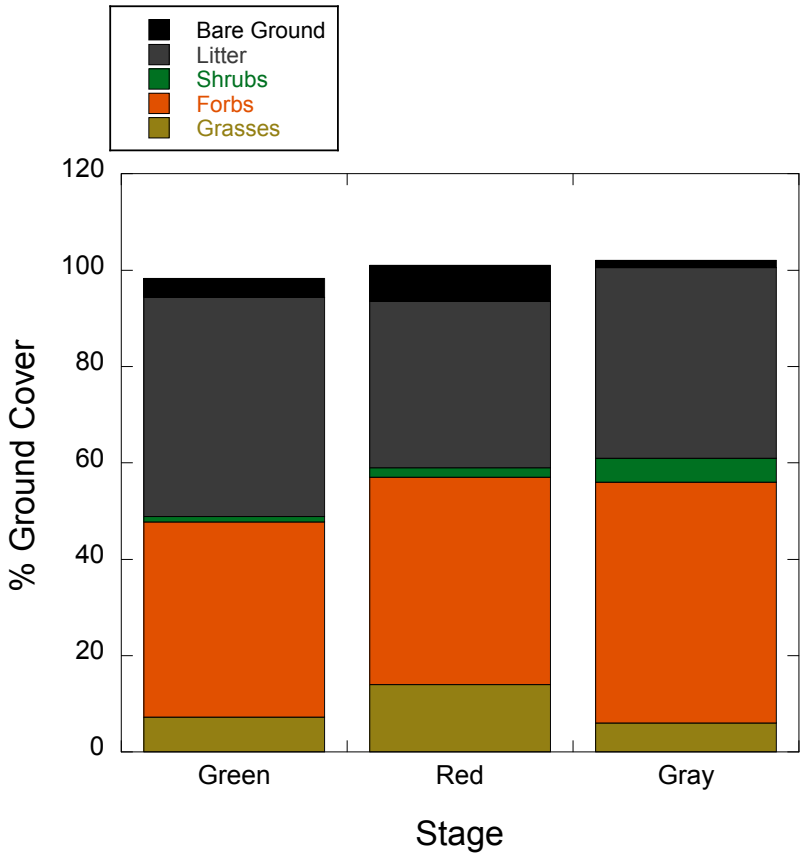
Bare Ground
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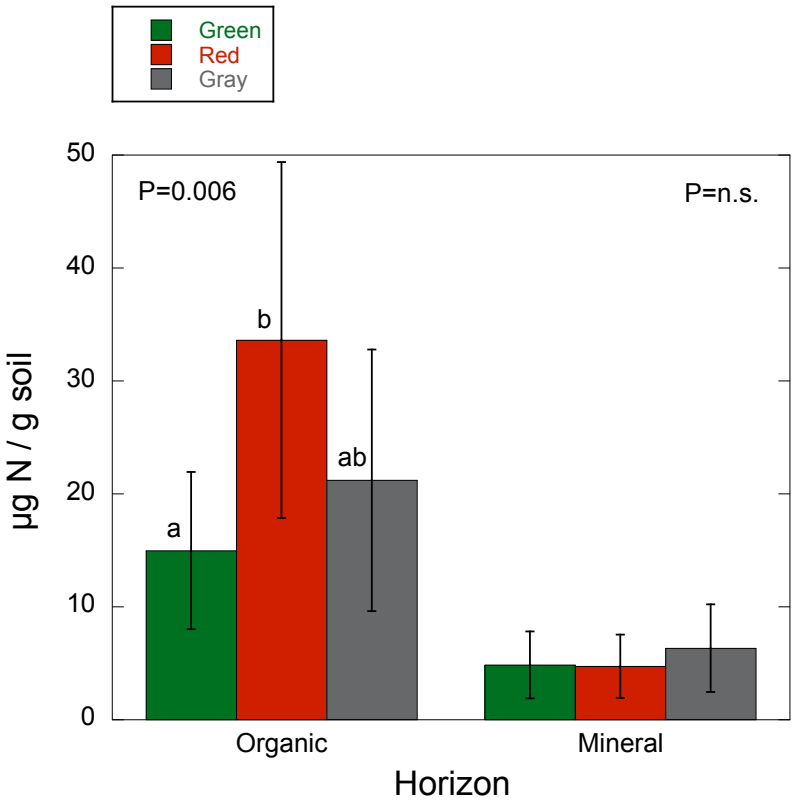
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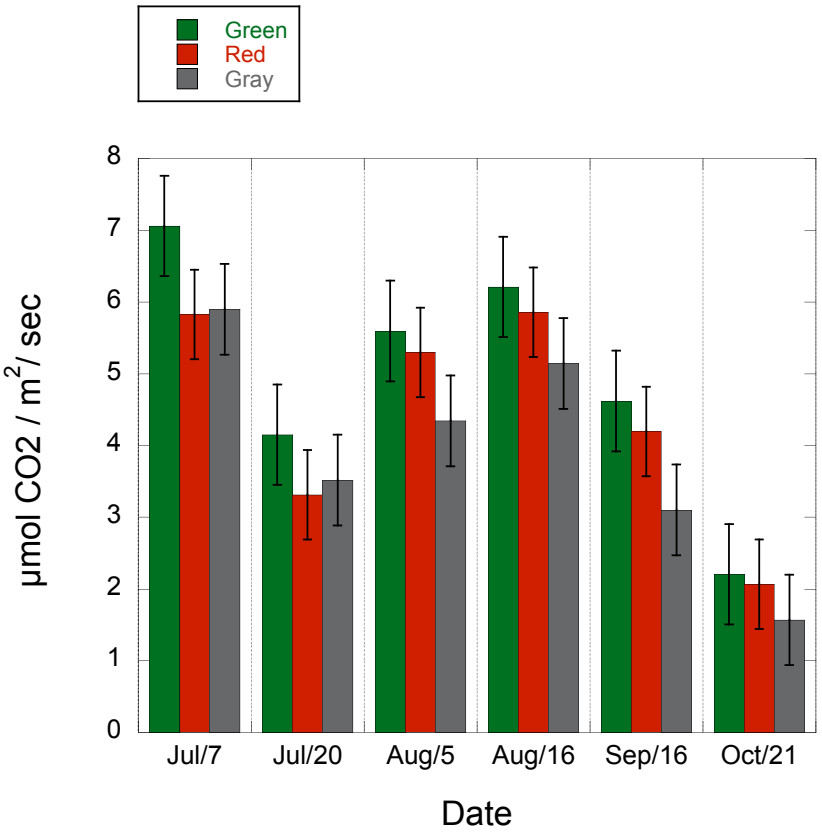
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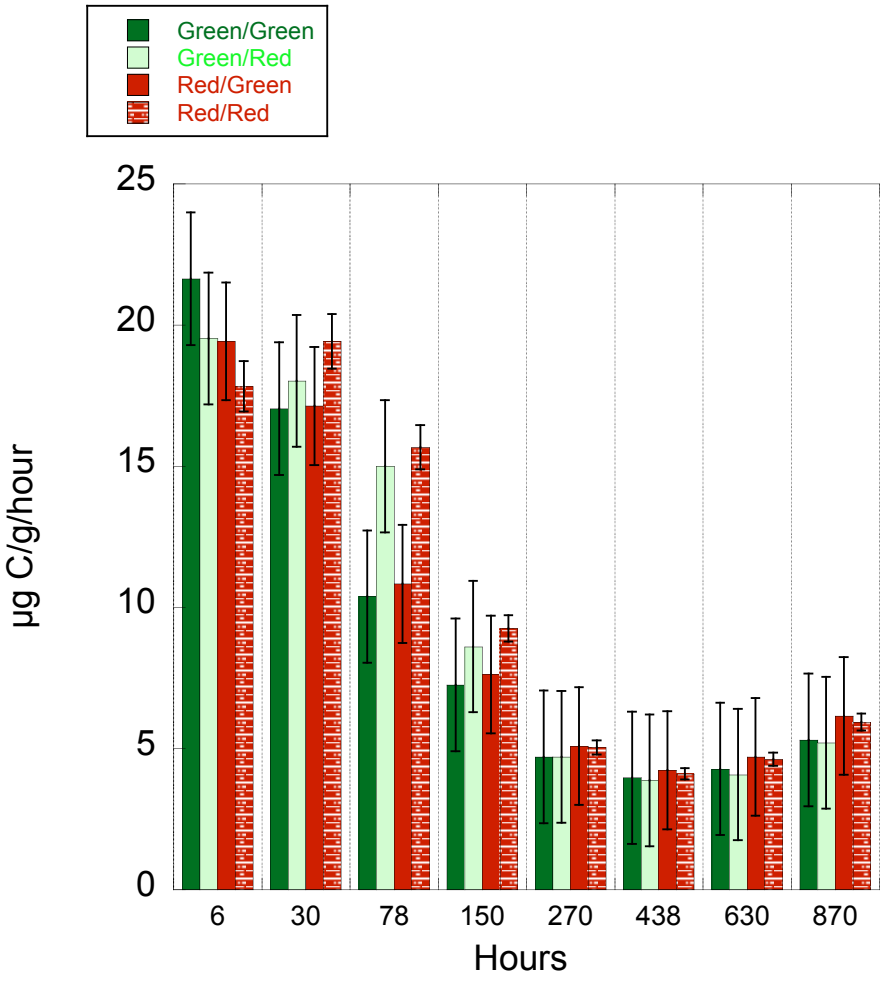
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630
870
Hours