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Fire exclusion forest dynamics and nitrogen cycling in low elevation forests of western Montana

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Fire Exclusion, Forest Dynamics and Nitrogen Cycling in Low Elevation Forests of Western Montana

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PRESENTED IN PARTIAL FULFILLMENT
FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

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ABSTRACT

MacKenzie, M.Derek, Ph.D., April 2004 Forestry

Fire Exclusion, Forest Dynamics and Nitrogen Cycling in Low Elevation Forests of Western Montana

Chair: Thomas H. DeLuca THD

Co-chair: Anna Sala AS

Little is known about the effects of fire exclusion on forest dynamics and nutrient cycling in the dry inland Northwest. Historically, low elevation forests of western Montana burned more frequently and with a lower severity than today. I established a chronosequence of sites in western Montana, from 2 to 130 years since fire to look at the long-term effects of fire exclusion and performed experiments to determine the mechanism of change. Research objectives included an analysis of: 1) forest dynamics and diversity; 2) forest floor nitrogen (N) mineralization; 3) mineral soil N mineralization and microbial activity; 4) seasonal N mineralization and microbial activity with increasing time since fire; and experiments to determine 1) the plant-specific effects on N mineralization and microbial activity; and 2) the influence of fire produced charcoal on N cycling. Stand basal area increased primarily as a result of increasing Douglas-fir establishment and growth. Understory composition shifted from grass and forb dominated, to a mixed understory of grasses, forbs and shrubs. Increases in above ground biomass resulted in an increased forest floor thickness, with higher contents of total carbon (C), total N, potentially mineralizable N (PMN) and total phenols. Biodiversity increased rapidly and then plateaus 25 years after fire. Mineral soil N decreased rapidly and also plateaus between 25 and 50 years since fire. Together these results appear to be evidence for the ecological 'foot-print' of the natural disturbance regime. The rate of decomposition decreased the metabolic quotient increased indicating microbial stress. Total phenols may interfere with organic matter mineralization either directly, by inhibiting microbes or indirectly, by precipitating humic compounds and slowing the N cycle. Total phenols increased in the forest floor and were negatively correlated with nitrification in the mineral soil suggesting ecosystem allelopathy. Experimental analysis of different plants indicated that ericoid shrubs had a negative influence on N cycling, but charcoal additions offset the effect. In summary, this work suggests that fire drives N cycling for approximately 25 years in this ecosystem, as mitigated by the influence of charcoal. After this period, it seems that woody plants begin to drive N cycling with allelochemicals.

DEDICATION

This work is dedicated to my family, my parents Mike and Sandi, and my brother Graham. Thank you for encouraging me to achieve all my goals in life.

ACKNOWLEDGMENTS

I would like to thank my two co-supervisors, Tom DeLuca and Anna Sala. Tom has been an inspiration to me as an academic supervisor and as a friend. He accepted me into his life and his family, and it has been a great privilege to get to know both. Missoula truly became a home away from home because the DeLuca's were a surrogate family second to none. As an academic Dad, Tom has been the best; by his very nature he creates a learning environment that is both fun and challenging.

Anna took me in when I first got to campus, while Tom was on sabbatical. The year I spent with her lab was very rewarding; as were her words of wisdom and the kick in the pants she gave me to start the initial phase of my research. Tom and Anna come at research from very different, but complimentary perspectives and working with them together has given me the best of both.

The rest of my committee members have also been instrumental in my successes, so I would like to thank Matthias Rillig, Ray Callaway and John Goodburn. Many graduate colleagues have contributed time in the field, collecting samples and discussing various ecological topics, including: Michael Gundale, Valerie Kurth, Rachel Loehman and Damien Dubrowin. I have also had many research assistant and laboratory technicians that I would like to thank, including: Jen Shoumaker, Eric Sawtelle, Nathan Hilliard, Sthephen (Pete) Grum, Tricia Burgoyne, Brad McWilliams and Tracey Graafstra.

Lastly, this project would not have been possible were it not for the financial support of a McItire-Stennis fellowship, an NSF grant, a USDA grant and the support of the College of Forestry and Conservation.

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INTRODUCTION

Little is known about the effect of fire exclusion on forest dynamics and subsurface processes in the dry inland Northwest. Fire has been excluded from much of this region during the last 100 years due to Euro-American settlement. Historically, ponderosa pine (*Pinus ponderosa* P.&C. Lawson) forests appeared to experience low severity fires with a return interval of 20 to 50 years. Low severity fire regimes promoted open stands of mature ponderosa pine with an understory of grass and forb species. Today, many ponderosa pine stands around western Montana have not burned for over 130 years and the visual evidence based on historic photograph collections is impressive. Photographic evidence suggests that ponderosa pine forests are being encroached on a large scale by Douglas-fir (*Pseudotsuga menziesii* [Biessn.] Franco) in the overstory, and by woody shrub species in the understory. Fire exclusion may have caused these forests to miss 3 to 4 disturbance events that would have effectively re-set forest successional "clocks".

Fire exclusion raises several questions about the way ecosystem function may be changing in ponderosa pine stands in the dry inland Northwest. The focus of my dissertation is to address several specific research objectives related to fire exclusion in low elevation forests of western Montana. These objectives include analyzing the effect of fire exclusion on: 1) forest dynamics and diversity; 2) forest floor N mineralization; 3) mineral soil N mineralization and microbial activity; 4) seasonal N mineralization and microbial activity; 5) plant type specific effects on sub-surface processes and 6) the effect of fire deposited charcoal on N turnover and microbial activity.

Dissertation Overview

This dissertation was written in partial fulfillment of the Ph.D. requirements at the University of Montana and is comprised of the usual accompanying material, a review paper and four research papers that address the objectives above. The title of each paper is given below with a brief overview of its continents.

Paper 1: Forest fire exclusion and potential modifications to forest dynamics and nitrogen cycling in the dry interior Northwest.

This paper provides the basic background and perspective for the research. It reviews the current understanding of the N cycle and recent developments, including recent evidence that some plants have the ability to access organic N, or to produce allelopathic chemicals, both of which indicate that plants are not just passively involved in N acquisition. It also examines the evidence for forest dynamics both pre- and post-Euro-American settlement, and the effect of fire as a vector of natural disturbance. In the context of these two driving factors, fire exclusion has the potential to change N mineralization by allowing post-disturbance plant communities to take a more active role in the N cycle.

Paper 2: Forest structure and organic horizon analysis along a fire chronosequence in the low elevation forests of western Montana.

For this paper, a chronosequence of time since fire was established using 25 different poderosa pine/Douglas-fir sites in western Montana, located at various positions on the landscape. The forest floor is a integral part of forest nutrient cycling, but one that is commonly missed. The organic horizon is also significantly reduced after fire and yet N mineralization has been measured to be high after fire. Vegetation structure and composition, and forest floor biochemistry were studied to examine whether and how stand dynamics and organic horizon nutrient cycling change with time since fire. This paper was submitted for publication to the Journal of Forest Ecology and Management January 10th, 2004.

Paper 3: Fire exclusion, nitrogen mineralization and biodiversity in low elevation forests of western Montana.

As a continuation of the research addressed above, a sub-set of fire chronosequence sites, with similar environmental characteristics (slope, aspect and elevation), were identified and analyzed for plant divertiy and mineral soil nutrient cycling. Mineral soil biochemistry was analyzed to determine if the hypothesized reduction of available N with increasing time since fire occurs and whether this reduction can be correlated to microbial activity and increases in alleochemicals on site. Plant diversity was measured to examine whether shifts in plant community composition are related to or responsible for the expected changes in N cycling. This paper was submitted for publication to the Journal of Ecological Applications May 10th , 2004.

Paper 4: Seasonal Nitrogen Availability and Microbial Activity in Fire Excluded Low Elevation Forests of Western Montana.

The same sub-set of fire chronosequence sites was examined for seasonal levels of inorganic N, ninhydrin reactive N, anthrone reactive C and soluble phenols. These compounds were extracted from ionic and non-ionic resin capsules installed at three different times during the year and left to incubate for several months. Ninhydrin reactive N, anthrone reactive C and soluble phenols all represent indicators of microbial activity. Analyzing these compounds with resin capsules is a novel technique in soil science representing *in-situ* incubations. As a novel techinique in forest soil research it merits further investigation.

Paper 5: Comparing litter quality and N mineralization in the understory of Ponderosa pine/Douglas-fir forests.

Two different plant types (*Artostphylos uva-ursi* and *Carex spp.*) were selected to assess biochemical microsite differences created by plants. Leaf extract allelopathy was analyzed in a bioassay of seed germination (*Populus tremuloides*). Factorial combinations of glycine (labile organic N source) and charcoal were applied to greenhouse and field incubations of litter and mineral soil samples from the two plant types. These treatments were designed to determine if the rate of mineralization is substrate limited or inhibited by the production of allelochemicals. It was also designed to examine how different plant functional groups affect N mineralization.

Forest fire exclusion and potential modifications to forest dynamics and nitrogen cycling in the dry interior Northwest

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Abstract: The exclusion of fire over the last 100 years has changed forest dynamics in the inland dry Northwest. Low elevation forests used to be maintained by a low severity fire regime with a return interval of 10 to 50 years. Fire is known to promote N mineralization and modify plant community dynamics in the short-term, but the effect on of excluding fire from ecosystem processes, well beyond natural successional trends, is completely unknown in this ecosystem. This paper attempts to synthesize what is known currently about ecosystem function from this ecosystem and others, and to produce hypotheses for the effects of fire exclusion that can be tested by observations and experiments. To this end, three subjects are reviewed in detail, including: the N cycle, fire exclusion in low elevation forests and the potential for allelopathic interactions on fire excluded sites. Fire exclusion is believed to increase woody plant dominance on these sites and decrease N availability. However, very few data exist from natural systems and the mechanisms by which fire exclusion may reduce N availabiltiy including allelopathic interactions are poorly understood.

Keywords: Fire history, N fixation, Allelopathy, Euro-American settlement, Fire regime, Microbial uptake, Organic N uptake

INTRODUCTION

Fire is a significant natural disturbance in most western ecosystems including that of the dry inland Northwest. Fire exists on the landscape in many different forms, including high severity fires that kill all plants on site at one end of the spectrum and low severity fires that kill the understory, but maintain the mature trees at the other end of the spectrum. Low elevation forests of the inland Northwest were characterized by frequent low severity fires until approximatly 100 years ago during settlement. The potential for fire exclusion to affect forest dynamics and sub-surface processes is great, but has only been studied in the short-term to date. Fire acts to shape forest structure and nutrient cycling, two important components of ecosystem function. As a disturbance regime, fire acts to re-set successional clocks and drives forest structure based on the intensity of disturbance. Ecosystems tend to adapt to the most prevalent disturbance regime and reflect the disturbance type in their species composition and diversity. Therefore, removing disturbance has the potential to change successional pathwasy by allowing plant communities to develop beyond disturbance regulated parameters. This is also true of sub-surface processes including microbial activity and nutrient turn-over. Specific types of disturbance have different effects on sub-surface processes, but in general stimulate nutrient mineralization by adding organic matter, increasing light interception and increasing water resources. In the absence of disturbance, sub-surface processes may decline for a number of different reasons including allelopathic interactions between plant communities, litter quality and the microbial community. The potential for allelopathic properties to develop in ecosystems pushed beyond their disturbance-maintained equilibrium has been documented in other ecosystems and will also be reviewed here.

This review is organized into three parts, each based on one of the three main objectives. The objectives of this review include: 1) outlining the N cycle; 2) summarizing forest dynamics and fire exclusion in the ponderosa pine-Douglas-fir forests of western Montana; and 3) reviewing allelopathy and its potential to affect ecosystem function in fire excluded sites.

THE NITROGEN CYCLE

Nitrogen Fixation

Nitrogen is an important plant nutrient and one of the most limiting in terrestrial ecosystems (Vitousek and Howarth 1991). It is abundant in the atmosphere, where N_2 gas represents 78% of atmospheric gases, however in this form it is unavailable to plants and first must be fixed to a bioavailable form (Vitousek and Howarth 1991). Nitrogen-fixation is carried out by several different abiotic processes (lightning, chemical), but of specific interest to this paper are the biological processes (Fig. 1). Several different genera of symbiotic and free-living micro-organisms are capable of fixing N (Paul and Clark 1996), including 40 genera of Cyanobacteria and 4-5 genera of Rhizobia. The process requires large amounts of energy to break the triple covalent bonds between the two N atoms of atmospheric N_2 gas (Stevenson and Cole 1999). This energy is delivered from plants to symbiotic N-fixers in the form of reduced C compounds; in the case of free-living N-fixers, reduced C is produced by decomposition of labile C pools from soil organic matter (SOM). The process is limited by the availability of the key enzyme nitrogenase and, as it is energy costly, N-fixation is very efficient. Nitrogen fixation is potentially the most important life maintaining biological process next to photosynthesis (Stevenson and Cole 1999). Reduced atmospheric N is generally not lost from the site of fixation, but rather immobilized by microbes and made available to host plants only slowly (Sculten and Schitzer 1998). Non-symbiotic plants can only obtain fixed N that has entered the N cycle at some point and become available through N mineralization (Fig. 1).

Nitrogen Mineralization

Heterotrophic soil microbes mineralize soil organinic matter (SOM) as a source of energy, to secure C skeletons for building biomass and to access NH_4^+ for metabolic purposes (Stevenson and Cole 1999). Any N that is in excess of demand is released into the soil medium. Plants have access to the N that is in excess of microbial requirements for growth. As an average, soil microbes require 1 N for every 6 C that they assimilate into biomass (4:1 bacteria and 8:1 fungi) (Paul and Clark 1996). However, the energy to produce heterotrophic microbial biomass comes from SOM oxidation, where C is respired three times as fast as it is assimilated. Therefore the average microbial requirement of C and N is more like 18 to 1 (12:1 bacteria and 24:1 fungi) (Sculten and Schitzer 1998, Stevenson and Cole 1999). In soils with a ratio of less than 18:1 there should be net mineralization of N which could be available to plants for uptake. In soils with a C to N ratio more than 30:1 net immobilization of N by the microbial community will limit plant available N.

Organic N is mineralized by the microbial community to form many different inorganic ions, but many of these represent losses of N by denitrification and will not be discussed here. There are 3 inorganic N ions that important for plant nutrition. These ions are mineralized in succession and include: ammonium (NH_4^+), nitrite (NO_2^-) and nitrate (NO_3^-) (Stevenson and Cole 1999). Ammonification is the process by which organic N is mineralized to NH_4^+ which can be used by plants or microbes, as mentioned above. Ammonium in excess of plant and microbe nutritional requirements may be oxidized as a source of energy by a specific group of microbes (chemo-autotrophs) in a process called nitrification. Nitrite is produced first by a genera of bacteria called Nitrosomonas and rapidly converted to NO_3^- by a genera of bacteria called Nitrobacter (Paul and Clark 1996, Stevenson and Cole 1999). Heterotrophic nitrification may also occur, but at this time its ecological significance is not well understood (Stevenson and Cole 1999). Nitrite is toxic to most organisms because it is a strong oxidant and interferes with the transfer of electrons. It is rapidly oxidized to NO_3^- by the microbial community, therefore only NH_4^+ and NO_3^- accumulate in most soils and are the predominant forms of inorganic N used by plants (Raven 1992). This overall process is called mineralization and results in the release of inorganic N, while the same reactions in the reverse direction are called immobilization and result in the conversion of inorganic N into organic N by plants and microbes (Brady and Weil 1999).

Plant Uptake – Inorganic N

Nitrogen is the most important macronutrient after C, H, and O. It is used in many biochemical processes at the cellular level and is a key constituent of amino acids, DNA, RNA, chlorophyll and hormones to name a few (Stevenson and Cole 1999). The accepted doctrine in plant biology today is that plants must rely on soil micro-organisms to release inorganic N through the mineralization of SOM (Keiland 1994, Lipson et al. 1999, Hodge et al. 2000). Plant uptake of inorganic N is then dependant on the ratio of C to N, where only N in excess of microbial demand is available. Ammonium is the preferred form of N uptake in most ecosystems based on availability, but in some cases NO_3^- is preferred (Stevenson and Cole 1999). Nitrate in the plant must be converted to NH_4^+ before it can be incorporated into plant biomass in the form of glutamine. This is an energy expensive process called assimilatory NO_3^- reduction and is catalyzed by three different enzyme systems, the first of which, nitrate reductase is the rate limiting step (Stevenson and Cole 1999). As such, NO_3^- has a metabolic cost associated with it that NH_4^+ does not.

As an anion, NO_3^- is not part of the cation exchange capacity (CEC) of soils and is subject to loses by leaching (Fig. 1). Another significant loss of NO_3^- occurs in saturated soils where the NO_3^- ion is used as an electron acceptor in the absence of oxygen. This process is called dissimilatory NO_3^- reduction or denitrification (Stevenson and Cole 1999)(Fig. 1). For these two reasons, NO_3^- may be considered a liability in the context of N-fixation, a high energy consuming process.

When measured in soil, the pools of NH_4^+ and NO_3^- are very small and transient and do not tell us much about the internal and external N cycle (Fig. 1). The difference between gross and net N mineralization gives a better estimate of mineralization, immobilization and turnover (Hart et al. 1994, Stark and Hart 1997), but in some ecosystems gross N mineralized still does not account for the total plant biomass being produced (Keiland 1994). Many studies in ecology have shown that net N mineralization, as measured in-situ, is not enough to account for the biomass accumulating on certain sites (Keiland 1994, Nasholm et al. 1998, Lipson et al. 1999, Lipson and Nasholm 2001, DeLuca et al. 2002). The conventional model does not seem to account for N turnover and this evidence seems to suggest that something between the internal and external N cycle is being short-circuited. Recent findings suggest that plants have evolved a means of accessing organic N reserves in SOM (Keiland 1994, Nasholm et al. 1998, Lipson et al. 1999, Lipson and Nasholm 2001).

Organic N in Mineral Soil

As we have seen from the previous sections, the most important reactions for N, in terms of bioavailability, are fixation, mineralization and immobilization. Nitrogenase is the enzyme that catalyzes the fixation reaction among symbiotic and free-living micro-organisms and has been called the most important biochemical after chlorophyll (Brady and Weil 1999). Mineralization of SOM makes N available to plants and microbes as inorganic N. It is immobilization of N, however, that reduces N to organic forms and removes it from labile pools.

The composition of N compounds in the soil are on average 80% organic, being made up of proteinacious material (40%), amino sugars (6%) and heterocyclic N (35%), while the remaining N is inorganic and mostly found as NH_3 (19%) (Sculten and Schitzer 1998). These different compounds are determined by different techniques of analysis on the hydrolyzable and non-hydrolyzable portions of SOM. While neither of these pools are bioavailable they do shed light on the nature of the SOM as it relates to the internal N cycle (Fig. 1). These pools also indicate that all forms of organic N are available to mineralization, but cycle through at different rates due to molecular complexity (Sculten and Schitzer 1998). Heterocyclic N, representing the old and stabilized SOM, is far less available than NH_4^+ or labile pools of amino sugars (Fig.1). The question is 'What is the availability of proteinacious material that represents 40% of SOM?'. Proteins and amino acids (AA) dominate organic N sources and are affected by three processes in the soil: proteolysis or the enzymatic decomposition of proteins to constituent AA, reactions that bind proteins and AA to the soil matrix and competition between plants and microbes (Lipson and Nasholm 2001). Therefore some proteinacious material is tied up in the stabilized SOM, but proteolysis makes some available to microbes for mineralization. Is it available to plants?

Plant Uptake - Organic N

Recent work has confirmed that many plants can take up organic N (Hodge 2000; Lipson and Nasholm 2001). These findings put conventional thinking into contention. If plants can access organic N then they can short circuit the N mineralization process. Most terrestrial ecosystems can be thought of as overflowing with organic N and therefore should not be N limited. However, most organisms are still limited by N availability and of the extent to which plants take up organic N, may explain why N limitations still exist.

Keiland (1994) determined that N mineralization in arctic environments was not sufficient to account for the amount of biomass produced, but that organic N was plentiful. Therefore, he measured the uptake of three amino acids with ^{14}C labeled substrate and compared it to ammonium uptake as ^{14}C labeled methyl amine. The results showed that various different types of plants including grasses, forbs, deciduous and evergreen shrubs all took up amino acids, but only glycine was taken up in the same quantity as ammonium. There were also some differences between plant functional groups where deciduous shrubs took up more organic N than evergreen shrubs.

Some problems exist with the methods of this study. The use of labeled C to identify amino acid uptake may underestimate the total amount due to root respiration, because the carboxyl group is more readily cleaved off than the other C bonds (Nasholm et al. 1998). Also, the ^{14}C method must be performed in the lab due to radioactivity and therefore is partially removed from the reality of field interactions. Last, the use of methyl amine as a surrogates for NH_4^+ is inappropriate as some degree of mineralization will have to occur and therefore uptake rates will not be similar. It would be better to label the N and use NH_4^+ directly. Still, the work served to inspire more work into plant uptake of organic N as a potentially important ecological process and created much debate.

The problem with using a N labeled substrate alone is that it would not be possible to differentiate between N taken up as amino acids or as ammonium in the plant (Nasholm et al. 1998). By using a double labeled amino acid and looking for both C and N signatures in the plant we can tell if the organic molecule was taken up whole or as mineralized N. In a recent study, Nasholm et al. (1998) used double labeled C and N to measure amino acid uptake as compared to ammonium uptake in Boreal forests. By looking at the excess amounts of these isotopes in plants they found significant differences and concluded that organic N uptake can be a significant part of plant N nutrition in Boreal forests. Again, glycine was found to be used by plants directly, instead of first passing through the N cycle. However, a large portion (64%) of the added substrate was in SOM compared to the 27% taken up by plants indicating high microbial competition. Microbial competition is one of the headline debates in current plant organic N use studies. Another is the microbial mediated transfer of nutrients to plants.

Interactions Between Plants and Microbes

Symbiosis with N-fixing species of bacteria is obviously a evolutionary adaptation that plants and microbes have developed to gain access to N, where some of the N is delivered to plants in an organic form and so outside of conventional wisdom on plant available N. Some researchers have suggested that plants gain organic nutrients from other microbial associations and many studies have looked at the acquisition of organic nitrogen by mycorrhiza (Abuzinadah and Read 1986, Keller 1996, Hodge et al. 2000, Emmerton et al. 2001). Mycorrhiza are a symbiotic association between plants and fungi, similar to the symbiotic relationships between N-fixing bacteria and plants, but where plants exchange sugars for nutrients and water (Paul and Clark 1996). The mycorrhizal association developed through evolutionary feedbacks that give plants a competitive advantage in nutrient acquisition as facilitated by the fungi (Paul and Clark 1996). Mycorrhizal fungi occur ubiquitously in nature and have several different growth forms usually associated with different plant functional groups.

Ectomycorrhiza penetrate plant cells and have been shown to take up organic nitrogen very efficiently (Keller 1996). Ericoid mycorrhiza, associated with the family Ericaceae, also penetrate plant cells and have been shown to take up organic N effectively (Emmerton et al. 2001). The debate is whether or not plants are gaining organic N intracellularly delivered directly by the fungi and therefore avoiding the problem of active transport across the cell membrane of a large molecule, or is N being delivered to plants in an inorganic form, already mineralized by the fungi (Abuzinadah and Read 1986, Nasholm et al. 1998). Delivery as an inorganic source would explain the existence of a N tracer by itself in plants, but recent work shows that plants seem to be acquiring the intact organic N molecule (Nasholm et al. 1998), most likely from the fungi. It has also been shown that endomycorrhiza and non-mycorrhizal species take up organic N suggesting that some plants are taking organic N from the soil and have the ability to transport these larger molecules across membranes (Nasholm et al. 1998).

In summary, many interactions among the biotic and abiotic factors of the N cycle make it very complex. Nitrogen has many valence states making it attractive two many different types of organisms, it is an essential nutrient and is required in high concentrations. Nitrogen is also the catalyst for many inter-trophic interactions between plants and microbes. In the following sections I will examine how fire exclusion may affect N cycling in the low elevation forest of western Montana and whether or not allelopathy may play a role in N mineralization in these systems.

FIRE EXCLUSION

Historic Fire Regime

Before Euro-American settlement (≈1900), the return interval for low intensity fires in the ponderosa pine-Douglas-fir ecosystems of the dry inland Northwest was on the order of 10-50 years (Arno et al. 1995, Arno et al. 1997, Barrett et al. 1997). These low intensity fires were characterized by understory burning that removed most of the competing grasses, shrubs, smaller trees and some of the larger trees. Ponderosa pine is a shade intolerant, early seral species that has high fire resistance due to very thick bark. It can co-dominate with Douglas-fir, a more shade tolerant, late seral species with low fire resistance. There is good evidence to suggest that the frequent occurrence of low intensity fires in the understory of these forests allowed ponderosa pine to maintain dominance in the canopy (Arno et al. 1995, Arno et al. 1997, Barrett et al. 1997). It is believed that the forest structure of pre-settlement times consisted of an open parkway with widely spaced, uneven-aged ponderosa pine trees in the canopy and a mixture of forbs, grasses, shrubs and seedlings of both ponderosa pine and Douglas-fir, recovering from the last fire event, in the understory.

During post Euro-American settlement (1900 to present) the structure of ponderosa pine-Douglas-fir forests has been changing due to forest harvesting techniques and the exclusion of low intensity fires (Brown et al. 1994, Arno et al. 1995, Arno et al. 1997, Barrett et al. 1997). Lower elevation forests represent an easily accessible resource that was exploited thoroughly in western Montana. It is difficult to find any old-growth Ponderosa pine forests that have not experienced some level of anthropogenic disturbance (Arno et al. 1995, Arno et al. 1997). These studies indicate that harvesting the largest pine trees accelerates forest succession towards Douglas-fir dominated forests. Fire exclusion is also having a negative effect on the dominance of Ponderosa pine in these lower elevation forests. Brown et al. (1994) calculated that fire is almost three times less common on the northern Rocky Mountain landscape than it was in pre-settlement times, and that the ratio of low intensity fires to stand replacing fires has changed from 100:0 in pre-settlement times to 20:80 today. The resulting stand structure found in many low elevation forest is comprised of closed canopy conditions where Ponderosa pine and Douglas-fir are co-dominants and there has been a shift in understory species to more shade tolerant shrubs (Arno et al. 1995, Arno et al. 1997, Naumburg and DeWald 1999).

Computer simulations have shown that a fire return interval of 20 years restricts Douglas-fir from the canopy, while simulations of fire exclusion show that by 100 years since fire, Douglas-fir enters the canopy and by 200 years, has out-competed Ponderosa pine for canopy dominance (Keane et al. 1990). Post-fire exclusion stand structure has resulted in increases in basal area (BA) and understory biomass, and is thought to have decreased in nutrient availability (Arno et al. 1995, Arno et al. 1997, Barrett et al. 1997). These changes are thought to have a long-term negative effect on overall stand productivity (Covington and Sackett 1992, DeLuca and Zouhar 2000) and ecological function, however no studies have attempted to quantify long-term understory differences and sub-surface processes with increasing time since fire for low elevation forests of the dry inland Northwest.

Forest Dynamics

Short-term changes in stand structure with fire exclusion can affect ecosystem functioning in Ponderosa pine/Douglas-fir forests (Busse et al. 1996, Naumburg and DeWald 1999, Newland and DeLuca 2000). As time since fire increases, overstory density increases and these forests move from an open to a closed canopy. The overstory changes have an affect on understory structure and composition, resulting in different ecophysiology.

Naumburg and DeWald (1999) showed that a reduction of light in the understory had a negative effect on graminoid species presence and abundance. Graminoid distribution is directly influenced by two factors, tree density and seasonal distribution of direct sun. With increases in tree density and decreases in direct sun, graminoid presence and abundance decreased with time since fire. Newland and DeLuca (2000) have also shown decreases in the number of species and abundance of N-fixers with increasing time since fire. N-fixing plants may have an important role in N availability after fire and may have an effect on long-term productivity, but need disturbance and open space to establish. Changes in the amount of forest floor litter, which increase with time since fire (DeLuca et al., 2002), and the loss of seed banks and microbial communities may affect the colonization rate of N-fixers even if fire is re-introduced. Busse et al. (1996) studied site productivity in Ponderosa pine forests when understory vegetation was removed for a 35 year period. They showed that the presence of understory vegetation affected both N and water availability and found that understory removal treatments increased BA and growth rates for the first 20 years, but that the last 15 years of study showed no difference between treatments, with the overall growth rate of all treatments declining in the last 5 years of study. These results have significant implications for long-term productivity.

Nutrient availability, particularly N availability, and soil water availability have been identified as being key factors for long-term stand productivity (Pritchett and Fischer 1987). Current results on soil N will be considered in the context of overstory and understory changes caused by fire and fire exclusion in the Ponderosa pine-Douglas-fir system.

Forest Soil Nitrogen

Ponderosa pine-Douglas-fir forests have low N mineralization and low inorganic N accumulation and are gennerally N limited (DeLuca and Zouhar 2000). The short-term effects of fire on soil N include: increased N mineralization, increased nitrification and increased microbial activity (Wells et al. 1979, Pritchett and Fischer 1987, Neary et al. 1999). However, other studies indicate that the effect of fire on soil N cycling may be limited to a very short time after burning (Covington and Sackett 1992, DeLuca and Zouhar 2000, Newland and DeLuca 2000) and some of the long-term results on N availability and productivity may have been overestimated (Stark and Hart 1997). Fire exclusion is speculated to change soil N availability by increasing the amount of forest litter towards high C:N ratios, increasing terpenoid concentrations and increasing the amount of microbial immobilization (Covington and Sackett 1992, DeLuca and Zouhar 2000).

Changes in the labile N pool, both before and after fire, have been the focus of many contemporary studies. Covington and Sackett (1992) found that soil inorganic N pools increased after burning and that this increase was positively correlated to pine establishment and growth, as well as herb growth. They assessed soil concentrations of NH_4^+ and NO_3^- before and after burning, and one year later. They found that increases in NH_4^+ were positively correlated to the amount of forest floor consumed. Ammonium concentrations which were initially high, decreased after one year, while NO_3^-

concentrations lagged behind and were still elevated after one year. Increased rates of nitrification in the short-term may have implications for long-term net ecosystem production as nitrate may be lost through leaching and dinitrification. DeLuca and Zouhar (2000) investigated the effect of prescribed fire on soil N availability and found that it was initially high following fire. They also found increasing nitrification and microbial immobilization, and decreasing potentially mineralizable N. However, after two years of study, they found no detectable differences in N availability between pre- and post-burn site conditions. It is not well understood if these results will continue to affect the future productivity of these stands, but it is hypothesized from data collected 12 years after fire that decreases in substrate quality and decreases in N mineralization may cause substantial decreases in productivity (Monleon et al. 1997).

High rates of gross nitrification have been measured in a variety of western forest ecosystems and suggest that microbial immobilization of labile N may be severly underestimated (Stark and Hart 1997). Nitrate assimilation by microbes was thought to be minimal in mature, undisturbed conifer forests, but Stark and Hart (1997) showed that microbial biomass is a net sink for NO_3^- and promotes N retention in mature ecosystems. Rapid microbial immobilization of inorganic N may cause the over-all organic N pool to grow, but the N may be only slowly available to plants. The questions that arise from this finding include: 1) is gross nitrification high followed by rapid immobilization in the ponderosa pine/douglas-fir ecosystem, or as an alternative, 2) is nitrfcation being inhibited by poor substrate quality and allelochemicals. There is evidence for some plant initiated allelopathic effects on N cycling and the microbial community when the natural fire regime is excluded.

ALLELOPATHY

Allelopathic Potential

Different types of plant mediated interference on nutrient cycling and plant development echanisms have been observed and tested in many ecosystems. These interference mechanisms are generally referred to as allelopathy and have involved everything from constitutive chemical defenses that interfere with herbivory, to root exudates that interfere with alternate species root development, to allechemicals produced by leaf litter that interfere with microbial activity (Wardle et al. 1998). Allelopathy has been examined at the population level by examining the interactions of one plant species on another species of plant or animal, however the causal relationship of these interactions have been difficult to prove. Wardle et al. (1998) suggests that the theory of allelopathy is more suited to application at the ecosystem level where different species may exercise similar characteristics and thus have an effect on the overall ecosystem function.

Ecosystem Function

Many studies have examined allelopathic interactions between plants and ecosystem function presumably mediated by the production of secondary metabolites (Northup et al. 1995, Jaderlund et al. 1997, Wardle et al. 1998, Hattenschwiler and Vitousek 2000). Secondary metabolites are produced from the products of photosynthesis, but are not used in the synthesis of biomass or for energy transfer, which represent primary metabolism (Raven 1992). Plants produce many different kinds of secondary metabolites which vary from simple storage compounds to constitutive or inducible defense compounds. Phenolic compounds constitute a specific type of secondary metabolite produced in the shikimic acid pathway and have often been linked to allelopathic interference (Hattenschwiler and Vitousek 2000). Phenols are a large group of compounds that in some combinations have an aromatic ring with a hydroxyl substitution. Phenols may include various other substitutions and can be further classified as polyphenols (multiple rings), low molecular weight compounds and high molecular weight compounds or condensed tannins (Hattenschwiler and Vitousek 2000). These compounds have been documented to effect the N cycle in different ways.

Effect on the N cycle

Many studies suggest that phenolic compounds reduce litter quality and are involved in humus formation, both making decomposition of organic substrates harder (Northup et al. 1995, Jaderlund et al. 1997, Northup et al. 1998). In general, a negative correlation exists between organic N concentrations and inorganic N concentrations for various different ecosystems ranging from pygmy pine to boreal forests. This phenol-induced relationship has been discovered for both forest floor extracts and leaf extracts from certain understory species and is thought to be a successional feature which causes the replacement of one species by another with increasing time since disturbance and potentially as an anthropogenic feature caused by fire suppression in the boreal forest (Jaderlund et al. 1997). It is also possible that phenol-induced decreases in available N represent a means by which fixed N is retained in forest ecosystems, as SOM. Another possibility is that the production of phenols may have developed as an evolutionary drive toward the inhibition of nitrification, where NO_3^- leaching or denitrification is prevented.

Some studies have speculated that forest ecosystems may inhibit nitrification as a means of avoiding the energy required to reduce nitrate in the plant (Stevenson and Cole 1999). The reduction of NO_3^- occurs in leaf chloroplasts and is limited by the availability of the enzyme complex NO_3^- reductase which can be measured as an estimate of the costs involved with nitrate uptake from the soil (Raven 1992). Maintaining organic N on a site would become its own evolutionary force towards organic N uptake. One last possibility exists. If some plants can sense different volatile compounds as a means of inter communication in the face of herbivory (Shonle and Bergelson 1995), maybe plants can also sense different N oxides as they are released by nitrification (leaky pipe theory) or as the products of denitrification (Stevenson and Cole 1999). An ability to sense these losses and the cost involved with N fixation would lead to adaptations that reduce the occurrence of N losses.

CONCLUSION

Fire and macro-climate are major drivers of plant community dynamics and sub-surface processes and therefore have a top down effect (Fig.2). If the natural fire regime is left intact, these factors should remain the dominant drivers of ecosystem function. However, secondary or response factors, such as plant succession, litter quality and N mineralization have the potential to directly or indirectly influence ecosystem processes in the face of fire exclusion (Fig. 2). Although fire exclusion is a top down factor at the landscape level, it will stimulate changes that initiate bottom up effects at the stand and microsite level where the most drastic outcome of fire exclusion are registered as changes to litter quality and N cycling.

One last question remains, 'is N availability greater with frequent low intensity fires even though the total N pool is smaller due to frequent volatilization?' Fire clearly influences short-term changes in plant communities and sub-surface processes. However, no studies exist to date that examine these changes in the long-term. Therefore, scientific study is required to analyze the time frame in which these potentially drastic and significant changes occur in the inland forests of the dry Northwest.

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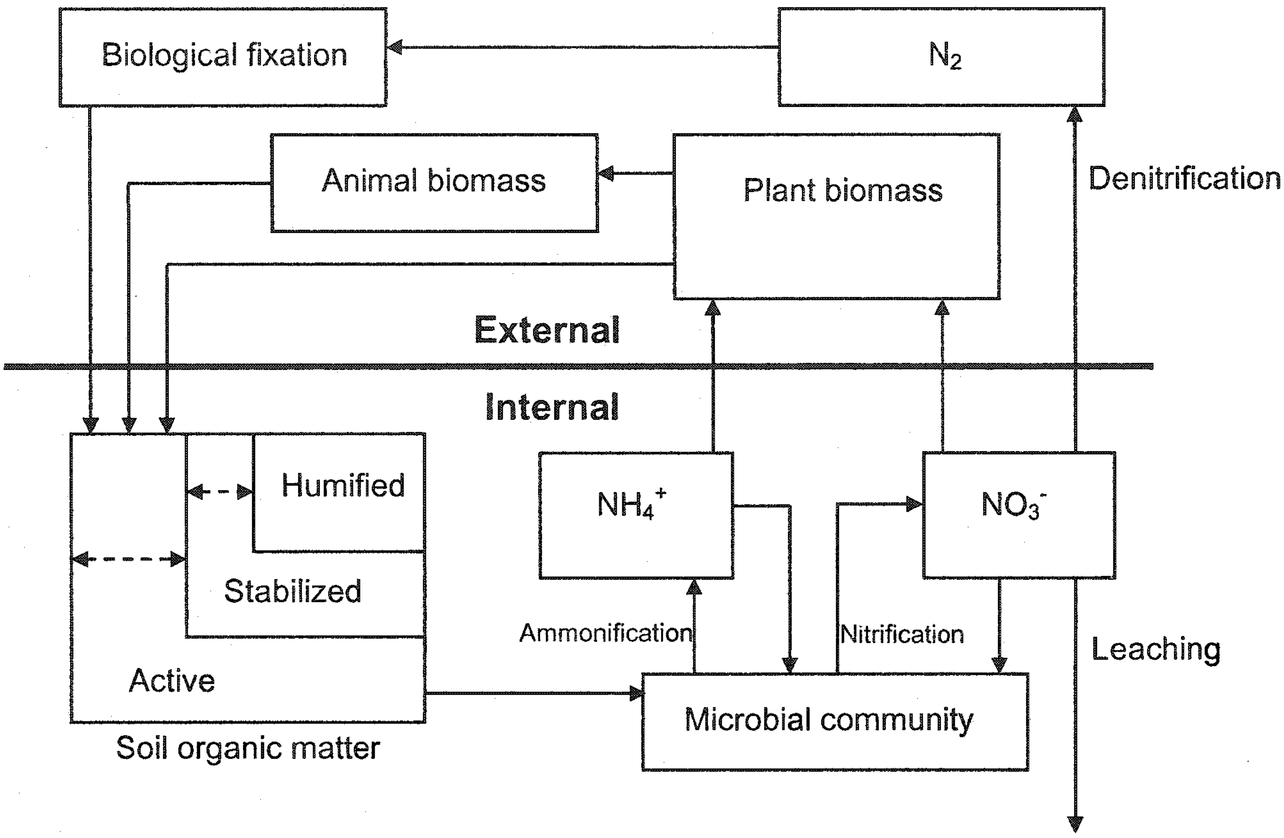
Biological fixation
N₂
Animal biomass
Plant biomass
Denitrification
External
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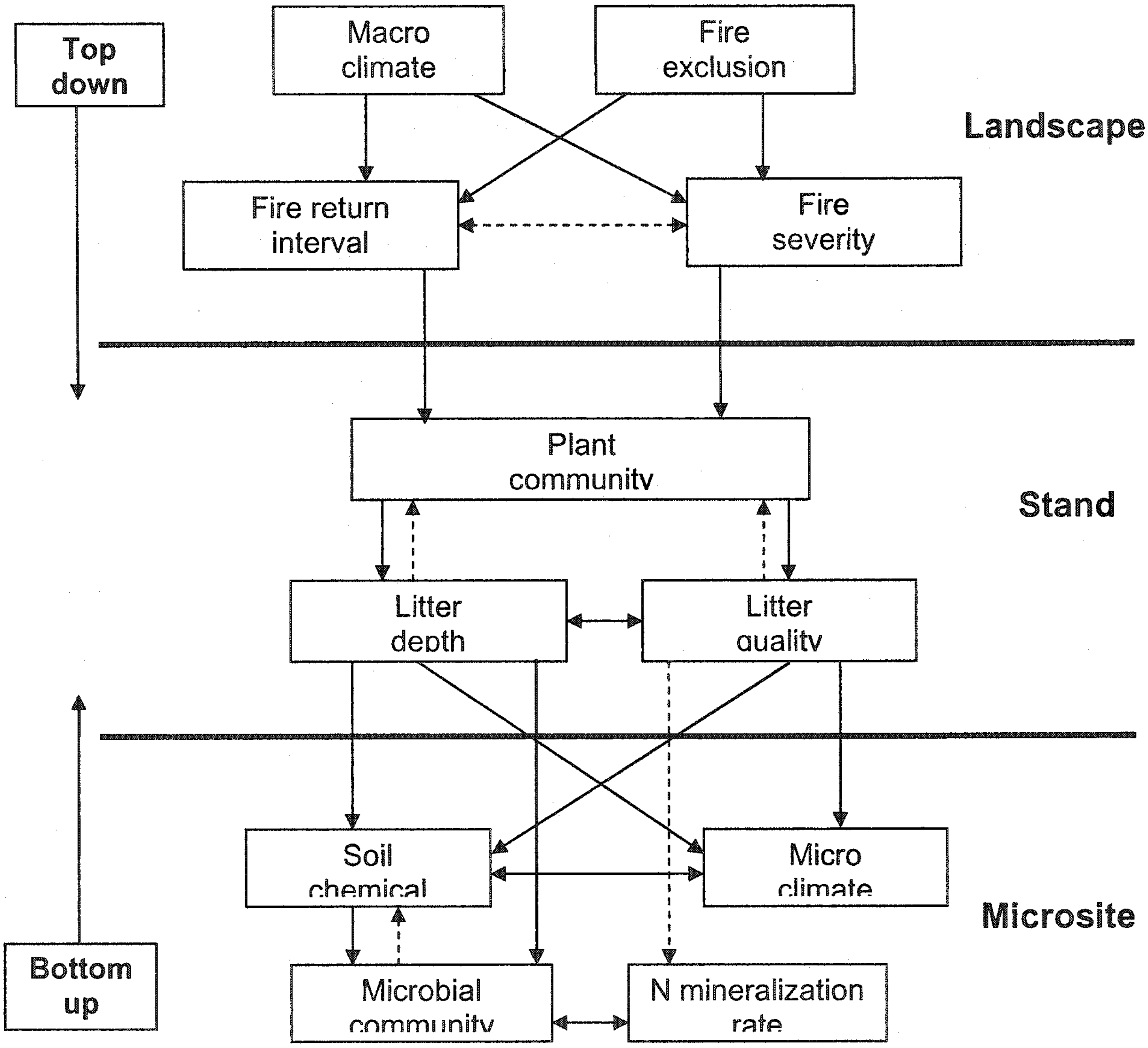
Humified
Stabilized
Active
Soil organic matter
NH₄⁺
NO₃⁻
Ammonification
Nitrification
Leaching
Microbial community


Top down
Macro climate
Fire exclusion
Landscape
Fire return interval
Fire severity
Plant community
Stand
Litter depth
Litter quality
Soil chemical
Micro climate
Microsite
Bottom up
Microbial community
N mineralization rate

Forest structure and organic horizon analysis along a fire chronosequence in the low elevation forests of western Montana

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Abstract: Although fire consumes much of the forest floor, few studies have examined the change in forest floor characteristics with increasing time since fire. Mixed forests of ponderosa pine (*Pinus ponderosa* Doug. Ex. laws) and Douglas-fir (*Pseudotsuga mensizii* (Mirb.) Franco) in the inland Northwest once burned with greater frequency than today. Fire exclusion over the last 100 years is believed to have caused a shift in forest structure, forest floor decomposition and nitrogen availability. However, no research has clearly demonstrated this in forests of the dry inland Northwest. The objective of this study was to determine how fire exclusion has shaped forest structure and understory composition in ponderosa pine/Douglas-fir forests and how these changes have altered forest floor mineralization. Stand level and understory vegetation characteristics, organic horizon depth and biochemistry were analyzed along a 132 year chronosequence at 25 sites in second growth forests of Western Montana. Principle components analysis confirmed that time since fire was significantly correlated to most biotic variables, including nutrient availability and indicating that the disturbance regime is tightly coupled to ecosystem function. Douglas-fir basal area, total shrubs and forest floor thickness were observed to increase with time since fire. Graminoids and forbs did not change significantly with time since fire. There was a significant increase in the content of total C, total N, NH_4^+ and potential mineralizable N (PMN) in the forest floor, while NO_3^- content decreased significantly with time since fire. Total phenols increased significantly and were positively correlated with forest floor thickness, total shrub cover, PMN and NH_4^+, but not correlated with NO_3^- content, suggesting that phenolic compounds accumulate with time and may affect N transformations.

Keywords: Fire chronosequence, fire exclusion, forest structure, secondary succession, forest floor chemistry, phenolic compounds, principle components analysis

INTRODUCTION

There is little known regarding the effect of fire exclusion on plant communities, forest floor decomposition and nutrient dynamics in the inland Northwest. A wealth of evidence indicates that low elevation ponderosa pine (*Pinus ponderosa* Doug. Ex. laws) and Douglas-fir (*Pseudotsuga mensizii* (Mirb.) Franco) forests burned more frequently than they do presently (Arno et al., 1995; Arno et al., 1997; Barrett et al., 1997; Brown et al., 1994). Fire exclusion over the last 100 years is believed to have caused a shift in forest structure, including increases in forest density by the recruitment of Douglas-fir into the canopy and changes in understory composition (Barrett et al., 1997; Brown et al., 1994; Keane et al., 1990; Keane et al., 1996). In spite of the importance of ponderosa pine forests of the Northwest, little research has examined the effects of fire exclusion on forest structure and nutrient cycling in these forests. In ponderosa pine forests of the

Southwest, fire maintained, grass dominated understories are replaced by pine needles and bare ground when fire is excluded (Covington and Sackett, 1986; Covington and Sackett, 1992). We believe that an alternate succession takes place in the inland Northwest where grass dominated, fire dependent understories are replaced by shrubs with increasing time since disturbance (Arno and Allison-Bunnell, 2002; Arno et al., 1995; Arno et al., 1997; Newland and DeLuca, 2000). Studies to date in the inland Northwest have shown that the introduction of fire promotes grasses and forbs in the short-term (Choromanska and DeLuca, 2001; Naumburg and DeWald, 1999: DeLuca, 2000 #157; Newland and DeLuca, 2000). However, few studies have quantified changes in understory species that occur with time when fire is excluded. Changes in overstory and understory species composition due to fire exclusion are likely associated with changes in forest floor properties, which in turn affect N cycling in the mineral soil (Pritchett and Fischer, 1987; Stevenson and Cole, 1999).

A change in fire frequency would have a significant effect on N cycling (Neary et al., 1999). Many studies have documented short-term (1 to 15 years) changes in nitrogen cycling in the mineral soil after fire (Choromanska and DeLuca, 2001; Covington and Sackett, 1992; DeLuca and Zouhar, 2000; Hart et al., 1994; Newland and DeLuca, 2000). Overall these studies suggest that fire causes a thermal ammonification of organic N in mineral soil that persists for 2 to 5 years and results in a large measurable pulse of NH_4^+ in excess of biological needs. As the pH is also raised temporarily by ash deposits, the excess NH_4^+ is quickly nitrified. However, after approximately 5 years, NH_4^+ and NO_3^- concentrations are no longer elevated, but rather near or below pre-fire levels. The return to pre-fire levels are likely associated with increased plant uptake, microbial immobilization and potentially with increased N retention in the forest floor. Forest floor thickness is expected to increase, while it's quality should decrease with time since fire (Prescott et al., 2000; Wardle et al., 2003; White, 1994; Zackrisson et al., 1997). Increases in lignified materials, phenolic compounds and tannins should reduce forest floor mineralization and result in decreased N availability. These changes to forest floor quality may have a significant affect on N availability in mineral soil. Although forest floor properties are expected to experience substantial changes with time since fire and to have a major influence on mineral soil N cycling few studies have documented such changes.

Here, we studied changes in forest structure and forest floor properties from a chronosequence of low elevation, second growth ponderosa pine-Douglas-fir forests in western Montana. Physical and chemical analyses of the organic horizon were made in an effort to establish how fire exclusion changes the decomposable substrate and nutrient potential. Our specific objectives were to: 1) quantify changes in basal area and understory vegetation with increasing time since fire; and 2) quantify changes in forest floor thickness and biochemistry with increasing time since fire.

MATERIALS AND METHODS

Study Sites

A chronosequence of 25 sites with increasing time since fire were selected from GIS fire history maps of Lolo National Forest (LNF) and Bitterroot National Forest (BNF) in western Montana. These maps were produced by the regional Forest Service offices and were characterized by different themes including fire polygons marking the perimeter and date of historical fires. The maps document fire locations dating back to 1880 (LNF) and 1870 (BNF) that were selected as the oldest fire dates for this study.

Topographic maps overlaid with fire polygons and forest types were used to identify potential low elevation sites with ponderosa pine and Douglas-fir as the dominant species. GPS coordinates at the center of the polygons were recorded to locate stands in the field and minimize the chance of surveying outside the burned area. Selected potential sites on the map were ground-truthed and either accepted or rejected based on species composition, evidence of burning and amount of harvesting. Sites where ponderosa pine/Douglas-fir were not a dominant component (greater than 80% of overstory species), with no evidence of burning or with excessive harvesting (more than 5% tree removal) were rejected. Fire dates and harvesting activity were corroborated from local Forest Service officers and from visual estimates of cut stumps, charred trees and presence of charcoal in the soil.

In order to increase the number of sampled sites, we did not discard sites based on aspect, slope and elevation (within the ponderosa pine/Douglas-fir elevation range). We were able to identify 14 stands with fire dates greater than 1900 in 11 geographical areas in western Montana (Table 1). In each area we also sampled an adjacent site not burned since 1900, resulting in a total of 25 sites. By selecting at least one recent fire (> 1900) and one old fire (> 1900) within each general area we intended to eliminate any potential environmental bias of sampled sites (e.g. old fires located in distinctly different areas than more recent fires).

Vegetation and Forest Floor Sampling

Two 100 m long transects separated by 50 m were established parallel to each other at a random location near the center of each fire polygon. With a stratified random sampling system, eighteen 0.5 m^2 rectangular quadrats were located along each transect to study understory vegetation. Plots were located by selecting two numbers between 1 and 10 for each 10 m section of transect. The first number determined the distance along the transect and the second number determined the distance perpendicular to the transect (alternating above and below). Vegetation was recorded in each quadrat by ocular estimation of percent cover by functional groups. The functional groups included: graminoids, forbs, ericoid shrubs, deciduous shrubs, evergreen shrubs, non-vascular plants, bare ground and woody debris. At every third vegetation plot we collected data on basal area (BA, total for all sites and by species for a subset of sites), forest floor thickness, slope, aspect and elevation. We obtained point estimates of BA using a 10 factor prism (Barnes et al., 1988). Forest floor thickness was measured by removing a portion of the forest floor to mineral soil which was kept for chemical analysis.

Chemical Analysis

Total carbon (C), total nitrogen (N), total phenols and inorganic N were analyzed for the forest floor by different methods. Forest floor samples were air dried and ground using a shatter box to pass a 0.01 mesh for total C and total N analysis. Total C and total N were measured from forest floor material as indicators of potential N availability and changes in litter quality over time. Content was calculated by multiplying the concentration data by an aerial factor, calculated as forest floor thickness times an average forest floor density of 0.16 $g.cm^{-3}$, which is representative for forests in this area. Total C and total N analyses were performed on a Fissions Elemental Analyzer (Milan, Italy). Air-dried forest floor samples were used to analyze total phenols by the Prussian blue method (Stern et al., 1996). Total phenols were extracted from the forest floor material by shaking 25 g of forest floor with 50 ml of 50% methanol for 16 hours. Extracts were then filtered on a vacuum manifold with Whatman 42 filter papers and analyzed by spectrophotometry with (+)-catechin as the standard. Air-dried forest floor samples were also used to analyze inorganic N in the O horizon. Because the forest floor samples were air dried, we performed an aerobic incubation to measure the amount of ammonification and nitrification. We also performed an anaerobic incubation to determine the potential mineralizable N (PMN) as an index of N availability over time. Samples were incubated for ten days at 60% water holding capacity (WHC) to re-establish mineralization but to avoid analyzing the N spike that would result from the consumption of labile organic matter that results from drying and storing the samples. Twenty five grams of sample were placed in a 250 ml French square bottle, wet down with 13 ml of water and incubated at 25 °C for 10 days for available N analysis. Five grams of sample were placed in a centrifuge tube and wet down with 3 ml of water and incubated at 25 °C for ten days for PMN analysis. After 10 days, we added 15 ml of water to the PMN samples and replaced the headspace with N_2 gas to create anaerobic conditions and re-incubated the samples for 14 days at 25 °C. Samples were shaken for 30 min. and filtered on a vacuum extraction manifold with Whatman 42 filter papers. The extracts were analyzed for NH_4^+ and NO_3^- by segmented flow colorimetry with a Bran-Luebbe Auto Analyzer III (Chicago, IL).

Statistical Analysis

Principle components analysis allowed us to reduce a very large multi-variate data set to a group of factors influenced by the correlation of data points to different components (Wilkinson, 1997). Each factor explains a certain percentage of the variation within the whole data set. Pearson correlation with uncorrected probabilities were also calculated to show which variables significantly influenced these factors (Wilkinson, 1997).

Vegetation data were normalized to the amount of bare ground per plot and averaged per site because we were specifically interested in the relative changes of functional groups with time since fire. By normalizing the vegetation data, we were able account for some of the moisture differences between sites in terms of vegetation potential. Basal area, forest floor and biochemical data were also averaged per site. Linear regression analysis was applied to average values for understory functional groups, BA, forest floor thickness and biochemistry with time since fire. Because we had some replicated fire ages (Table 1) we averaged the corresponding site values and show error bars representing the standard error of the mean. In all cases, the assumptions for linear regression were tested, including normality, constant variance and independence (Wilkinson, 1997).

Pearson correlations with uncorrected probabilities (Wilkinson, 1997) were performed for certain variables. Correlations were used to measure the relationship between variables where the causality of the interaction was unknown. Total phenols were correlated to total shrubs, forest floor thickness and biochemistry data. In all cases where probability data are reported in this study we used an alpha of 0.100 to determine significant relationships.

RESULTS AND DISCUSSION

Ecosystem Analysis

Results from principle components analysis revealed strong correlation's between most biotic variables and time since fire indicating that fire exclusion affects ecosystem function. Principle components axis 1 (PCA 1) accounted for 41.8 % of the variation, while PCA 2 accounted for 20.6 % of the variation (Fig. 1). Those variables circled by the vertical ellipse vary significantly with PCA 1 and those circled by the horizontal ellipse vary significantly with PCA 2 at the 95% confidence interval based on uncorrected Pearson correlation probabilities (Wilkinson, 1999). Variables that are significantly correlated to PCA 1 include: BA, forest floor thickness, total C, total N, total phenols, total shrub, and available N.

PCA 1 was strongly related to time since fire, while PCA 2 was strongly related to the other abiotic variables including: elevation, slope and aspect as shown by regression analysis (data not shown). Ericoid and deciduous shrubs varied significantly with PCA 2, evergreen shrubs did not vary significantly with either PCA, while total shrubs varied significantly with PCA 1. These results suggest that, unlike Boreal systems, where ericaceae shrubs are driving ecosystem function (DeLuca et al., 2002; Zackrisson et al., 1997), it is likely that in this ecosystem all woody growth (shrubs and trees) are affecting ecosystem function. Total phenols, total C, total N and PMN were clumped together and significantly correlated to PCA 1 at the opposite end from total shrubs. Perhaps this implies a connection between the overall accumulation of organic chemistry and woody growth, but it is difficult to say with out further investigation.

Forest floor NH_4^+ and NO_3^- contents were correlated to PCA 1 indicating that available N in the forest floor is a function of time since fire. However, the mechanism shaping the availability of NH_4^+ and NO_3^- seems to be opposed, as NH_4^+ is positively correlated and NO_3^- negatively. Vervaet et al. (2002) found that N mineralization rates were well correlated with total N and the C/N ratio from forest floor samples analyzed with PCA, but they did not include differences in environmental characteristics within their PCA. By including large scale temporal and spatial heterogeneity we have addressed which factors vary with fire exclusion at the landscape level in the dry inland Northwest. As the variability of these factors depends on TSF we performed linear regression analysis to determine the strength of each relationship.

Overstory structure

We found that fire exclusion had dramatic effects on forest structure regardless of the substantial variability in our data set created by the large differences in aspect, slope and elevation. Total BA increased with increasing time since fire (Fig. 2), as is to be expected of forest dynamics in second growth forests (Barnes et al., 1988). Most of the increase in total BA was due to a significant increase in Douglas-fir BA (Fig. 2), while ponderosa pine BA did not vary with increasing time since fire (data not shown). The trends for both total BA and Douglas-fir BA area exhibit log-linear increases with the curve flattening between 20 to 30 years after disturbance. This probably represents the period of canopy closure from which subsequent increases in basal area over time occur at a slower rate and which is known to affect other ecosystem properties (Barnes et al., 1988). Therefore, this 20 to 30 year period may be of significance in other data sets.

Increases in BA were due to increases Douglas-fir growth in these inland forests (Fig. 2). Frequent low intensity fire preferentially kills thin barked, fire sensitive Douglas-fir and maintains open ponderosa pine forests. The fact that ponderosa pine BA did not increase with time is probably because the high light requirements of this species are not compatible with the low light environment in denser canopies that develop with the absence of fire. As a typical shade tolerant species, ponderosa pine has a low proportion of foliage relative to sapwood and requires high light to compensate for respiratory loses and sustain positive growth. Douglas-fir on the other hand is more shade tolerant, has a much larger proportion of leafs relative to sapwood and can tolerate lower light intensities (Callaway et al., 2000; Wagner, 1986). Further, increases in BA and tree density due to fire exclusion in the inland NW have been shown to decrease soil moisture availability during the summer (A. Sala unpublished data) and exacerbate water stress. As opposed to the drought tolerator strategy of Douglas-fir (Stout and Sala, 2003), drought avoidance in ponderosa pine involves some significant costs including strong stomatal closure, large amounts of sapwood relative to foliage as a water storage system and thick bark (Pinol and Sala, 2000; Stout and Sala, 2003). When fire is excluded, ponderosa pine loses competitive advantage relative to Douglas-fir. Open stands of ponderosa pine are created by two things: a lack of sufficient water for other species to survive or frequent fire ignitions, be they human or natural in origin.

Understory Cover

Fire exclusion also had some effects on understory cover as shown by the percent cover of four different functional groups with increasing time since fire (Fig. 3). There is a negative trend for grasses or forbs, but the slope was not significantly different from zero in either case. Total shrub cover and ericaceous shrub cover however, increased significantly with time since fire (Fig. 3). These data show a shift from dominance by grasses and forbs to co-dominance between grasses, forbs and shrubs very quickly post-disturbance. It does not represent a successional replacement of herbaceous species by woody species, as shrubs are present immediately after disturbance, regaining ground cover from intact root balls (Arno and Allison-Bunnell, 2002). All four functional groups considered here have the ability to maintain co-dominance in the forest understory. Grasses and forbs have rapid growth and nutrient turnover (Kaye and Hart, 1998; Naumburg and DeWald, 1999; White et al., 1991) and shrubs potentially alter their environment to maintain co-dominance. Ericaceous shrubs were singled out because they have been implicated in shaping environmental function through allelopathic interactions (Hattenschwiler and Vitousek, 2000; Nilsson et al., 2000; Northup et al., 1998; Wardle et al., 1998). The presence of allelochemicals and their correlation with forest floor biochemical properties are addressed in section 3.6.

Understory recruitment after frequent low severity fire in Southwestern ponderosa pine forests has been shown to consist predominantly of bunch grasses (Covington and Sackett, 1992; White, 1985; White et al., 1991). This is also the common perception held for ponderosa pine forests of the dry inland northwest. However, this presumption is errant due to the distinctly different climate, fire frequency and species composition of inland NW forests (Agee, 1993; Arno and Allison-Bunnell, 2002) as well as the data presented here. Forests of the inland Northwest that have enough moisture to support Douglas-fir growth, characteristically have more woody shrub cover in the understory (Agee, 1993; Arno and Allison-Bunnell, 2002). Understory vegetation is consumed by low severity fires, but the nature of this fire regime causes consumption to be patchy at best (Arno and Allison-Bunnell, 2002). As grasses and forbs typically grow and senesce faster than shade tolerant shrubs, it seems obvious that differences in cover should change with time since fire, but the length of this succession is not known or well understood for the dry inland northwest. This data set shows that the length of time required to reach co-dominance is short and that the competitive exclusion of grasses and forbs by shrubs does not happen in this time frame.

Forest Floor Properties

Forest floor thickness increased significantly with time since fire (Fig. 4). It should be noted that the total forest floor thickness that accumulated over this chronosequence is still quite shallow (average 6-6.5 cm) compared to other ecosystems. The forest floor of these sites was mostly characterized by undecomposed material ($O_{i/e}$) and very little (< 10 %) humic material (O_a). The shallow forest floor thickness, even after 130 years of fire exclusion, is representative of the extremely dry climate in western Montana, with only 20 inches of rain annually in low elevation forests, mostly falling as snow (Nimlos, 1986). The predominance of $O_{i/e}$ material justifies the use of 0.16 g cm^{-3} for forest floor density and results from this study indicate that forest floor density does not change significantly with time since fire (data not shown). The increase in forest floor thickness exhibits a log-linear relationship similar to the one for total BA and Douglas-fir BA, however the forest floor curve flattens farther along the chronosequence, somewhere between 25 and 50 years. This suggests that the accumulation of organic material on the forest floor is related to something other than canopy closure and will be discussed further in the following sections.

Both total C and total N content (kg ha^{-1}) increase slightly with time since fire (Fig. 5). However, total C and total N concentrations (g kg^{-1}) do not exhibit a significant increase with increasing time since fire (Fig. 5). Given that fire consumes a portion of the forest floor and that through time organic material accumulates, as shown by Figure 4, it is not surprising that the overall nutrient content also increases. With increasing content, but no change in concentration of these two nutrients, we have evidence to suggest that the type of litter inputs to the system do not change drastically over this time period. This was unexpected as we recorded significant increases in shrub cover and therefore expected changes to C and N concentrations and litter quality. A basic result of the total C and N data is to calculate the C to N ratio as a means of addressing litter quality. The C to N ratio exhibited a significant trend with time since fire, increasing from 20:1 after fire, to 30:1 with 130 years of fire exclusion (data not shown). This suggests a decline in litter quality and a reduction in available N as speculated, but as the ratio remained between 20:1 and 30:1 the data provides only weak evidence as such. Therefore, a more direct analysis of N availability was examined on re-incubated forest floor samples.

Nitrogen Availability

Fire exclusion was found to have a significant effect on N availability. Forest floor N content was measured after a 10 day aerobic incubation (NH_4^+ and NO_3^-), and again after a 14 day anaerobic incubation (PMN). The NH_4^+ and PMN contents increased significantly with time since fire, however, NO_3^- content was found to decrease significantly with time since fire (Fig. 6).

The significant increase in NH_4^+ accumulation (during a 10-d aerobic incubation) with time since fire was not expected. The data suggest that a surplus of moderately labile organic matter develops above the major plant rooting zone and probably accumulates during the dry summer and winter months, only to be rapidly mineralized to NH_4^+ in times of snow melt and spring rain. This may explain why grasses and forbs are able to maintain their presence with increasing time since fire. They are adapted to pulses of increased nutrient availability and take full advantage of the post thaw spring pulse, dying out later in summer and fall (DeLuca et al., 1992; Fierer and Schimel, 2002). Shrubs and trees on the other hand do not rely on pulses of nutrients and instead acclimatize to the overall low nutrient availability that predominates in this ecosystem (Choromanska and DeLuca, 2001; DeLuca and Zouhar, 2000; Newland and DeLuca, 2000). There is also evidence that woody species can exist on NH_4^+ alone, whereas grasses prefer NO_3^- and may experience NH_4^+ toxicity (Persson et al. 2003). As it seems that none of this NH_4^+ reaches the soil, as indicated by studies showing pre-fire NH_4^+ levels within 5 years post-disturbance (Covington and Sackett, 1992; DeLuca and Zouhar, 2000; Hart et al., 1994), perhaps the increase of NH_4^+ in the forest floor is evidence of a plant mediated shift in N cycling.

The decrease in NO_3^- with time since fire indicates that something is interfering with nitrification as the stands continues into late secondary succession. This is a common phenomena in many forest ecosystems as they progress into late secondary succession (DeLuca et al., 2002; Hattenschwiler and Vitousek, 2000; Northup et al., 1998; Stark and Hart, 1997), but the mechanism by which nitrification appears to almost cease with increasing time since disturbance is not commonly agreed upon. Reduced nitrification may be a function of rapid turnover of NO_3^- (Stark and Hart, 1997), or possibly inhibition of nitrification by allelopathic chemistry (Northup et al., 1998). However, it is not clear whether allelopathic chemistry directly affects the microbial community or increases the humification process, and the source of allelochemicals is not clear; are they plant derived of products of decomposition. This last question will be addressed in the next section.

The fact that NO_3^- decreases with time suggests that this is not a relic of the incubation process which would have resulted in increased NO_3^-. It is possible that dissimilatory NO_3^- reduction resulted in the formation of NH_4^+, but that would account for less than 10% of the ammonification recorded. While nitrification has been shown to decrease in late secondary succession forest soils (Hattenschwiler and Vitousek, 2000; Northup et al., 1998), little data are available for forest floor mineralization. The increase in PMN with time since fire corroborates the findings for NH_4^+ accumulation with time since fire.

The magnitude of the NH_4^+ and PMN data are similar, but are notably greater than values reported for mineral soil in this region (e.g. DeLuca and Zouhar). The extreme values may be representative of forest floor mineralization that have not been previously reported for this ecosystem or this time sequence. Mineralizable N is a factor related to accumulation of organic matter and the curves of both NH_4^+ and PMN mimic the forest floor thickness curve. Therefore, it is possible that the increases in N availability simply represent the post-disturbance system coming to equilibrium with leaf litter an decomposition. However, the change in PMN with time since fire indicates that early sites mineralize more N than later sites when compared to the NH_4^+ data. The early sites experience a 50% increase in PMN-N while the later sites experience only a 20 % increase in PMN-n when compared to NH_4^+-N on average. This may suggest that there is a shift in the chemical composition of the organic horizon that alters N mineralization dynamics with increasing time since fire.

Both ammonification and nitrification exhibit weak log-linear trends that flatten somewhere between 25 and 50 years (Figure 6). It is interesting to note that the historic fire return interval for these forests was on the order of 25 to 50 years. This is good evidence that the disturbance regime is tightly coupled to ecological function. The amount of time required before nutrient availability begins to plateau is perhaps a universal trend related to time since fire. DeLuca et al. (2002) presented chronosequence data for northern Sweden in which nitrification decreased significantly with increasing time since fire. The relationship also produced a log-linear trend, but the curve flattened farther out along the time since fire axis, somewhere between 80 and 120 years. This time frame is similar to the historic fire regime of Boreal forests. Again, this is good evidence that ecological function is tightly coupled to the disturbance regime and differs from one regime to another. One possible mechanism for the tight coupling of ecosystem function to the disturbance regime is the production and maintenance of allelopathic chemistry.

Allelochemicals

Phenols have been identified as allelochemicals capable of interfering with the digestive tracts of herbivores, inhibition of seed germination in grasses and forbs, and as inhibitors of nitrification in forest soils (Hattenschwiler and Vitousek, 2000). Total phenols were measured on forest floor samples and increased significantly with time since fire (Fig. 7). With significantly decreasing NO_3^- contents in the forest floor it seems possible that phenols may be interfering with nitrification in this ecosystem as well. Total phenols also exhibited a log-linear trend with the curve flattening between 25 and 50 years suggesting a relationship between forest floor decomposition, N availability and phenol content.

At this point it is not clear if the accumulation of phenols is a selectively adapted feature of the plant community or a secondary function of woody decomposition. Phenols are produced as secondary metabolites by the shikimic acid pathway in plants, but can also be synthesized *de novo* as a by product of decomposition (Hattenschwiler and Vitousek, 2000). Ericoid plants have been shown to produce large quantities of phenols in some ecosystems (Choromanska and DeLuca, 2001; DeLuca et al., 2002; Wardle and Nilsson, 1997; Zackrisson et al., 1997), but whether or not they represent a large enough percent of the understory vegetation to drive ecosystem function in this ecosystem is beyond the scope of this paper. Total shrub cover increases significantly and could be driving the accumulation of phenols in this ecosystem.

In an effort to show whether total phenols were plant-mediated allelochemicals or a by-product of woody decomposition total phenols were correlated with total shrub cover and forest floor thickness (Table 2). Both Forest thickness and total shrub cover were significant and positively correlated to total phenol content. There is, however, an element of auto-correlation with forest floor thickness and total phenol content, as thickness was used to convert phenol concentration to content. However, a strong correlation exists between forest floor thickness and total phenol concentration as well (data not shown). It is not clear from these correlation's whether or not shrubs are increasing total phenols and forest floor thickness or vice versa. Therefore, these results do not help to determine the origin of phenols in the forest floor and more experiments will have to be performed.

The N availability data suggested that there may have been some chemical interference with N mineralization. Total phenols were significantly correlated with PMN, weakly correlated with NH_4^+ and not well correlated with NO_3^- (Table 2). It is possible that the positive correlation reflects the increasing organic matter accumulation on these sites. Low molecular weight phenols have been identified as potential food sources for microbes (Hattenschwiler and Vitousek, 2000; Northup et al., 1998) and may simply reflect metabolic currency for both aerobic and anaerobic conditions. This does not readily explain why NO_3^- decreases with time since fire, but could be a function of either inhibition or immobilization. Regardless, it is clear that there are dramatically different mechanisms responsible for the NH_4^+ and NO_3^- trends.

CONCLUSION

By exploring forest floor properties and N mineralization in the context of ecosystem function we have addressed an overlooked topic in forest soil science today. Many studies have characterized forest floor physical parameters with increasing secondary succession, but few have studied the biochemical nature of the organic horizon. Both PCA analysis and regression analysis imply that time since fire is having the greatest impact on forest structure and nutrient cycling for these sites even though there is large divergence in other environmental characteristics such as slope, aspect and elevation. With increases in BA and understory cover it is not surprising to find an increase in the forest floor thickness. While the quantity of the forest floor increases and therefore total C and N contents, the quality appears to decrease. Several lines of evidence support this idea: 1) a slight but significant decrease of the C/N ratio over time; 2) an increase of phenolic compounds; and 3) a decrease of NO_3^- after aerobic incubation. The increase of NH_4^+ seems counter intuitive but might indicate that a proportionally larger fraction of less labile N accumulates in the forest floor over time, which is released only after an incubation period. In nature such incubation periods may be equivalent of wet warm spring conditions which may create pulses of N to mineral soil that maintain grasses (Fierer and Schimel, 2002). Decreasing nitrification and increasing phenol could be related to increased immobilization of NO_3^- or to some form of chemical interference of nitrification. The effect high levels of NH_4^+ and total phenols in the forest floor needs further investigation on mineral soil properties.

This ecosystem is somewhat unique in that it was shaped historically by frequent low severity fire. These low severity disturbances acted to maintain ponderosa pine/Douglas-fir forests in a perennial state of rejuvenation. The data from this study indicate a tight coupling of ecological function to the historic disturbance regime as shown by the repetition of a log-linear trend which begins to flatten between 25 and 50 years, similar to the historic fire return interval. These results are another example of how ecosystem properties have 'after-life' characteristics created by the disturbance regime that has maintained the ecosystem (Nilsson et al., 2000; Zackrisson et al., 1996). These 'after-life' properties have different phenology at different sites, as was shown by the Boreal forest example of DeLuca et al. (2002) and represent real properties of forest systems.

Management of these second growth ponderosa pine/Douglas-fir forests should include some component of the natural disturbance regime to maintain forest health. Selective harvesting with low severity understory burning would likely promote rapid nutrient turnover (DeLuca and Zouhar, 2000) and increased tree growth (Arno and Allison-Brunell, 2002). In this way, a natural mosaic of stands in different stages of recovery would be established and promote forest health and biodiversity (Barnes et al., 1988). There is also mounting evidence that landscape heterogeneity will promote resistance to premature disturbance and disease (Pickett and Cadenasso, 1995).

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Table 1: Chronosequence sites found in Western Motanta with fire year, time since fire (TSF) and environmental factors including, aspect, slope and elevation.

Site	Fire year	TSF	Slope (°)	Aspect	Elevation (m)	Soil Taxonomy
Alberton	1988	14	29.2	S	1339.4	Xeric Dystrocryepts
	1880	122	22.8	SE	1520.8	
Blodett Canyon	2000	2	8.3	S	1047.8	Lithic Dystrusepts
	1988	14	14.0	S	1456.2	
	1988	14	5.0	S	1409.6	
	1870	132	11.7	S	1295.0	
Clearwater	1988	14	5.0	S	1248.9	Typic Dystrocryepts
	1880	122	11.0	SE	1299.1	
Lake Como	1975	27	14.3	E	1407.1	Typic Dystrocryepts
	1870	132	16.7	E	1404.0	
Lick Creek	1993	9	12.3	NE	1264.6	Typic Dystrusepts
	1870	132	21.7	E	1266.7	
Lake Como N	1924	78	28.3	NW	1288.0	Typic Dystrocryepts
	1870	132	13.8	NW	1222.1	
Lake Como S	1924	78	19.5	E	1516.4	Typic Dystrocryepts
	1870	132	15.3	NE	1500.8	
Lost Horse	1915	87	12.7	SE	1273.8	Typic Dystrocryepts
	1870	132	11.3	SW	1246.2	
Miller Creek	1959	43	28.2	SW	1175.5	Typic Dystrusepts
	1880	122	26.0	SW	1205.9	
Nine Mile	2000	2	13.7	S	1056.4	Typic Dystrocryepts
	1910	92	10.3	SE	1089.6	
	1880	122	16.8	S	1137.5	
Willow Creek	1996	6	33.3	S	1590.6	Lithic Dystrusepts
	1870	132	33.7	S	1668.4	

Table 2: Pearson correlation analysis with uncorrected probabilities. Total Phenols (μg capsule $^{-1}$) in forest floor material were correlated to the variables listed below.

Variable	r^2	p-value
Forest Floor Depth (cm)	0.674	0.002
Total Shrubs (% cover)	0.626	0.003
NH_4^+ (ug capsule $^{-1}$)	0.304	0.099
NO_3^- (ug capsule $^{-1}$)	-0.243	0.148
PMN (ug capsule $^{-1}$)	0.655	0.005

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Figure 1: Principle component analysis was applied to biotic factors of the fire chronosequence data set. These include: graminoids cover (G), forbs (F), deciduous shrub cover (DS), evergreen shrub cover (ES), erricaceae shrub cover (E), total shrub cover (TS), basal area (BA), forest floor depth (LFH), total C (TC), total N (TN), C to N ratio (C/N), ammonium (NH_4^+) and nitrate (NO_3^-). PCA 1 is related to TSF and PCA 2 is related to environmental parameters. The bi-directional elipses show factors that were significantly ($p<0.05$) related to each axis by Pearson correlation and the bi-directional rectangles show factors that were significant at $p<0.100$.

Figure 2: Change in basal area ($m^2\ ha^{-1}$) with time since fire for both the total BA of all canopy species together (a) and Douglas-fir BA (b) as measured on fire chronosequence sites in western Montana.

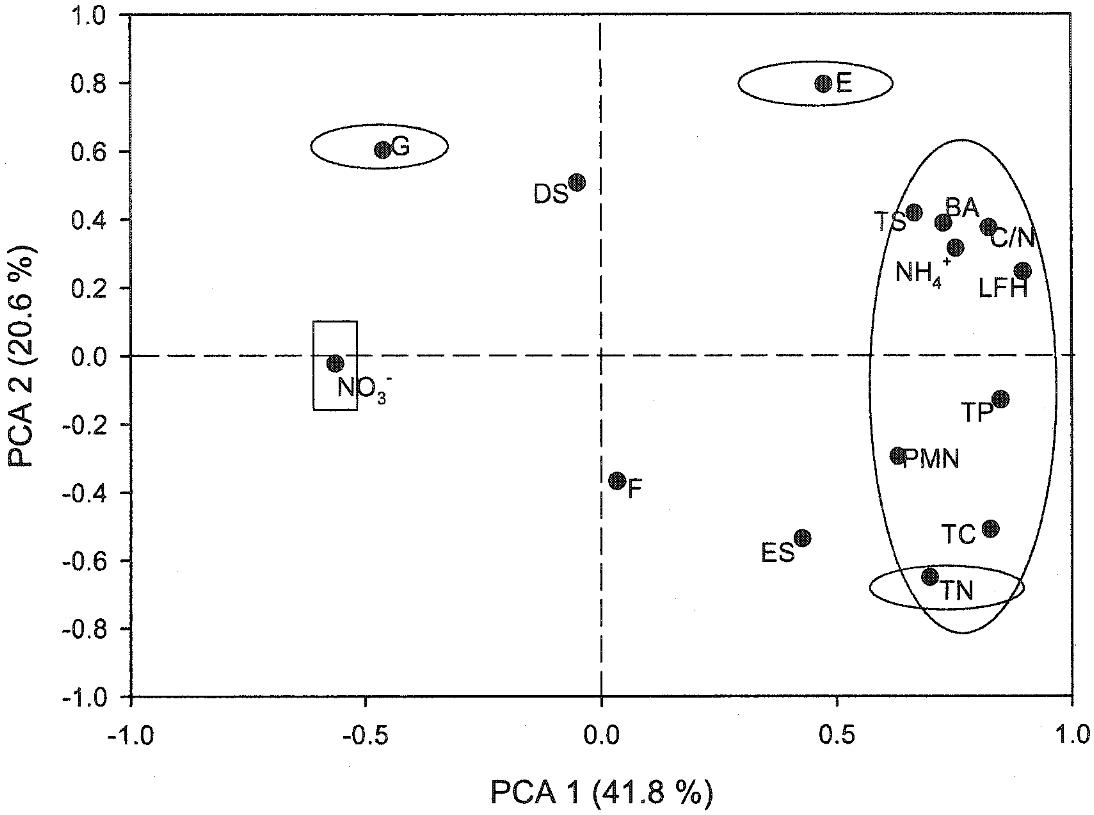
Figure 3: Changes in graminoid (a), forb (b), ericaeceous shrubs (c) and total shrubs (d) with time since fire for the chronosequence sites in western Montana.

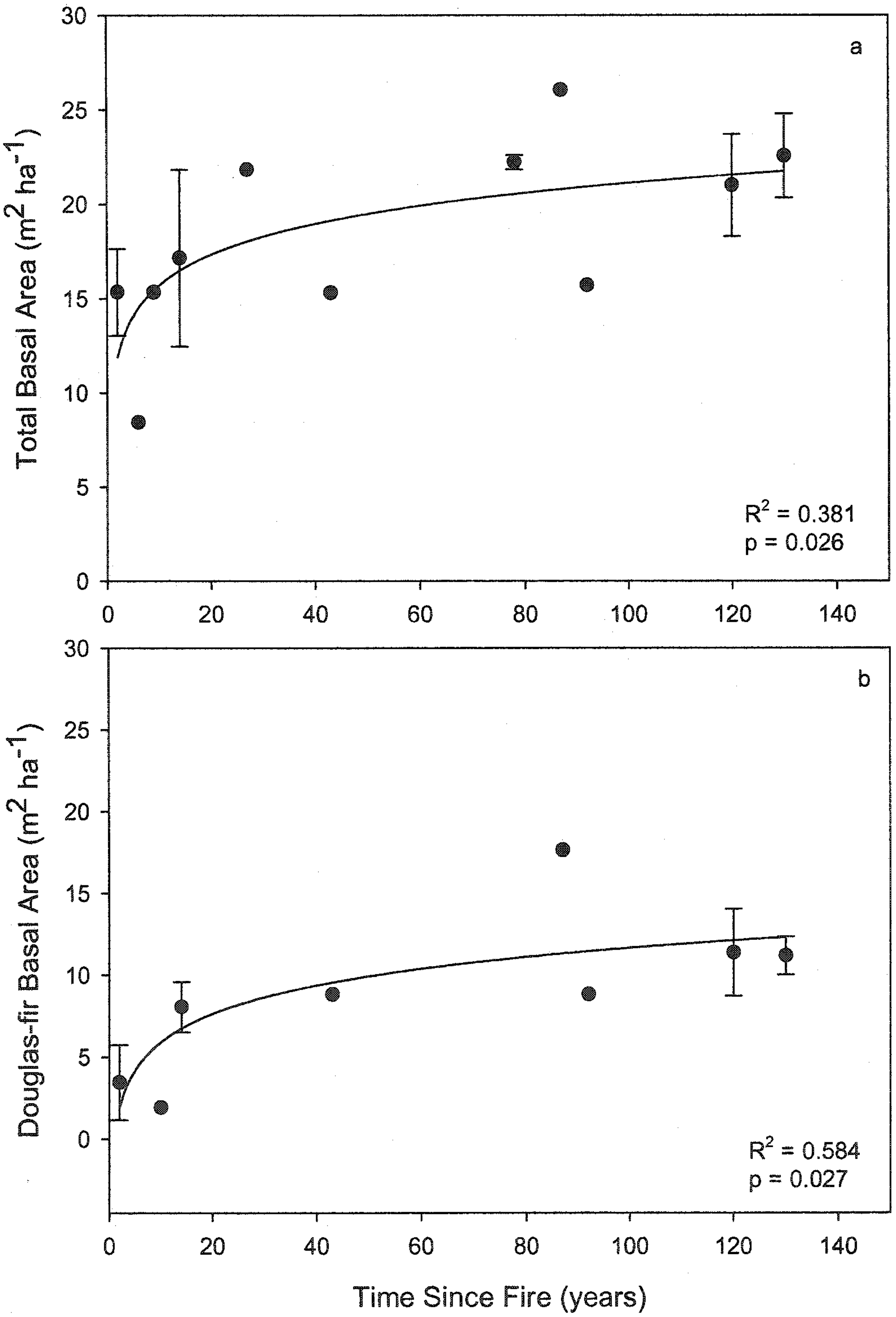
Figure 4: Change in depth of organic horizon with time since fire for the chronosequence sites in western Montana.

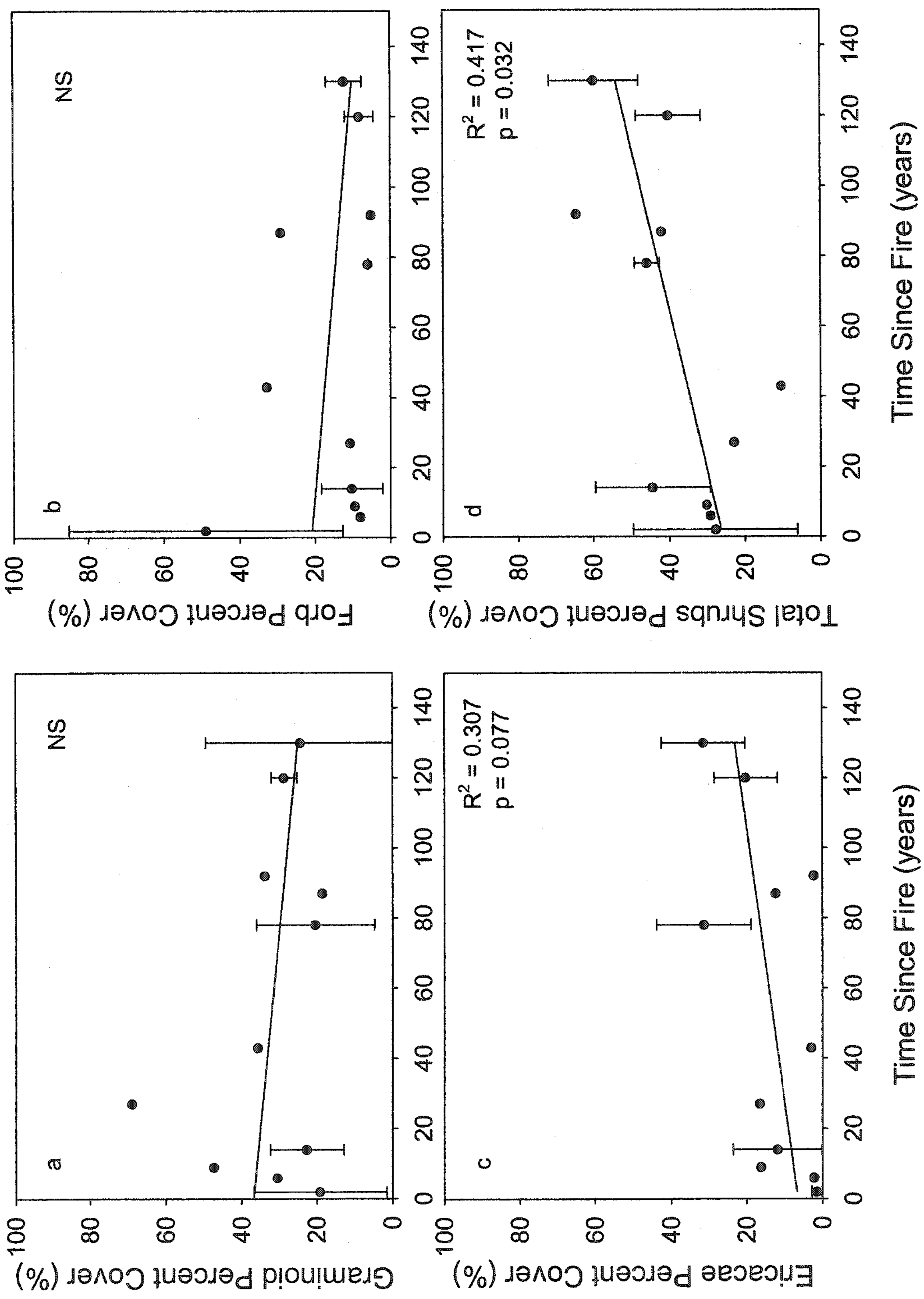
Figure 5: Change in total carbon (a) and total nitrogen (b) content ($kg\ ha^{-1}$), and concentrations ($g\ kg^{-1}$) of total C (c) and total N (d) with increasing time since fire in western Montana.

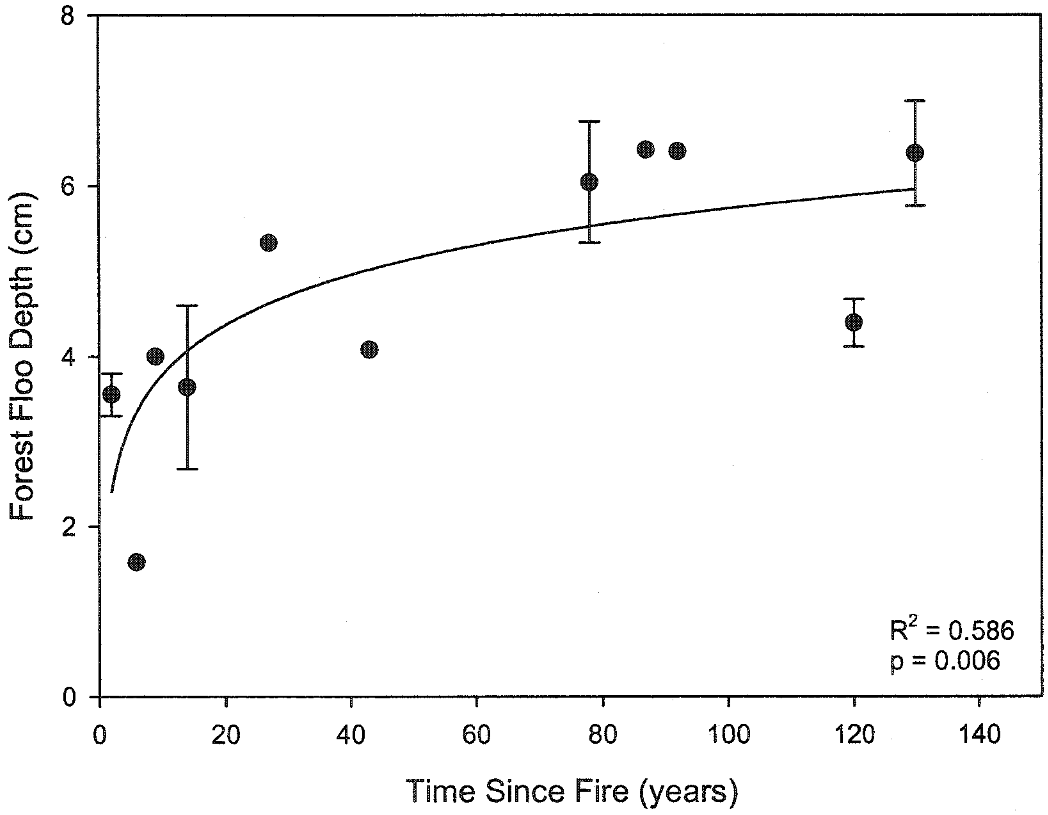
Figure 6: Ten day aerobic incubations of forest floor material were analyzed for NH_4^+ (a) and NO_3^- (b) contents based of forest floor densities ($ug\ cm^{-2}$) and forest floor PMN (c) as determined by using a 14-d anaerobic incubation for the chronosequence sites in western Montana.

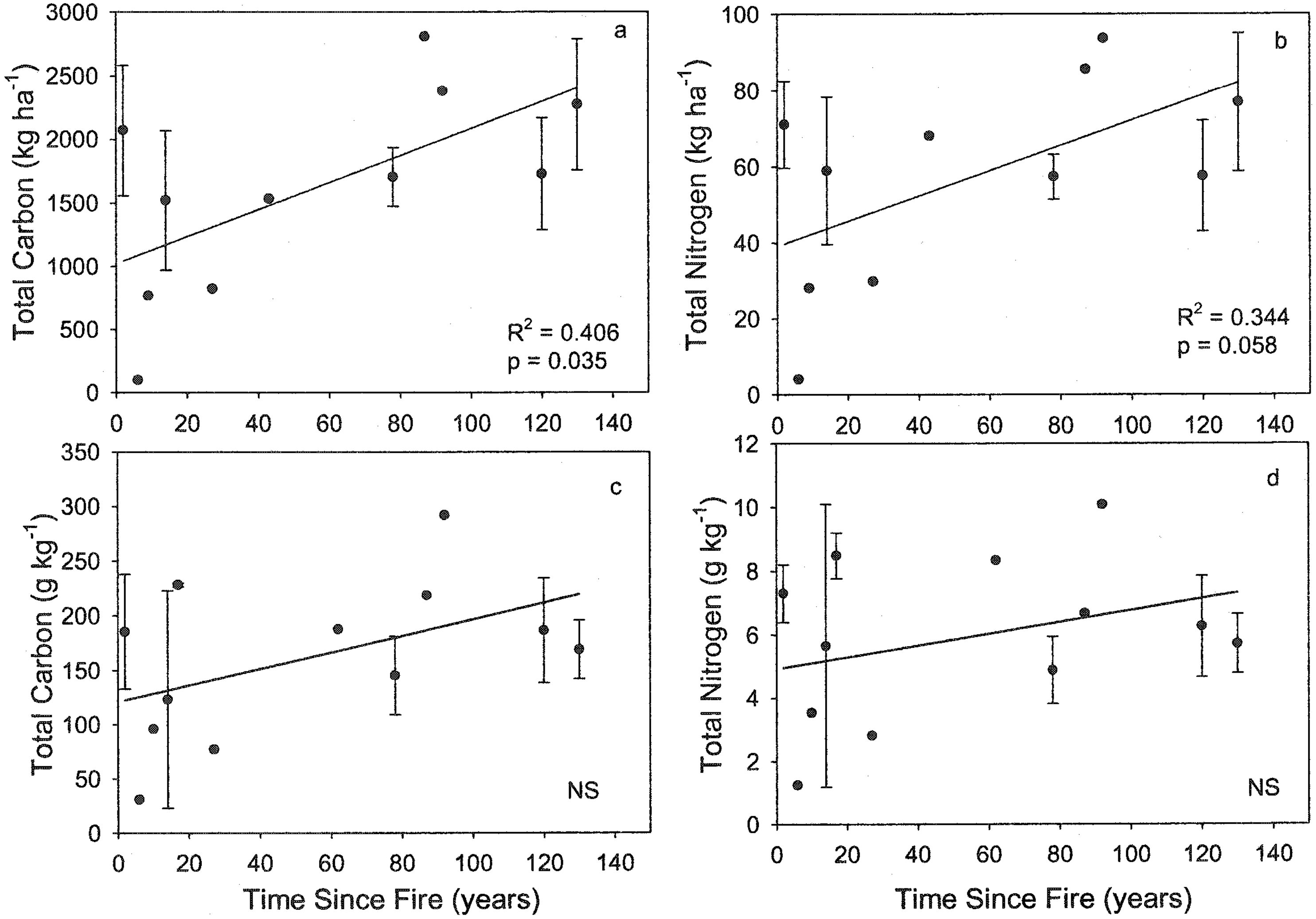
Figure 7: Change in total phenols extracted from forest floor material using 50 % aqueous methanol (mg cm^{-2}) with time since fire for the chronosequence sites in western Montana.

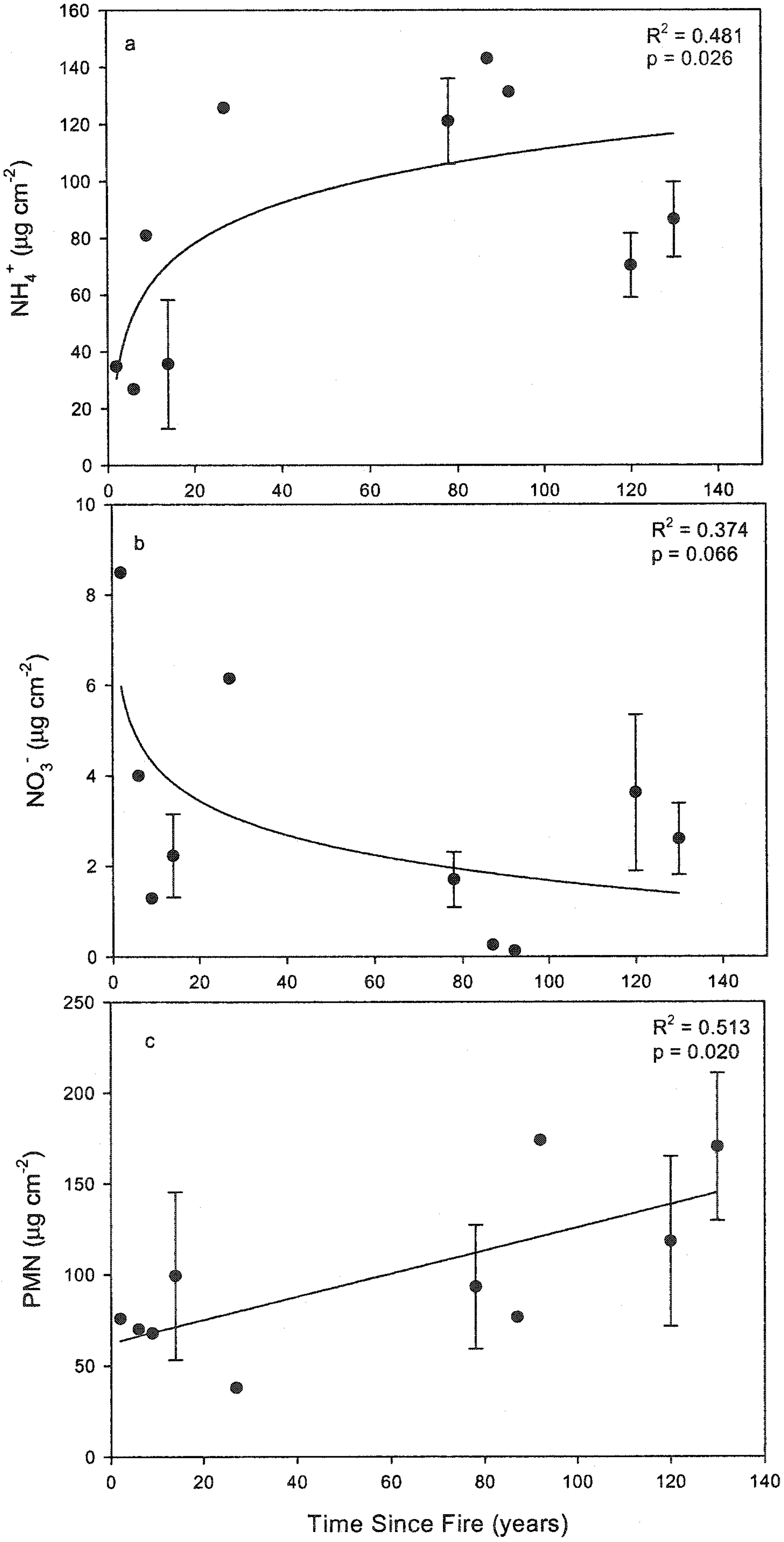

1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
PCA 2 (20.6 %)
-1.0
-0.5
0.0
0.5
1.0
PCA 1 (41.8 %)
E
G
DS
TS
BA
C/N
NH_4^+
LFH
NO_3^-
TP
PMN
F
ES
TC
TN

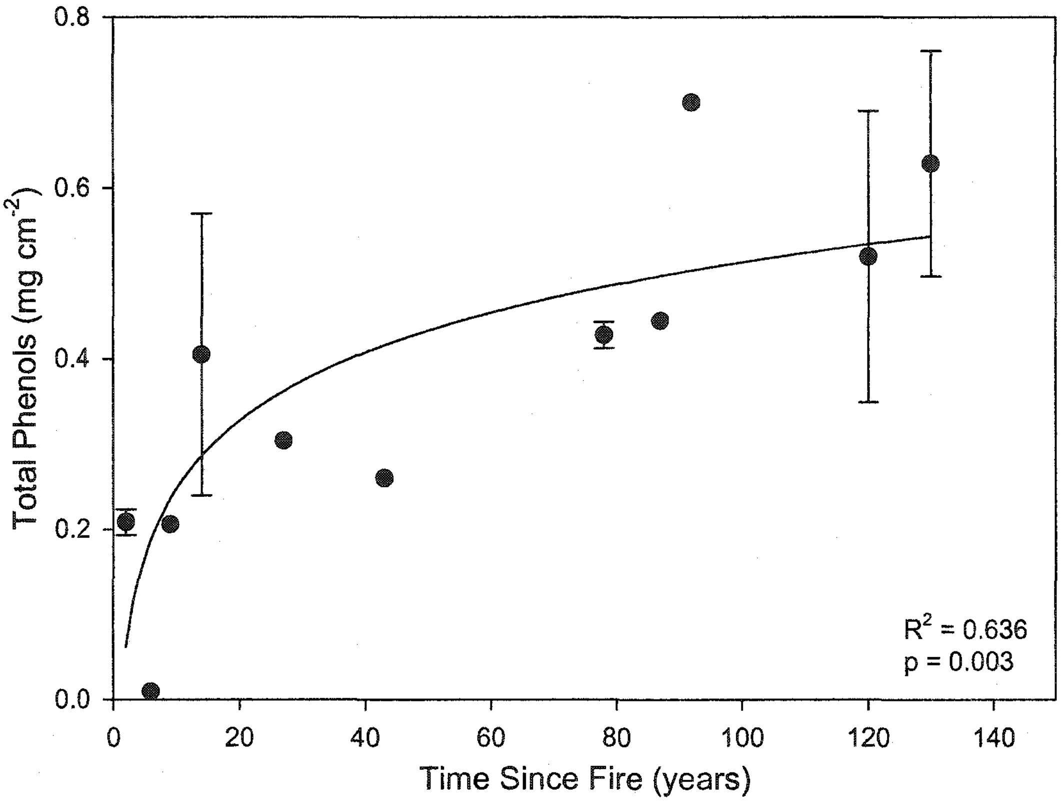

30
25
20
15
10
5
0
Total Basal Area (m^2 ha^{-1})
a
R^2 = 0.381
p = 0.026
0
20
40
60
80
100
120
140
30
25
20
15
10
5
0
Douglas-fir Basal Area (m^2 ha^{-1})
b
R^2 = 0.584
p = 0.027
0
20
40
60
80
100
120
140
Time Since Fire (years)


a
NS
Graminoid Percent Cover (%)
b
NS
Forb Percent Cover (%)
c
$R^2 = 0.307$
$p = 0.077$
Ericacae Percent Cover (%)
d
$R^2 = 0.417$
$p = 0.032$
Total Shrubs Percent Cover (%)
0
20
40
60
80
100
120
140
Time Since Fire (years)


8
6
4
2
0
Forest Floo Depth (cm)
0
20
40
60
80
100
120
140
Time Since Fire (years)
R² = 0.586
p = 0.006


a
b
c
d
Total Carbon (kg ha^{-1})
Total Nitrogen (kg ha^{-1})
Total Carbon (g kg^{-1})
Total Nitrogen (g kg^{-1})
$R^2 = 0.406$
p = 0.035
$R^2 = 0.344$
p = 0.058
NS
NS
0
500
1000
1500
2000
2500
3000
0
20
40
60
80
100
0
50
100
150
200
250
300
350
0
2
4
6
8
10
12
0
20
40
60
80
100
120
140
Time Since Fire (years)
Time Since Fire (years)


a
R² = 0.481
p = 0.026
NH4+ (µg cm-2)
0
20
40
60
80
100
120
140
160
b
R² = 0.374
p = 0.066
NO3- (µg cm-2)
0
2
4
6
8
10
c
R² = 0.513
p = 0.020
PMN (µg cm-2)
0
50
100
150
200
250
0
20
40
60
80
100
120
140
Time Since Fire (years)


0.8
0.6
0.4
0.2
0.0
Total Phenols (mg cm^{-2})
0
20
40
60
80
100
120
140
Time Since Fire (years)
R^2 = 0.636
p = 0.003

Fire exclusion and nitrogen mineralization in low elevation forests of western Montana

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Abstract: Little is known about fire exclusion effects on N cycling in the low elevation forests of western Montana. Ponderosa pine forests are thought to have been maintained historically by frequent low-intensity wildfires resulting in open, uneven aged stands of grass dominated understory. One hundred years fire exclusion has apparently caused a shift in forest structure toward greater domination by shade tolerant plants including Douglas-fir and various shrubs. To date it is not clear how this long-term change in fire frequency has influenced ecosystem function in terms of N availability and plant-soil-microbe interactions. A fire chronosequence approach was used to study how fire exclusion shapes soil processes and plant diversity. A total of ten sites with increasing time since fire were examined for soil biochemical properties including decomposition, available N, microbial biomass and respiration, and total phenols. Tongue depressors and cotton strips were installed to estimate cellulosic and ligno-cellulosic decomposition rates over an 18 month period. The sites were also monitored for a two month period with ionic and non-ionic resin capsules for net N mineralization and labile C pools. Tongue depressors and cotton strips were found to have reduced rates of decomposition with increasing time since fire. Mineral soil NH_4^+ and NO_3^- did not change significantly with time since fire. However, mineral soil mineralizable N, as estimated by anaerobic incubation and resin sorbed NH_4^+ and NO_3^- decreased significantly with time since fire. An increase in the metabolic quotient and a decrease in labile C pools suggested an increasingly difficult substrate for microbial decomposition. Total phenols showed no significant trend in the mineral soil, but were negatively related to net nitrification in a highly significant correlation. These results imply that microbes are being inhibited by both the availability of labile substrate, as well as by some chemical factor on site which may be mediated by vegetation. Biodiversity initially increased with the recovery of shrubs, but then remained constant with time. Our results point to an important role of shrubs as drivers of decreasing N turnover with increasing time since fire and suggest that the alteration of fire regimes in ponderosa pine ecosystems have substantial consequences on the N cycle via changes in vegetation.

Keywords: Chronosequence, nitrogen cycle, allelopathy, secondary succession, soluble sugars, resin capsules, metabolic quotient, total phenols, decomposition, biodiversity

INTRODUCTION

There is currently little known regarding the influence of time since fire on nitrogen (N) cycling and the factors that affect N cycling in low elevation forests of the dry inland Northwest. Recently the interior Northwest has experienced several years of catastrophic forest fire. In 2003 more than 300 thousand hectares burn in western Montana alone and in 2000 a similar area burned in western Montana and northern Idaho.

Fire exclusion is blamed for the large number of high severity fires that have begun to occur nearly every summer in this region. Vegetation shifts towards late secondary succession increases fuel loading, causing forests to become more susceptible to high severity fire and is the explanation given for the apparent increase in forest burning (Arno et al. 1995). This has lead to management strategies that propose to reduce live and dead fuel loading. However, late secondary succession also causes changes to nutrient availability (Oliver 1981, Vitousek and Howarth 1991, Callaway et al. 2000). To date little emphasis has been placed on quantifying long-term changes in nutrient availability with increasing time since fire. Many studies show that litter quality decreases with late secondary succession (Zackrisson et al. 1997, Nilsson et al. 2000) and it is possible that the allelopathic properties or 'after-life' of plant litter reduces nutrient availability for some time (Northup et al. 1998, Hättenschwiler and Vitousek 2000). The question remains whether major attributes of ecosystem function, including nutrient availability, allelopathic interactions and plant diversity are well coupled to the disturbance regime in the dry interior Northwest

Historic evidence from western Montana suggests that ponderosa pines were maintained by frequent low severity fires that burned every 10-50 years (Arno et al. 1995, Arno et al. 1997, Barrett et al. 1997). Many forests in the area have not experienced fire for over 130 years providing an excellent opportunity to study the effects of fire exclusion on N cycling. In a recent chronosequence study in western Montana (MacKenzie et al. *in press*), we showed that concurrent with increases in basal area, shrubs re-establish to co-dominance with grasses and forbs shortly after fire and 132 years is apparently not sufficient time for successional exclusion of herbaceous plants from these sites. These results contrast those of Southwestern ponderosa pine forests where fire maintained, grass-dominated understories are eventually replaced by pine needles and bare ground in the absence of fire (White 1985, Covington and Sackett 1986, White et al. 1991, Kaye and Hart 1998). We also showed that forest floor properties change dramatically with increasing time since fire, with increases in total C and total N, but decreases in available N and litter quality. We concluded that shrubs have the potential to drive nitrogen cycling in the absence of fire, through plant chemistry. In the context of plant community ecology, the potential for biodiversity to increase with increasing time since fire is high due to lower resource availability (Tilman 1987). However, in the context of ecosystem function, the shift from a disturbance controlled nutrient cycle to a vegetation controlled nutrient cycle, may be detrimental to productivity and biodiversity in the long-term.

The purpose of this study was to examine how fire exclusion has modified decomposition rates, N cycling, secondary metabolites in the mineral soil and plant diversity. The specific research objectives included: 1) to quantify the rates of decomposition; 2) to quantify N availability with time since fire; 3) to determine if differences in N availability are a function of microbial immobilization or phyto-toxic inhibition of microbial activity; and 4) to quantify how plant diversity is related to increasing time since fire. By looking at these characteristics we hope to determine how tightly forest N cycling and vegetation patterns in low elevation forests of the dry interior Northwest are coupled to the historic disturbance regime.

MATERIALS AND METHODS

Study Sites

Studies were conducted at 10 sites in western Montana of varied time since fire (2, 24, 45, 78, 87, 92, 122, 132 years since fire). Sites were selected from a previous chronosequence study (MacKenzie et al. *in press*) that had similar environmental characteristics including: slope, aspect, elevation, and soils at the sub-group level (Table 1). All sites were located in the Bitterroot National Forest, the Lolo National Forest, and the Blackfoot Clearwater Game Reserve (Fig. 1). Fire dates were determined by the Forest Service (data not published) and ground truthed by visual evidence of burning when possible. At each site, 100 m transects were laid out for sampling, perpendicular the main slope. Within each 10 m section of transect, two random numbers were drawn, the first number representing the distance along the transect and the second number representing the distance to a point, alternating above and below the transect, from which samples were collected. These sampling locations were marked for future reference.

Decomposition Analysis

To measure the rate of cellulose and ligno-cellulose decomposition, one tongue depressor and one cotton strip were installed at the forest floor/soil interface and left to decompose for 18 months at 10 sampling points per site. Cotton strips are primarily made-up of cellulose, while tongue depressors are made-up of both cellulose and lignin. It takes only one enzyme complex to decompose cotton strips making them more labile, whereas it takes a suite of enzyme systems to decompose tongue depressors, making them less labile (Paul and Clark 1996, Stevenson and Cole 1999). Therefore, cellulosic materials (cotton strips) should decompose far more rapidly than lignified material (tongue depressors) and perhaps decompose at different rates if the microbial community shifts to favor decomposers of high lignin, low cellulose material. Alternatively, change in the rate of decomposition will be the same for both materials if the soil environment becomes loaded with allelochemicals (Paul and Clark 1996, Zackrisson et al. 1996). Loss of mass from the tongue depressors was determined gravimetrically by the difference between the initial and final oven dried weights (60 ° C for 24 h.). However it was not possible to determine the final oven dried weight of the cotton strips as decomposition of the cloth made it difficult to effectively clean the strips of soil and fungal matter. Cotton strips were placed on a template of their original size with a grid pattern dividing the template into 10% sections and a visual estimate of the percent lost was calculated. This value was then converted to mass lost from the initial oven dried weights. The rate of decomposition was calculated by diving the mass lost by the amount of time and was reported as g per year.

Soil Sampling

In March of 2002, approximately ten soil samples were composited from the 0-10 cm depth at each of the ten sampling points for each site. Samples were collected with a standard, 2.5 cm diameter soil sampling probe and chilled on ice until they could be processed in the lab, usually the day after sampling. Sub-samples were weighed out for different analyses including, field moisture, pH, total carbon (C), total nitrogen (N), available N, potential mineralizable N (PMN), microbial biomass, microbial respiration and total phenols. Ionic and non-ionic resins were installed to monitor NH_4^+ and NO_3^-

mineralization over a 2 month period (Kjønaas 1999, Morse et al. 2000). Polyester capsules containing 10 ml (approximately 1g dry weight) of mixed bed ionic resins (PST-2, Unibest, Bozeman, MT) were installed just below the forest floor/soil interface at each of the ten sampling points per transect for analysis of amino-N, NH_4^+ and NO_3^- (Keeney and Nelson 1982). Capsules were covered with 1 to 2 cm of soil to secure them in place. Fishing line and flagging tape were attached to each capsule to aid in recovery. Non-ionic resin capsules (Unibest, Bozeman, MT) filled with approximately one gram dry weight (about 1100 m^2 of surface area) of XAD-7 resin (Rohm and Hass Inc., Philadelphia, PA) were installed the same way, adjacent to the ionic resin capsules, for analysis of anthrone reactive carbon (ARC) (Morse et al. 2000) and soluble phenols (Stern et al. 1996). In May of 2002, the resin capsules were removed and frozen until they could be analyzed.

Soil Chemical Analysis

Oven dried weight was determined by placing 30 g sub-samples in a forced air oven at 105 ° C for 24 hours. The difference between the field moist value and oven dried value were used to determine a moisture factor that would allow us to report chemical data on a per gram dry soil basis. Soil pH was determined in a 2:1 soil slurry with 0.01 M $CaCl_2$ (McLean 1982). A sub-set of soil samples were air dried and ground to pass a 0.01 mm mesh for total C and N analysis. Total C and N were determined by dry combustion and analyzed on a Fissions Analyzer (EA 1100, Milan, Italy). The C/N ratio was determined from this data by dividing the total amount of C by the total amount of N.

Total phenols were measured from air-dried samples by the Prussian Blue method (Stern et al. 1996). Twenty-five grams of air dried sample was extracted with 50 % methanol for 24 h. These samples were filtered with Whatman 42 filter paper, büchner funnels and a vacuum manifold. The extracts were then analyzed at 720 nm against a (+)-catechin standard.

Extractable NH_4^+ and NO_3^- were removed by shaking 30 g field moist soil in 50 ml of 2 M KCl for 30 min and filtering with Whatman 42 filter papers, büchner funnels and a vacuum manifold. The extracts were analyzed for NH_4^+-N and NO_3^--N on a segmented flow Auto Analyzer 3 (Bran and Luebbe, Chicago, IL). We used the salicilate-nitroprusside method for NH_4^+ and the cadmium reduction method for NO_3^- (Keeney 1982). These extracts were also used to determine the amount of amino N, as estimated by the ninhydrin reactive N (NR-N) method (Moore 1968) less the NH_4^+-N concentration for comparison with the fumigation results. To determine PMN, 5 g of field moist soil was immersed in 10 ml of deionized water in a 50 ml centrifuge tube. The head space was displaced with N_2 gas, the tubes sealed and incubated at 25 °C for 14 days. After 14 days, 10 ml of 4 M KCl was added to bring the total volume of extractant to 20 ml with a molarity of 2, shaken for 30 min. and filtered in the same manner as the other soil extracts. The extract was analyzed on a Technicon II autoanalyzer for ammonium with the same method as above. The unicubated ammonium data was subtracted from the incubated ammonium data to give the PMN per gram dry soil.

Microbial biomass was measured by the fumigation extraction method (Amato and Ladd 1988). Briefly, 30 g. of soil were fumigated with chlorophyll in an air-tight bell jar for 24 hrs. Fumigated samples were extracted in 50 ml of 2 M KCl with the same method as above and extracts were analyzed for Amino N by NR-N method (Moore 1968). Unfumigated data was subtracted from fumigated data to give organic N concentrations which were multiplied by 3.2 for Biomass N or 22 for biomass C (Amato and Ladd 1988). Microbial respiration was measured by the alkali trap/HCl titration method (Fierer and Schimel 2002). We used 50 g. of field moist soil, sieved to 4 mm to remove roots and allowed to respire in a mason jar for 3 days. Scintillation vials with 20 ml of 1 M NaOH were placed in the jars to trap CO_2 evolved. After 3 days, the traps were removed and the solution transferred to 250 ml Erlenmyer flasks containing 30 ml of 2 M $BaCl_2$ and a few drops of phenathalien. Titration to neutrality with 1 M HCl was performed and the volume of HCl used recorded. From this data the concentration of CO_2 evolved per g dry soil was calculated.

Resin Capsule Analysis

Ionic resins were extracted by 3 successive 10 ml rinses of 2 M KCl, each shaken for 30 min (Kjønaas 1999, Morse et al. 2000). Extracts form the three successive rinses were mixed and centrifuged at 3000 RPM for 10 min to remove soil particles, and NH_4^+, NO_3^- and NR-N analyzed as outlined above. Non-ionic resins were extracted with two different extractants in succession. Resin capsules were placed on top of 200 ml French square bottles under a partitioning pump that delivered 1 ml of extractant per minute for 30 minutes. The first extractant was distilled water for determination of ARC (Morse et al. 2000) and the second extractant was 50% methanol for analysis of soluble phenol (Stern et al. 1996). Studies have shown that distilled water does not remove phenols while 50 % methanol removes up to 95 % of the phenols sorbed (DeLuca, unpublished data).

Vegetation Survey

Understory vegetation was surveyed with 0.5 m^2 quadrats placed at 10 sampling points per transect. Each species was identified and the number of individuals recorded from within the quadrat. This included all grasses, forbs and woody species. Another 0.5 m^2 quadrat per sample was placed adjacent to the initial quadrat to pick up rare species of forbs. Woody species and trees were quantified in 10 additional 5 m radius plots. Again, species were identified and the number of individuals recorded. Vegetation data from both quadrat sizes was converted to number of individuals per m^2, per species. The Shannon index of biodiversity was calculated as:

$$H' = \sum_{i=0} pi \ln(pi) \qquad (1)$$

where H' is the Shannon index number and *pi* the proportion of individuals of each species to the entire number of individuals (Raven 1992).

Statistical Analysis

Systat 9.0 was used to perform statistical analysis of data sets collected from this study (Wilkinson 1999). Linear and log-linear regression analyses were performed to examine the relationship between biochemistry variables and time since time. In all cases tests were performed to ensure that the assumptions of regression analysis were met, including that the error terms were normally distributed, had constant variance and were independent of each other (Wilkinson 1999).

RESULTS AND DISCUSSION

Decomposition Analysis

Fire exclusion clearly influences biochemical processes including decomposition. The rate of decomposition decreased significantly for tongue depressors and cotton strips with increasing time since fire (Fig. 2). Both tongue depressor and cotton strip data exhibited linear decreases with time, however mass loss over time was greater for cotton strips than for tongue depressors (data not shown). It appears that the less recalcitrant substrate represented by cotton strips (ligno-cellulose) was mineralized to a greater extent than the recalcitrant material represented by tongue depressors (lignin). These data suggest that the shift in decomposition is due to an accumulation of recalcitrant and potentially inhibitory compounds rather than a shift in the microbial community as outlined earlier. In other words, the microbial community does not become better adapted to decompose the organic substrate, which is evidence for substrate limitation. This suggests that the amount of material the microbial community can mineralize is being inhibited by allelochemicals that most likely accumulate as the forest floor degrades. These results are in line with our previous study, which showed that total phenols in the forest floor were well correlated with forest floor thickness (MacKenzie et al. *in press*).

Nitrogen Availability

Fire exclusion showed dramatic effects on N mineralization when measured *in-situ* with ionic resin capsules. All forms of N measured, including NR-N, NH_4^+ and NO_3^- decreased significantly with increasing time since fire (Fig. 3). Ninhydrin reactive N is a measure of amino N, but not cyclic or polycyclic N and although a small fraction of the total dissolved organic N (DON) pool, NR-N represents the highly labile organic N (Moore 1968, DeLuca et al. 1992, Jones et al. 2004). Ninhydrin reactive N decreases with time since fire (Fig. 3 a) and is a solid indication of a shift in chemical nature of the products of decomposition. Available N also decreases with time since fire as shown by the very tight trends for NH_4^+ and NO_3^- (Fig. 3, b and c). Both of these trends followed log-linear decreases with inflexion point centered somewhere between 20 and 50 years since fire. As this time period reflects the historic fire return interval, we interpret this trend to be the result of a tight coupling of ecosystem function with the dominant disturbance regime. DeLuca et al. (2002) have shown similar log-linear decreases in nutrient availability with increasing time since fire in Boreal ecosystems. In this fire chronosequence however, their data showed that the inflexion point of the curve for decreasing resin collected NO_3^- concentrations occurs later, between 80 and 120 years since fire, which reflects the natural disturbance regime of Boreal forests (DeLuca et al. 2002).

Figure 3 also shows that the amount of NO_3^- that accumulates on resin capsules is similar in magnitude to the amount of NH_4^+. It is often assumed that nitrification is reduced as systems move into late secondary succession (Rice and Pancholy 1972, White 1988). Recent evidence suggests that a tight coupling exists in mature forest ecosystems where nitrification and NO_3^- uptake occur rapidly to reduce N loses (Stark and Hart 1997). There has also been a long debate about increasing allelopathic interference of nitrification with increasing time since disturbance (White 1988, McCarty and Bremner 1989, Northup et al. 1998, Hättenschwiler and Vitousek 2000). However, our data show that a significant amount of nitrification occurs immediately after fire and decreases at a similar rate as that of NH_4^+. It is difficult to say if the resin data shows inhibition of nitrification which may be related to the pool sampled by resin capsules. Resin capsules represent a unique measure between gross and net N mineralization. This is because available N flows or diffuses into resin capsules from the soil solution where it adsorbs to resin beads and is no longer readily available for uptake. By removing some of the inorganic N, we short-circuit N turnover, there-by making it difficult to say exactly what portion of the available pool we have sampled. It is also impossible to estimate the volume of soil sampled by these small diameter capsules, therefore the values are reported as N per capsule. The benefits of using resin capsules include 'real time' measurements of N availability, instead of 'point in time' measurements, and minimal disturbance to the soil environment during installation and removal (Kjønaas 1999).

Interestingly, soil grab samples did not exhibit any significant trends for either NH_4^+ or NO_3^- with increasing time since fire (Fig. 4). This is not surprising given that soil grab samples are a poor surrogate of N mineralization in forest environments where the rate of turnover can be rapid and the competition for N intense (Kronzucker 1997, Stark and Hart 1997). Soil PMN decreased significantly with increasing time since fire (Fig. 4), based on a 14 day anaerobic incubation. This gives an index of the amount of labile N that can be mineralized given optimal conditions, but reduces the amount of uptake and oxidation of N by other microbes (Keeney 1982). A decrease in PMN with increasing time since fire indicates that the amount of labile N that is easily mineralized by the microbial community of these sites is decreasing and is good evidence that the forest floor quality is changing and subsequently affecting nutrient turnover in the soil.

This finding is in contrast to reports from other ponderosa pine systems, where re-introduction of fire decreased the amount of PMN (Covington and Sackett 1992, Hart et al. 1994, DeLuca and Zouhar 2000, Newland and DeLuca 2000, Choromanska and DeLuca 2001). This discrepancy is difficult to reconcile, but may be a function of the nature of controlled fire vs. wildfire. Most of the studies cited above involve treatments of controlled burning, in some cases with multiple burning cycles, whereas the sites from this study were burned by wildfire and have only burned once in the last 130 years. Furthermore, Southwestern forests have very different understory vegetation, fire return interval and soil dynamics (White 1985, White et al. 1991, Hart et al. 1994). Another explanation is that the sites examined in this study have very few N-fixing species present and some cutting history. Fire exclusion has been shown to reduce the number of N-fixers (Newland and DeLuca 2000) and it is possible that removal of biomass from these sites in the past has reduced total N reserves in the soil.

Total C and N concentration (g kg^{-1}) in mineral soil, and the C to N ratio did not change significantly with increasing time since fire (Fig. 5). Fire consumes some portion of the forest floor and through the annual deposition of litter, an accumulation of organic material develops on the surface. The thickness of the forest floor and content (kg ha^{-1}) of total C and N increased significantly with time since fire (MacKenzie et al. *in press*). However, the concentration of total C and N in the mineral soil of these sites did not change with time since fire. The heat from a low severity fire does not penetrate soil to depth (Neary et al. 1999) so organic matter is not heated to the point of volatilization and therefore total C and N do not change from a fire perspective. However, long-term accumulation of organic matter should lead to higher concentrations of total C and N in the soil. Although a significant increase, the C to N ratio some of the older sites is around 30:1 which is high and likely leads to increased N immobilization (Stevenson and Cole 1999). Together, these results begin to indicate a transformation in the nature and structure of organic constituents. These changes may be directly influencing soil N cycling by influencing the microbial community.

The resin capsule N data clearly show that mineralization decreases with time since fire. Several possibilities exist to explain this phenomenon. First, rapid microbial immobilization of available C and N results in limited net mineralization (Bernston and Aber 2000, Vance and Chapin 2001). However, this would imply an increase in microbial biomass over time (see next section) Second, phenolic compounds created as secondary metabolites in plants may cause allelopathic interference in the microbial community resulting in reduced gross mineralization (Northup et al. 1998, Hattenschwiler and Vitousek 2000). In this scenario, phenolics would increase over time, which a previous study found to be the case in the forest floor (MacKenzie et al. *in press*). A combination of immobilization and inhibition exists, where phenolic compounds act as the pre-cursor to humus formation (Hattenschwiler and Vitousek 2000) and sorb proteinacious material, creating polyphenol protein complexes (PPC). This inhibits the microbial community by immobilizing available C and N. Evidence for this last possibility would be reduced decomposition of the same materials across all sites which we have shown with the decomposition results.

Microbial Activity and Soluble Organic Materials

Several indices of microbial activity were calculated from soil grab samples. Regression analysis showed that basal respiration rates (data not shown) and microbial biomass C had no relationship with time since fire, while the microbial metabolic quotient increased significantly with time since fire (Fig. 6). The metabolic quotient (Fig. 6 b) is calculated as biomass C divided by CO_2 respired per hour. Given that the size of the microbial community is not changing with time, these results indicate that the organic substrate is becoming more difficult to decompose. However, based on the biomass and respiration data, it is still not possible to say whether or not microbes are being directly of indirectly inhibited by the organic substrate. This indicates that the overall size of the microbial community is not changing through time and essentially discounts the theory that increasing microbial immobilization is responsible for the decrease in available N at these sites.

We used non-ionic resin capsules to assess bioavailable C by measuring ARC (Morse et al. 2000). The concentration of ARC that accumulates on the non-ionic resins decreased significantly with time since fire (Fig. 7 a). Soluble ARC represents free hexose sugars that are left behind by microbial digestion of organic matter and are positively correlated with microbial biomass and respiration (DeLuca 1998). This indicates a declining pool of highly labile C which can be thought of as a surrogate for microbial activity (DeLuca 1998) and suggests that microbes are being indirectly inhibited. Given that microbial biomass does not change, while the metabolic quotient increases and ARC decreases we can say that microbes are being indirectly inhibited by some organic constituent of the organic substrate. We also used non-ionic resin capsules to measure soluble phenols. Soluble phenols represent the low molecular weight category of phenols, which are most likely a microbial food source rather than allelochemicals (Hattenschwiler and Vitousek 2000). As such they might be used as another index of microbial activity, again measured *in-situ*. However, soluble phenols showed no significant relationship with time since fire (Fig. 7 b).

Ecosystem Allelopathy

In the strictest sense, allelopathy refers to plant-plant interference mechanisms that shape community dynamics, but recent arguments indicate that it is more appropriately applied to interference mechanisms at the ecosystem level (Wardle et al. 1998). These interference mechanisms are mitigated by the production of allelochemicals. Much evidence indicates that some phenolic compounds, produced as secondary metabolites by plants, are allelochemicals (Nilsson et al. 1998, Northup et al. 1998, Hattenschwiler and Vitousek 2000, Souto et al. 2000). We analyzed concentrations of total phenols from mineral soil samples and compared them to concentrations of forest floor total phenols from a previous study (MacKenzie et al. *in press*). Total phenols from the forest floor increase significantly with increasing time since fire, while total phenols from mineral soil did not (Fig. 8). However, total phenols from the forest floor follow a log-linear increase similar to the N availability data, with inflexion point between 20 and 50 years. We found total phenols in the forest floor to be positively correlated with a decrease in litter quality as mentioned previously (MacKenzie et al. *in press*). In this study, we found that mineral soil total phenols were negatively correlated ($p = 0.00$) to net nitrification as measured by ionic resin capsules (Fig. 9). This suggests that phenolic compounds are tightly coupled to the disturbance regime and interfering with N transformations in the absence of fire.

Although microbial biomass and respiration show no significant increase or decrease with time since fire, the metabolic quotient increased and ARC decreased, indicating microbial stress. Along with the decrease in decomposition, we have evidence that an increase in forest floor phenolic compounds results in a reduction in the rate of decomposition by hindering the activity of the microbial community. It is not clear whether this interference mechanism is through direct allelopathic inhibition of the microbial community or through indirect inhibition. Indirect inhibition may be the result of polyphenol protein complexes (PPC) (Hattenschwiler and Vitousek 2000), which sorb labile N compounds and may change the C to N ratio of the soil solution. We need to further investigate the explanation that polyphenolic compounds are sorbing proteinacious material and reducing N availability resulting in a reduction of microbial activity. It is also not clear from this data whether or not phenolic compounds are being produced directly by plants or by *de novo* synthesis in the forest floor as a by-product of lignin decomposition (Hattenschwiler and Vitousek 2000). To examine the impact of decreasing N availability and the potentially plant mediated accumulation of phenolic compounds we analyzed plant community composition to assess biodiversity on these sites.

Biodiversity

Existing literature suggests that a decrease in resource availability should result in an increase in plant diversity (Tilman 1987, Nilsson et al. 1999, Reich et al. 2001, Zak et al. 2003). However, it is not clear whether or not the plant community is driving resource availability or vice versa. We used the Shannon index (H') to calculate species diversity. The Shannon index sums the number of individuals as a proportion of the total so that rare species are not underestimated (Raven 1992). Table 2 shows that the youngest site, 2 years since fire, has the lowest H' with a value of 1.875 while the next site, 24 years since fire, has a value of 2.577. All other sites also have an H' above 2.0. Lower diversity immediately after fire when N availability is highest is in-line with current plant community theory (Tilman 1987). The lower diversity is primarily due to a higher proportion of graminoids immediately after fire, but the diversity shifts rapidly with the recovery of woody shrubs and plateaus after roughly 25 years when N availability also begins to plateau. Again, this is remarkably similar to the log-linear decrease of available N and the log-linear increase of total phenols. It constitutes further evidence that the recovery of woody shrubs may be driving N cycling on these sites. However, this evidence is indirect and requires further study to isolate the exact mechanism responsible for post-fire ecosystem function. Direct measurements of shrub produced secondary metabolites are needed for species in this area to determine if polyphenol production is an adapted trait or merely a biological mediated result. Evidence from Boreal systems indicates that polyphenol production may be an adapted trait in woody species of the ericaeceous family (Nilsson et al. 1998, Nilsson et al. 1999) and this family is of moderate importance in ponderosa pine forests of the dry inland Northwest as well (MacKenzie et al. *in press*).

CONCLUSIONS

The analysis of fire exclusion, set in the context of a fire chronosequence demonstrated a drastic decrease in N mineralization that plateaus with approximately 25 years since fire. The microbial community experiences increasing stress as indicated by decreasing indices of microbial activity and a reduction in the decomposition of cotton strips and tongue depressors (cellulose and ligno-cellulose respectively). These may be the result of increasing concentrations of phenolic compounds in the forest floor, although phenols did not increase in mineral soil. It is not possible to say whether or not phenolic compounds were acting as allelopathic agents, directly interfering with microbes or immobilizing labile organic N as polyphenol protein complexes and indirectly interfering with microbial decomposition. A question also remains about the origin of phenolic compounds in this environment, but the biodiversity data in context with previously collected data provides further evidence that they are most likely related to the recovery of shrubs on these sites.

Our previous study (MacKenzie et al. *in press*) showed that understory vegetation shifts towards co-dominance between herbaceous and woody plants. These results are paralleled here by the diversity data which indicates that biodiversity increases with decreasing resource availability, but remains constant with approximately 25 years since fire. Given the similar time frame for changing N availability and plant diversity, we can assume a tight relationship between plant/soil interactions. It is unlikely that one species of plant is driving the shift in chemical ecology, but rather a functional group, such as woody shrubs. Together, these data indicate that woody shrubs are potentially driving N cycling on these sites and that fire exclusion is changing the way this ecosystem functions by extending the successional sequence past the natural equilibrium evolved to over the last 10 000 years.

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Table 1: Fire chronosequence site despcriptions for 10 sites at 4 locations around western Montana including: time since fire (TSF-sampled in 2002), position on the landscape, pH, texture and soil classification. Landscape variables are the avaerage of 6 samples along two different transects, pH is the average of 10 samples along one transect and soil classification is from one soil pit per site.

Site name	Burn year	TSF (years)	Slope (°)	Aspect (°)	Elevation (m.)	pH	Texture
Ninemile	1880	122	10.3	137.0	1218.6	4.36	SL
	1910	92	16.8	175.3	1272.2	4.28	L
	2000	2	13.7	187.0	1181.5	4.85	SL
Clearwater	1880	122	11.0	141.3	1452.9	4.72	SL
	1957	45	8.8	163.1	1457.1	4.78	SL
	1988	24	5.0	172.7	1396.8	4.55	SL
Lost Horse	1870	132	11.3	207.0	1393.8	4.22	SL
	1915	87	12.7	143.8	1390.6	4.35	SL
Lake Como	1870	132	15.3	40.7	1678.5	4.27	SL
	1924	78	19.5	106.7	1696.0	4.26	SL

Table 2: Biodiversity for fire chronosequence sites in western Montana including: time since fire (TSF), species richness, density of individuals and Shanon index.

Site	TSF (years)	Species Richness (# of species)	Density (idividuals m^{-2})	Shannon Index (H')
NM1	2	22	641.6	1.875
CW1	24	29	541.1	2.577
CW2	45	29	459.5	2.191
LC	78	21	322.0	2.383
LH	87	28	402.3	2.425
NM2	92	31	340.4	2.704
CWUB	122	31	524.9	2.384
NMUB	122	31	652.5	2.241
LHUB	132	34	445.6	2.352
LCUB	132	36	322.0	2.295

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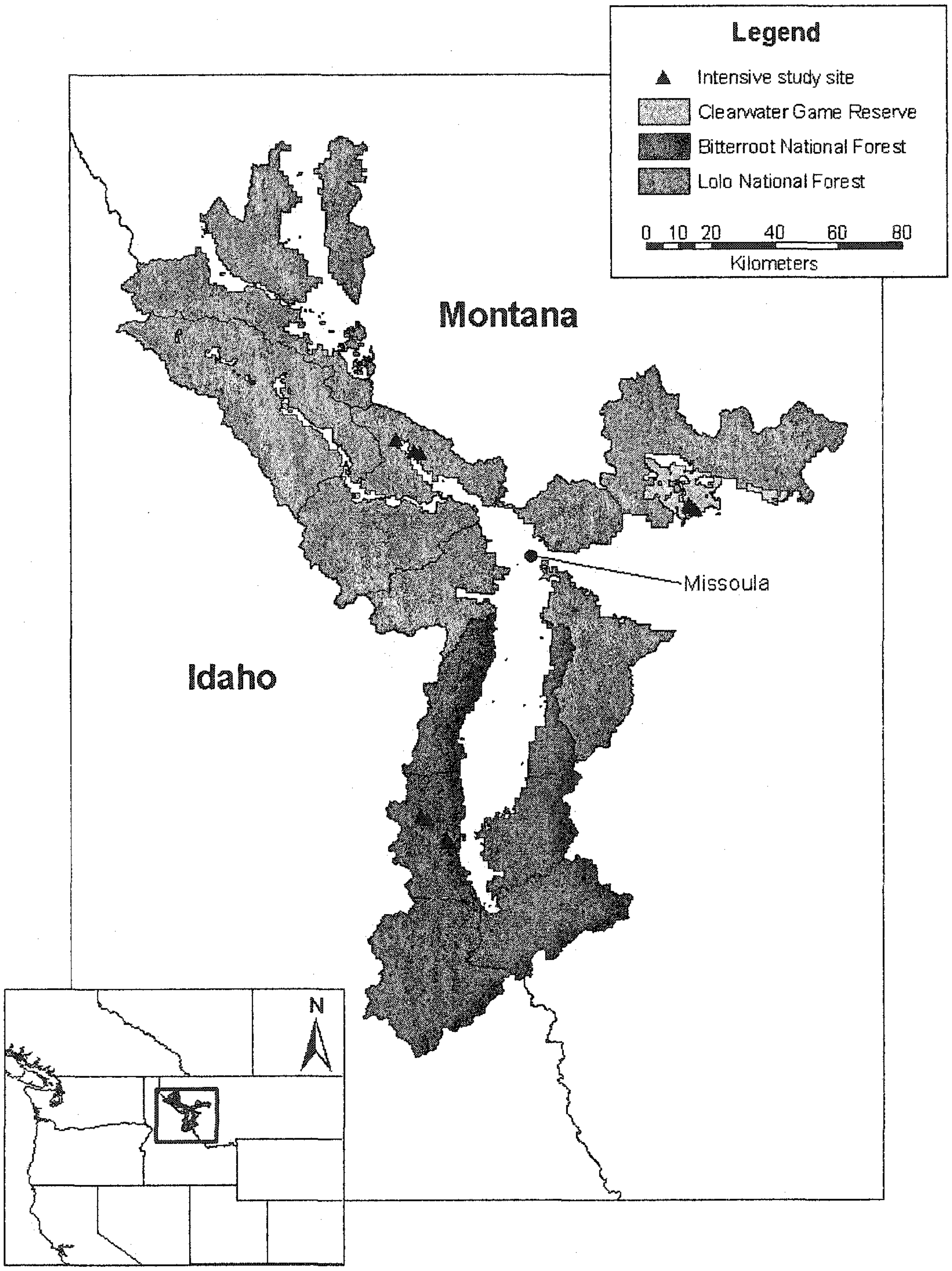
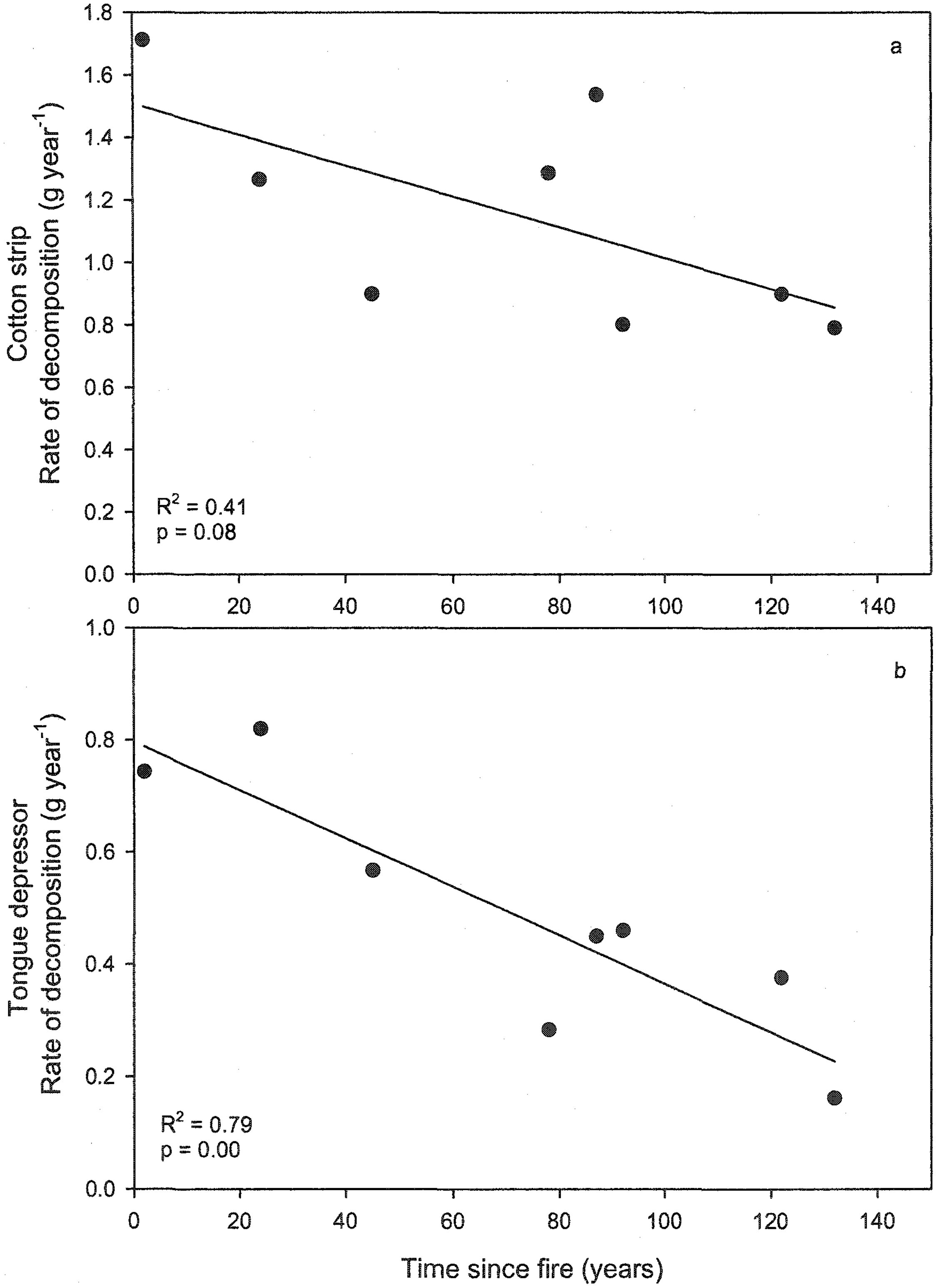
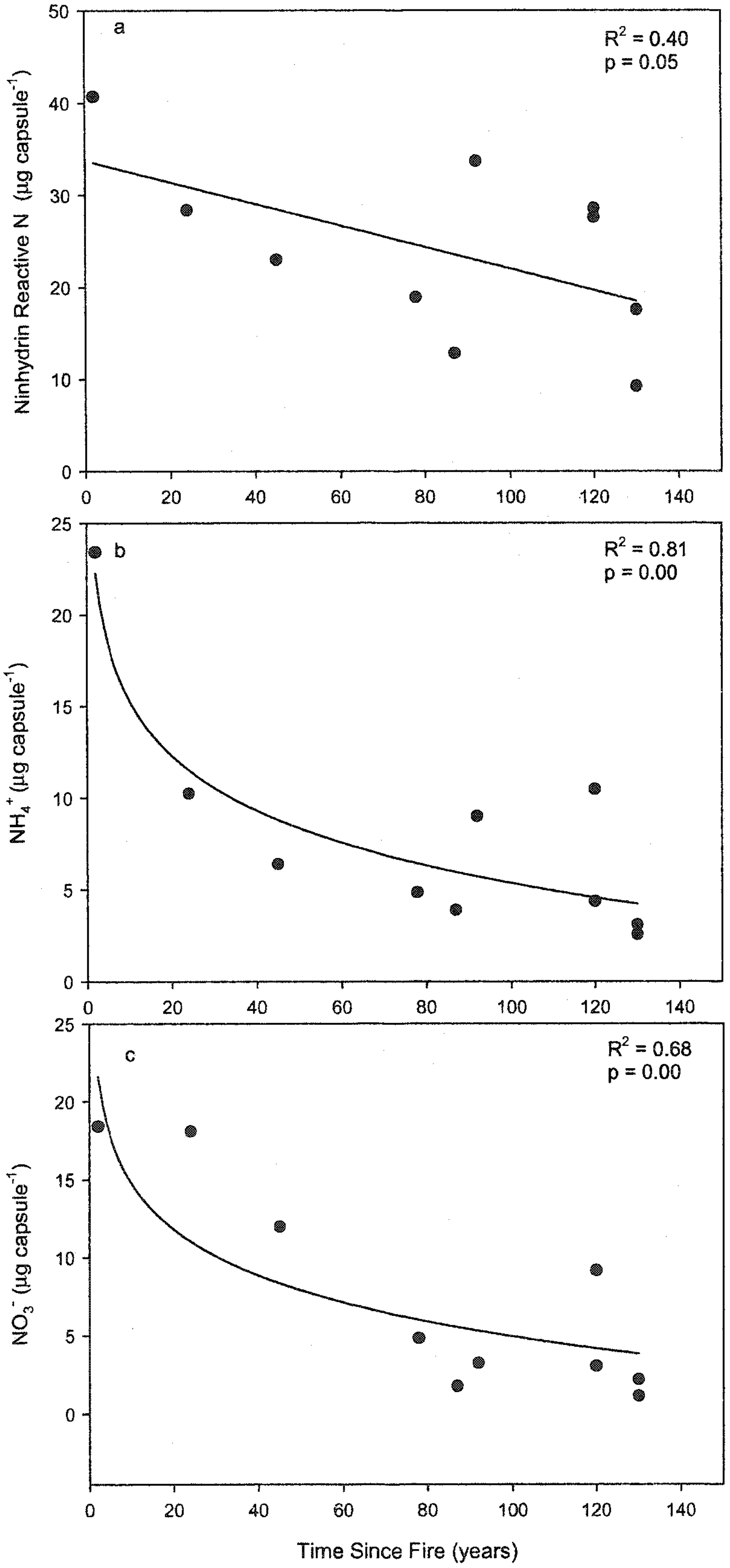
Figure 7: Sorption of free sugars (ARC, μg capsule^{-1}) and soluble phenols (mg capsule^{-1}) to non-ionic resins were measured on a fire chronosequence in western Montana. Regression analysis is reported for ARC (a) and soluble phenols (b) with time since fire (years).

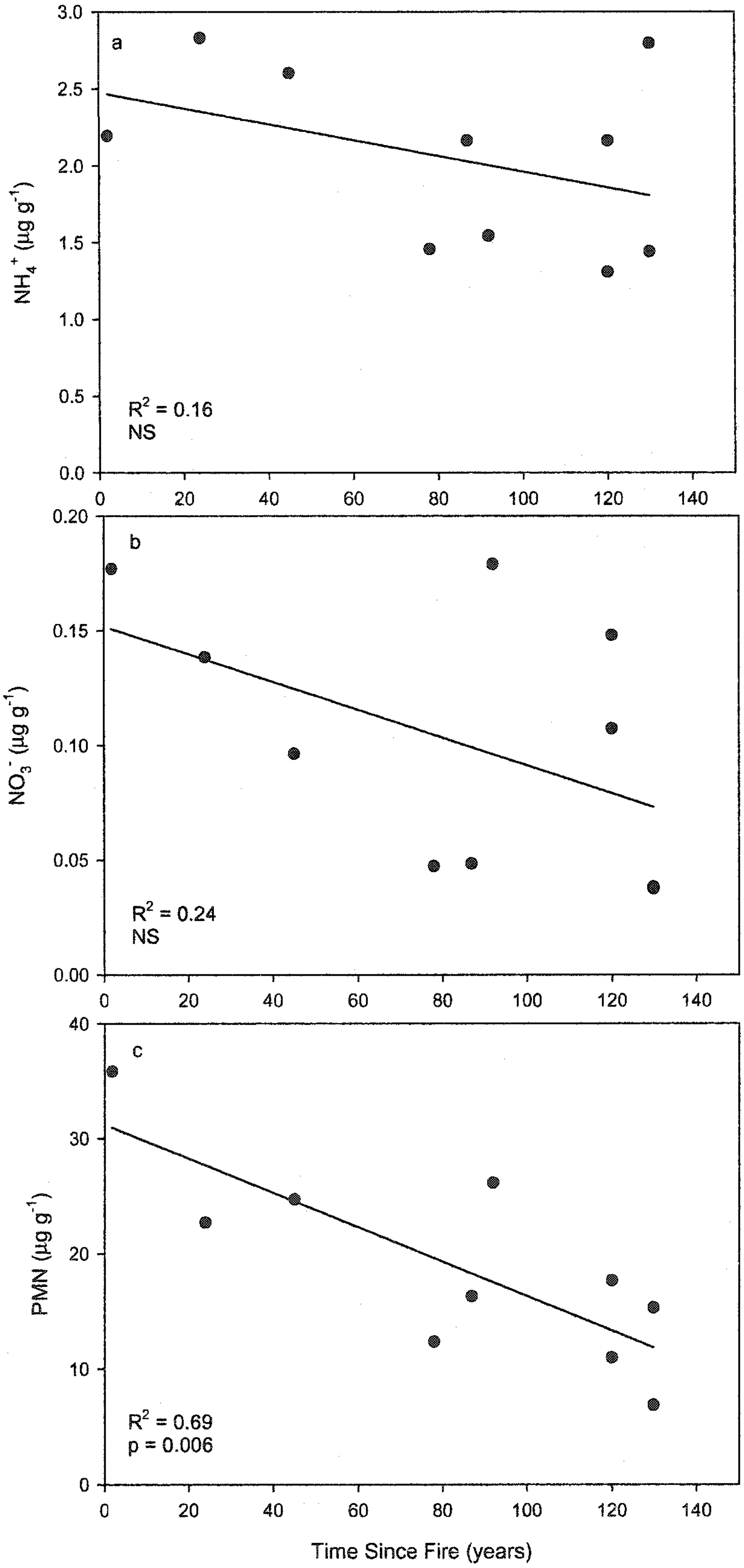
Figure 8: Total phenol concentration (mg g^{-1}) in forest floor (a) and mineral soil (b) samples from a fire chronosequence in western Montana and regression analysis was performed with time since fire (years).

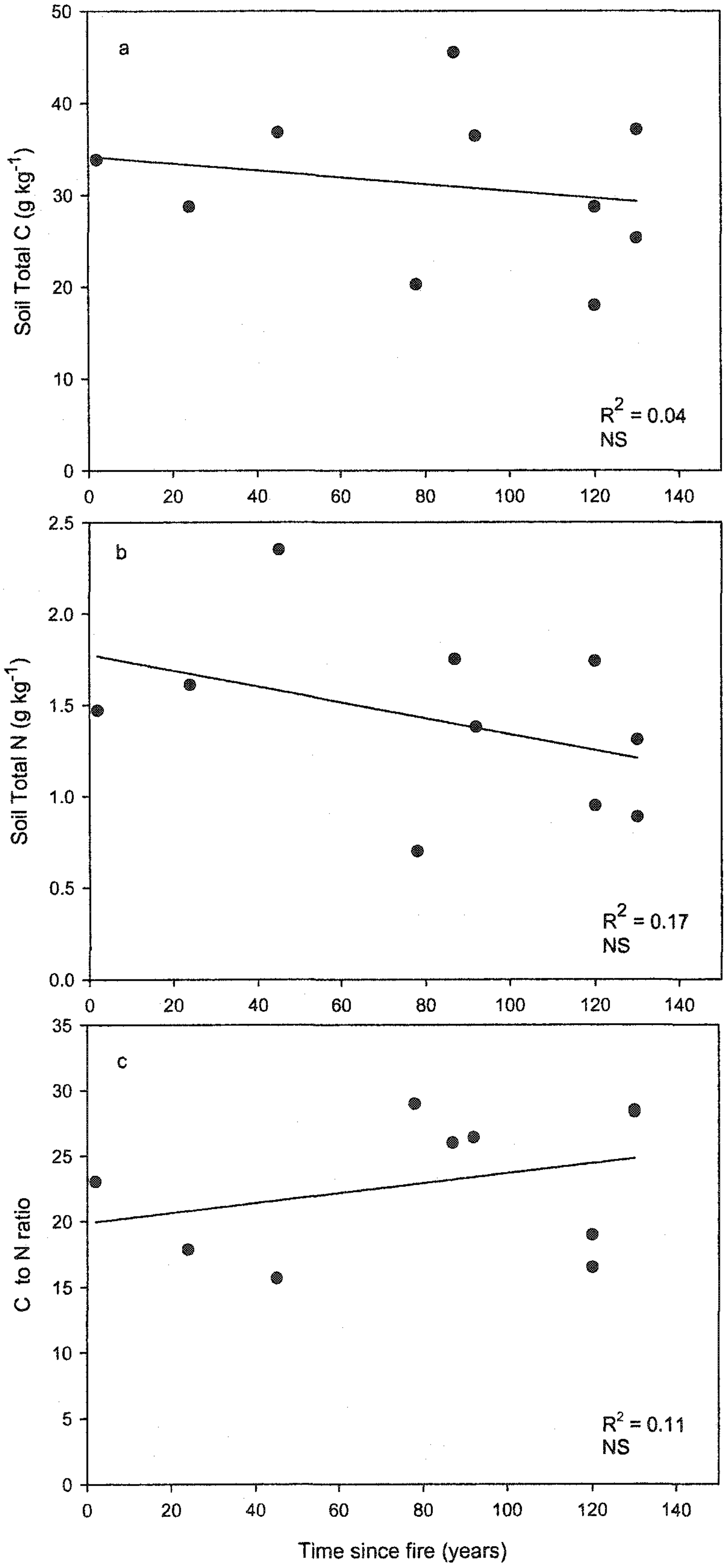
Figure 9: Net nitrification (ug capsule^{-1} month^{-1}) as measured by ionic resin capsules was compared to mineral soil total phenol concentration (mg g^{-1}) by uncorrected Pearson correlation for a fire chronosequence in western Montana.

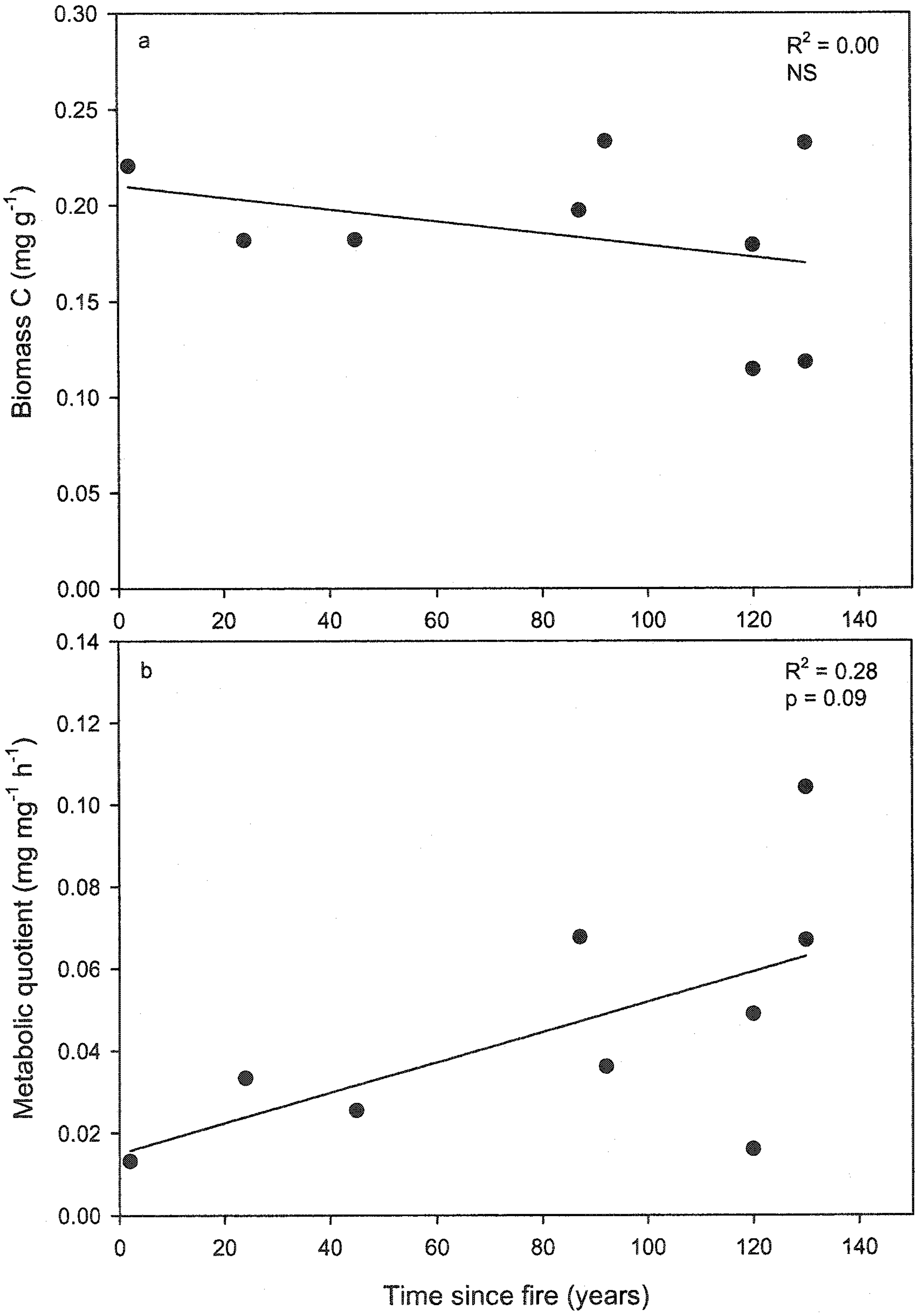

Legend
▲ Intensive study site
Clearwater Game Reserve
Bitterroot National Forest
Lolo National Forest
0 10 20 40 60 80
Kilometers
Montana
Idaho
Missoula
N

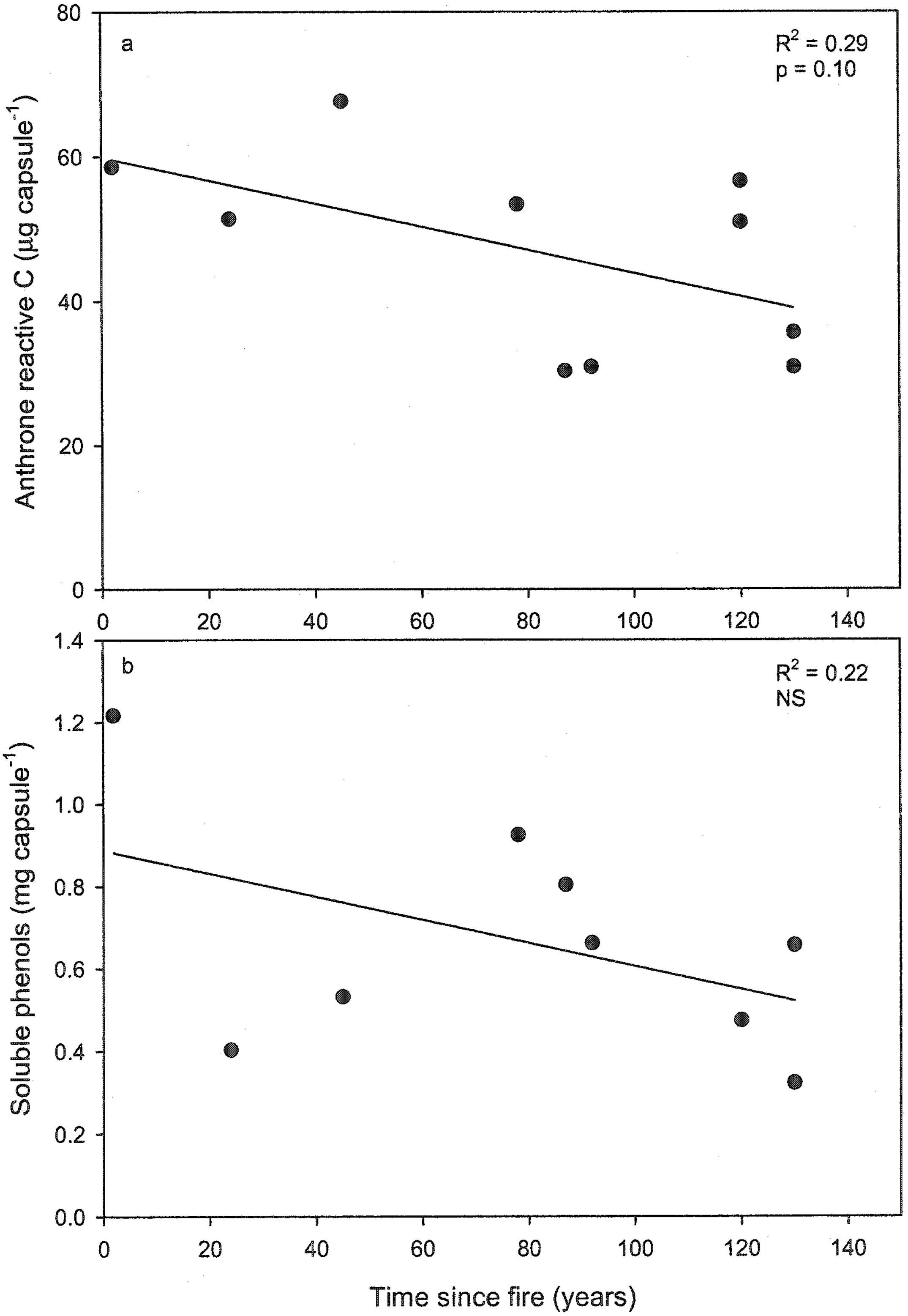

a
Cotton strip
Rate of decomposition (g year^{-1})
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
$R^2 = 0.41$
$p = 0.08$
0
20
40
60
80
100
120
140
b
Tongue depressor
Rate of decomposition (g year^{-1})
1.0
0.8
0.6
0.4
0.2
0.0
$R^2 = 0.79$
$p = 0.00$
0
20
40
60
80
100
120
140
Time since fire (years)

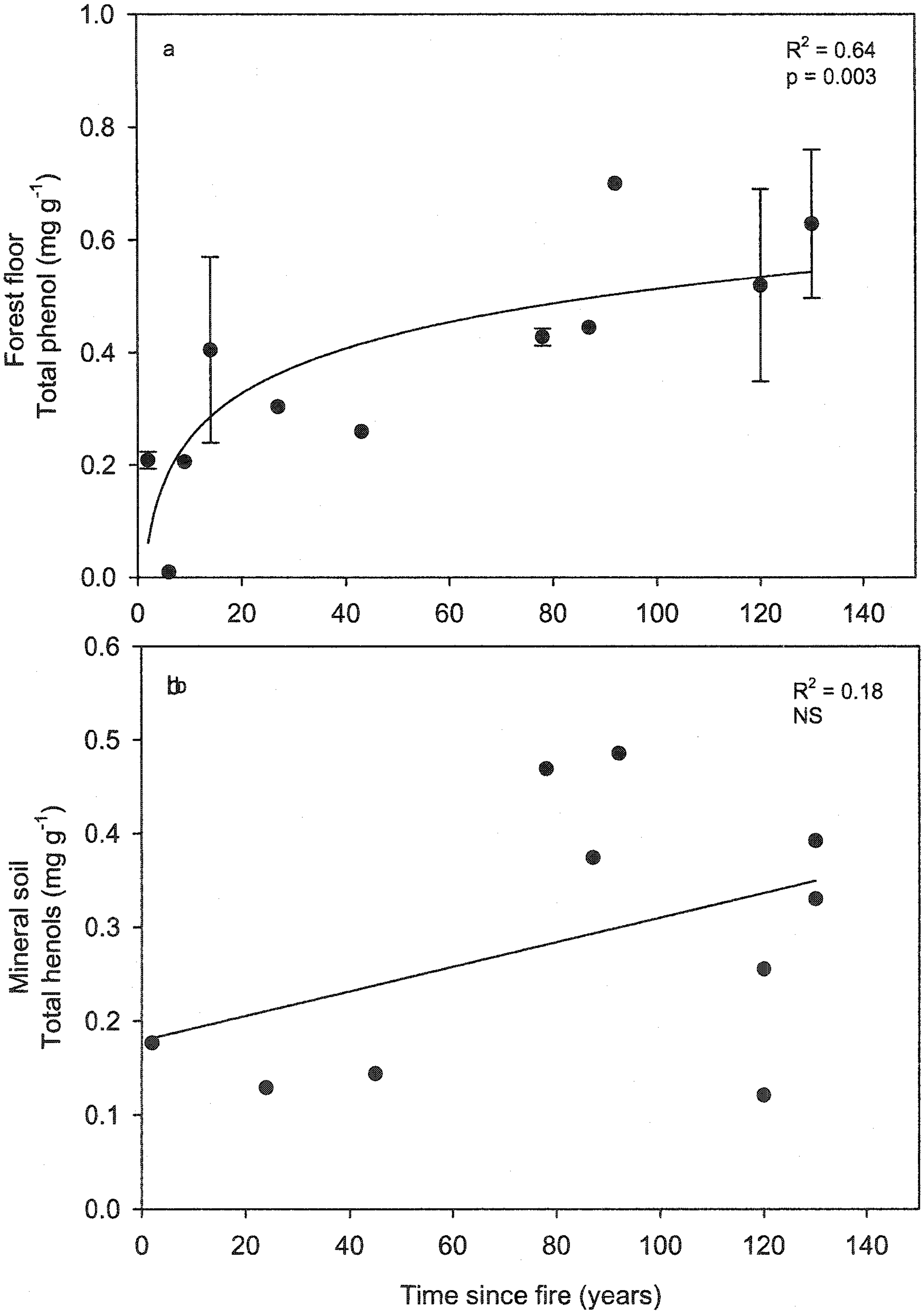

a
R^2 = 0.40
p = 0.05
Ninhydrin Reactive N (µg capsule^{-1})
0
10
20
30
40
50
0
20
40
60
80
100
120
140
b
R^2 = 0.81
p = 0.00
NH$_4^+$ (µg capsule^{-1})
0
5
10
15
20
25
0
20
40
60
80
100
120
140
c
R^2 = 0.68
p = 0.00
NO$_3^-$ (µg capsule^{-1})
0
5
10
15
20
25
0
20
40
60
80
100
120
140
Time Since Fire (years)

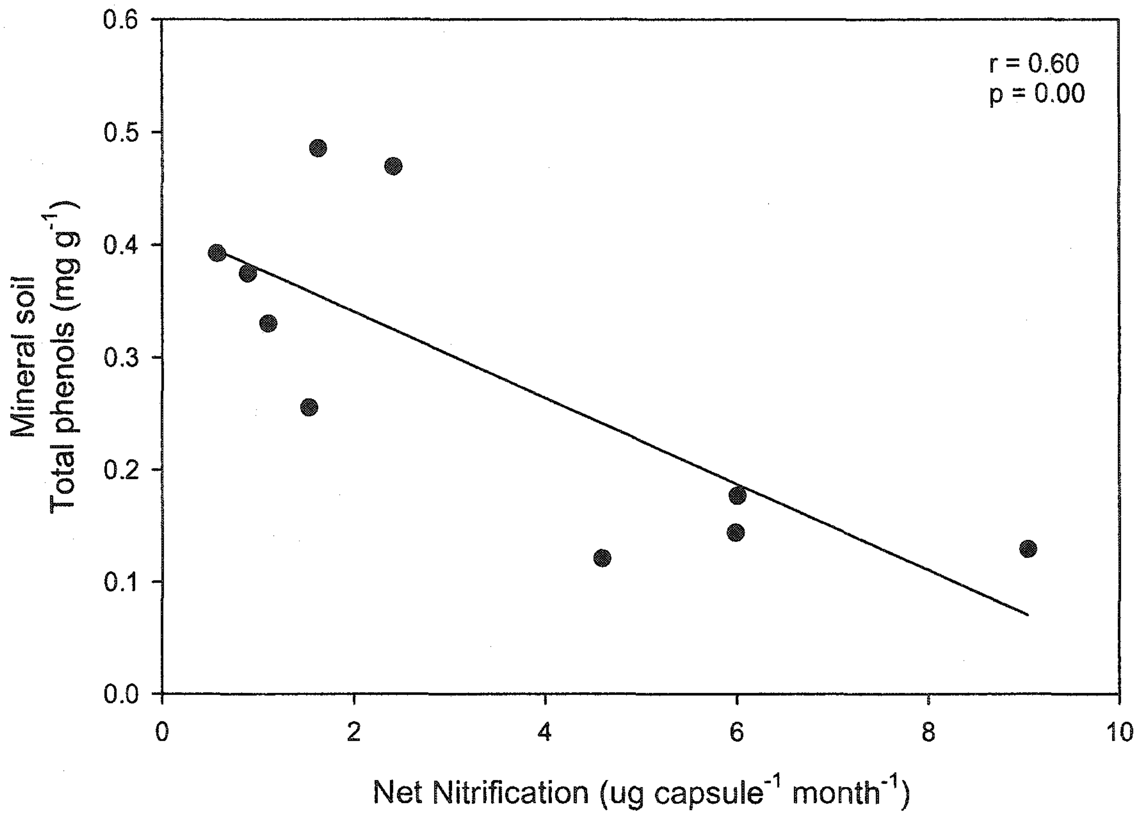

a
NH_4^+ (µg g^{-1})
3.0
2.5
2.0
1.5
1.0
0.5
0.0
$R^2 = 0.16$
NS
b
NO_3^- (µg g^{-1})
0.20
0.15
0.10
0.05
0.00
$R^2 = 0.24$
NS
c
PMN (µg g^{-1})
40
30
20
10
0
$R^2 = 0.69$
p = 0.006
0
20
40
60
80
100
120
140
Time Since Fire (years)


a
Soil Total C (g kg^{-1})
50
40
30
20
10
0
$R^2 = 0.04$
NS
0
20
40
60
80
100
120
140
b
Soil Total N (g kg^{-1})
2.5
2.0
1.5
1.0
0.5
0.0
$R^2 = 0.17$
NS
0
20
40
60
80
100
120
140
c
C to N ratio
35
30
25
20
15
10
5
0
$R^2 = 0.11$
NS
0
20
40
60
80
100
120
140
Time since fire (years)


a
R² = 0.00
NS
Biomass C (mg g⁻¹)
0.30
0.25
0.20
0.15
0.10
0.05
0.00
0
20
40
60
80
100
120
140
b
R² = 0.28
p = 0.09
Metabolic quotient (mg mg⁻¹ h⁻¹)
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
0
20
40
60
80
100
120
140
Time since fire (years)


a
R² = 0.29
p = 0.10
Anthrone reactive C (µg capsule⁻¹)
0
20
40
60
80
0
20
40
60
80
100
120
140
b
R² = 0.22
NS
Soluble phenols (mg capsule⁻¹)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
0
20
40
60
80
100
120
140
Time since fire (years)


1.0
0.8
0.6
0.4
0.2
0.0
a
$R^2 = 0.64$
p = 0.003
Forest floor
Total phenol (mg g^{-1})
0
20
40
60
80
100
120
140
0.6
0.5
0.4
0.3
0.2
0.1
0.0
b
$R^2 = 0.18$
NS
Mineral soil
Total henols (mg g^{-1})
0
20
40
60
80
100
120
140
Time since fire (years)


r = 0.60
p = 0.00
Mineral soil
Total phenols (mg g-1)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0
2
4
6
8
10
Net Nitrification (ug capsule-1 month-1)

Seasonal Nitrogen Availability and Microbial Activity in Fire Excluded Low Elevation Forests of Western Montana

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Abstract: Fire exclusion often results in decreased N mineralization due to poor litter quality and microbial stress. However, N mineralization is also affected by fluctuations in temperature and moisture. The objective of this study was to determine the simultaneous effect of time since fire and season on N mineralization and microbial indices. We installed ionic and non-ionic resin capsules along a fire chronosequence to measure N availability and microbial activity at three different times between November 2001 and August 2002. Ionic resin extracts were analyzed for NH_4^+ , NO_3^- and ninhydrin reactive N (NRN - amino N), a measure of microbial activity. Non-ionic capsules were analyzed for anthrone reactive C (ARC - hexose sugars), a measure of microbial activivty and soluble phenols (SP), a potential microbial food source. Repeated measures ANOVA indicated that all of these soil properties were significantly different during the measurement period, however regression analysis revealed that only certain variables showed a significant trend during specific seasons with increasing time since fire. All N measurements decreased significantly with time since fire during winter and spring sampling and were non-significant during summer sampling. Unlike summer concentrations of NH_4^+ and NO_3^-, NRN concentrations were highest in the summer, possibly due to disruption of microbial cells. Analysis of ARC indicated a similar trend for microbial activity, increasing over winter and spring, and crashing in the summer with the onset of hot, droughty weather. Soluble phenol data exhibited a peak in the spring sites of low and moderate time since fire, but is more difficult to interpret than ARC. Both ARC and SP need more study to determine how these variable correlate with the microbial community when collected on resin capsules. None of the biochemical variables analyzed here exhibited a significant trend during the summer sampling period indicating the effect of drought on this ecosystem and the importance of sampling time.

Keywords: Fire exclusion, N mineralization, microbial biomass, microbial respiration, seasonal N availability, dry inland Northwest, ponderosa pine, Douglas-fir

INTRODUCTION

Little is known about how N mineralization and microbial activity fluctuate with changing season and increasing time since fire in the dry inland Northwest. Fire exclusion in the ponderosa pine/Douglas-fir ecosystems of the dry inland Northwest has recently been shown to affect N mineralization adversely (MacKenzie et al. *in press, in review*) as hypothesized by Covington and Sackett (1992). Historically, these forests burned every 10 to 50 years with a low severity fire regime (Arno et al. 1995, Barrett et al. 1997, Arno and Allison-Bunnell 2002). This fire regime promoted an open canopy of ponderosa pine with an understory of grasses and forbs. Fire has been shown to promote N mineralization in the short-term (White et al. 1991, Kaye and Hart 1998, Newland and DeLuca 2000). However, Euro-American settlement of the west has drastically altered the natural fire return interval and many forests in this area have not burned for over 130 years. MacKenzie et al. (in review) showed that the extended absence of fire has caused N mineralization to asymtote at low concentrations due to poor litter quality and stressed microbes.

Ponderosa pine/Douglas-fir ecosystems of the inland Northwest receive most precipitation in the winter which becomes available during spring melt periods (Nimlos 1986). Nitrogen mineralization is influenced to a great extent by periods of wetting-drying and by cycles of freeze-thaw (DeLuca et al. 1992, Fierer and Schimel 2002). These phenomena influence N cycling primarily through their effects on microbial activity. Microbial cells are disrupted during cycles of freeze-thaw or wetting-drying which increases inorganic N availability and the amino N pool, represented by changes in ninhydrin reactive N (NRN) concentrations (DeLuca et al. 1992).

The objective of this study was to show how N mineralization and microbial activity were affected by the simultaneous effects of time since fire and season. To do this we used ionic and non-ionic resin capsules to study *in-situ* mineralization and biological activity. Ten field sites in western Montana, representing a chronosequence of time since last fire, had resin capsules installed at three different times of year. The specific research objectives were: 1) to analyze how inorganic N changes with season and time since fire and 2) to analyze how microbial activity changes with season and time since fire by examining different microbial indices including NRN, soluble anthrone reactive C (ARC) and soluble phenols (SP). We looked at microbial activity by measuring biologically available C as ARC and SP from non-ionic resin extracts. ARC has been shown to vary with microbial respiration and biomass (DeLuca 1998) and SP are low molecular weight C compounds that are most likely a microbial food source

(Hattenschwiler and Vitousek 2000). We hypothesize that the seasonal availability of inorganic N will decrease from winter to summer due to the on-set of hot, dry weather, as well as with increasing time since fire as indicated by our previous work (MacKenzie et al. *in review*). This study also showed decreasing NRN concentration with increasing TSF, indicating reduced labile substrate. However, NRN is also an index of microbial activity and it is possible that concentrations will increase with season as a function of the disruption of microbial cells, again as a result of the on-set of hot, dry weather. The other microbial indices considered here are ARC and SP which are both expected to decrease with time since fire as indicated by earlier work (MacKenzie et al. *in review*) and with season, as microbial activity ceases during hot, dry periods.

MATERIALS AND METHODS

Study Sites

Seasonal soil chemistry measurements were examined at 10 sites in western Montana of varied time since fire (2, 24, 45, 78, 87, 92, 122, 132 years since fire). These were the same sites used for intensive study of mineral soil properties and are described in more detail in MacKenzie et al (in review). All sites had similar slope (10-15 °), aspect (S to SE) and elevation (1200 -1350 m), and were within the Typic Dystrocrepts soil sub-group (Staff 1999) with silt to silty loam texture (Table 1).

At each site, a 100 m transect was laid out for sampling running perpendicular to the main slope. Within each 10 m section of transect, two random numbers were drawn, the first number representing the distance along the transect and the second number representing the distance to a point alternating above and below the transect, from which samples were collected. These sampling locations were marked for seasonal resin capsule installation and removal.

Macro-climate data was collected for western Montana (Montana region 01) for the year long period from November 2001 to November of 2002 (NOAA, 2004). Mean monthly precipitation (cm) and temperature (°C) data were collected for this period (Fig. 1). The climate of western Montana is characterized by hot summers with little precipitation, cold winters with moderate precipitation and wet springs with rapid warming. The seasons measured for this study were well within these parameters.

Seasonal Measurements

Ionic and non-ionic resin capsules were installed at the forest floor/mineral soil interface, left to incubate for several months, and removed for analysis at three different times from November 2001 to August of 2002. The winter sampling period was from November 1st, 2001 to May 1st, 2002, the spring sampling period was from May1st, 2002 to July1st, 2002 and the summer sampling period was from July 1st, 2002 to August 1st, 2002. Mixed bed ionic resin capsules were made of a polyester mesh material and contained 10 ml (approximately 1g dry weight) of resin (PST-2, Unibest, Bozeman, MT). Non-ionic resins were incased in the same polyester capsule (Unibest, Bozeman, MT), but filled with approximately one gram dry weight (about 1100 m^2 of surface area) of XAD-7 resin (Rohm and Hass Inc., Philadelphia, PA). Fishing line and flagging tape were attached to each capsule to aid in recovery.

Chemical Analysis

Ionic resins were extracted by 3 successive 10 ml rinses and 30 min of shaking with 2 M KCl (Kjønaas 1999). After each rinse the KCl was decanted into a centrifuge tube and after all 3 rinses the extractant was centrifuged at 3000 RPM for 10 min to remove soil particles. Ammonium, NO_3^- and NRN were analyzed on the extracts as outlined below. Non-ionic resins were extracted drop wise using eluents (H_2O and 50% MeOH) in succession (Morse et al. 2000). Resin capsules were seated on the openings of 200 ml French square bottles and placed under a 2mm nozzle connected by silicone tubing to a surplus partitioning pump that delivered 0.8 ml of extractant per minute for 35 minutes. The first extractant was distilled water and the second extractant was 50% methanol. We have found that distilled water removes few polyphenolic compounds while 50 % methanol removes up to 95 % of the phenols adsorbed (DeLuca, unpublished data).

Ionic resin extracts were analyzed for NH_4^+ and NO_3^- on a Technicon III auto analyzer (Bruam-Luebbe, Chicago, IL) using the salicilate-nitroprusside method for NH_4^+ and the NH_4Cl-Cd reduction method for NO_3^- (Keeney 1982). These extracts were also used to determine the amount of amino N by the ninhydrin reactive N (NRN) method (Moore 1968). Analysis of non-ionic resins included anthrone reactive carbon (ARC) (Morse et al. 2000) on the water extracts and soluble phenols (Stern et al. 1996) on the 50% methanol extracts.

Statistical Analysis

To analyze differences between sites (time since fire) and sampling time (season), we used the repeated measures analysis of variance (ANOVA) module in SYSTAT 9.0 (Wilkinson 1999). Repeated measures ANOVA was useful because it gave us the ability to compare multiple temporal samples for the same variable along our chronosequence of time since fire. Repeated measures ANOVA does this by contrasting variation in the between subject factors, in this case TSF, by the within subject factors, in this case season and the interaction between season and TSF (Underwood 1997, Wilkinson 1999). We also calculated R^2 and associated p-values by regression analysis to compare trends for individual season and TSF. This was performed as a means of post-hoc comparisons for the significant repeated measures test. In all cases assumptions of normality, constant variance and error independence were tested and met for both the repeated measures ANOVA and regression analysis (Wilkinson 1999). The significance of each case was assesed with an alpha of less than or equal to 0.100.

Contour plots are 2D graphs of three variables and were produced by Sigma Plot 8.0 (SPSS 2002) to demonstrate how different soil biochemical variables interact with seasonal data and TSF. Contour lines are produced to best represent the changes between data points and spatial or temporal factors (Lodewick and Whittle 1970). In this case, biochemistry variables were plotted against TSF and season. These plots enabled us to present a graphic extrapolation of how the biochemistry data might fluctuate as the year progressed and are meant to be a visual accompaniment to the repeated measures ANOVA and regression analysis.

RESULTS AND DISCUSSION

Nitrogen availability

Resin extracted NH_4^+-N and NO_3^--N varied significantly with both time since fire and season (Table 2). Overall, both variables decreased from winter to summer and with time since fire (Fig. 2). Furthermore, there was a significant time since fire by season interaction reflecting the fact that trends with time since fire varied depending on season. The contour plot shows that NH_4^+concentration is greatest after the winter measurement on the recently burned sites and decreased with both time since fire and season (Fig. 2). Regression analysis indicated that both winter and spring inorganic N concentrations decrease significantly with time since fire, but the summer measurement does not (Table 3). The contour plot for NO_3^- is similar to that for NH_4^+, with highest concentrations after the winter measurement on the most recently burned site and decreasing with both time since fire and season (Fig. 3). However, there is a NO_3^- concentration peak around 24 years since fire for the spring measurement, which may indicate a lag period between the highest rates of ammonification and nitrification. Regression analysis also showed that both the winter and spring measurements of NO_3^- decreased significantly with time since fire, but this trend does not appear during the summer measurement (Table 3).

The inorganic N flush that occurs during winter and spring seasons is substantial and likely represents the majority of plant available N for the entire year. The climate data for the area (Nimlos 1986) indicates that these seasons have the highest amount of precipitation which is most likely driving microbial mineralization. Our results are consistent with our initial hypothesis and the rported releases of inorganic N during cycles of freeze-thaw and wetting-drying (DeLuca et al. 1992, Fierer and Schimel 2002, Jones et al. 2004). It is interesting to note that although the spring period has more moisture and warmer temperatures than the winter, it has less available inorganic N. This must be a result of increased plant uptake and microbial turn-over (Aber et al. 1991). Both winter and spring N mineralization also decreased significantly with time since fire, further indicating the effect on N cycling of departing from the natural disturbance regime, as fire interval increases in these stands. Several studies have shown that litter quality and mineral soil organic matter quality decrease with increasing time since fire (DeLuca et al. 2002, MacKenzie et al. *in press, in review*). We can also say that there is a collective effect of season and time since fire on N mineralization.

Ninhydrin reactive N is a measure of amino acids and represents a significant portion of the DON pool (Sculten and Schitzer 1998, Jones et al. 2004). NRN varied significantly with time since fire and season, and there was also significant time since fire by season interaction (Table 2). Winter and spring concentrations of NRN exhibited significantly decreasing trends with time since fire (Table 3). However, in contrast to NH_4^+ and NO_3^- concentrations, they increased with season (Fig. 4).

Ninhydrin reactive N also decreased significantly with TSF for both the winter and spring seasons. However, the results were different form the inorganic N data in that the amount of NRN increased significantly by season, from winter to summer. The contour plots suggest that the decrease of inorganic N is in-line with the increase in NRN. Again, this is what we expected as the seasons progress towards summer and there is a significant decrease in precipitation and increase in temperature (Fig.1). The microbial community is experiencing stress by drying out and therefore less able to mineralize N. This accounts for the lower availability of inorganic N. The increase in NRN must be due disruption of microbial cells and the release of amino N into the soil matrix (Amato and Ladd 1988, DeLuca et al. 1992), also due to changing micro-climate. This is a different interpretation of resin collected NRN than in our previous study (MacKenzie et al. *in review*), where decreasing NRN with time since fire was seen as an indication of less labile substrate. However, this decrease may also be interpreted as a decrease in microbial activity, as it microbes that turnover the labile pools of N. In this data set we will interpret an increase in NRN with season is an indication of microbial activity that can be compared to resin collected ARC and SP concentrations.

Both inorganic N data and NRN data implicate winter and spring as the most important seasons for soil N measurements in this ecosystem. This is because time since fire trends are obscured during the summer sampling period when N mineralization essentially ceases due to a lack of moisture. We expect N mineralization to increase with the on-set of fall and winter precipitation, but did not mmeasure this period.

Microbial activity

Anthrone reactive C positively correlates with microbial biomass and respiration (DeLuca 1998), and as such represents a good surrogate measure for microbial activity. A benefit of this index is that it can be assessed by field incubation with non-ionic resin capsules. Anthrone reactive C varied significantly with time since fire and season, and had a significant time since fire by season interaction (Table 2). The contour plot shows that ARC concentrations increase with season (Fig. 5) similar to the NRN data. In contrast to our initial hypothesis ARC concentrations were overall higherin the summer, although there were winter peaks in 60 to 80 year old stands (Fig. 5). While ARC varied significantly with time since fire such variation was not linear and peaks of higher ARC concentrations occurred at intermediate times since disturbance.

If we compare the contour plots for NRN and ARC we see two distinct overlaps of high concentration during the summer measurements centered on 24 and 92 years since fire sites. These overlaps are difficult to explain if we believe that increased NRN indicates a disruption in microbial biomass and that increased ARC indicates an increase in microbial activity. However, it is possible that these two trends are both indicating declining microbial activity with season as indicated by the N mineralization data. Nitrogen mineralization drops in the summer months, while NRN and ARC accumulate. This may indicate either a disruption of microbial cells, spilling both amino N groups and hexose sugars into the soil medium or a lag between enzyme release and microbial death. The opposite seems to be true if we compare the winter measurements where there is an overlap of extremely high ARC concentration with low NRN concentration centered around the 87 years since fire site. In this case, high ARC with rapid microbial activity, suggested by low NRN and high inorganic N availability, indicates enzyme-released sugars. Whereas in the summer season, high ARC concentration with dormant microbes and consent plants indicates the release of non-structural carbohydrates (DeLuca 1998).

ARC will not be a very good surrogate measure for microbial activity if it has high concentrations during periods of both fast and slow microbial turn-over. As these data were generated with resin capsules, which lock compounds away from microbial turnover, unlike mineral soil, more analyses of the relationship between resin capsule ARC and microbial activity are needed. This study suggests that in conjunction with resin collected inorganic N and NRN, ARC measurements may be very useful for determining the prevalence and seasonality of hexose sugars and non-structural carbohydrates.

Soluble phenols varied significantly with season and time since fire, and there was a significant season by time since fire interaction (Table 2). However, SP did not vary linearly with time since fire in any season (Table 3). Soluble phenols concentrations peaked in the spring with highest values on the most recently burned sites (Fig. 6) with a second peak at the 78 years since fire site.

These results suggest that SP accumulate from winter to spring, when they are subsequently consumed (Hattenschwiler and Vitousek 2000) or adsorbed to hydrophobic surfaces (Piccolo et al. 1999). The NRN data seems to suggest that microbial activity declines in summer, therefore we expected SP to accumulate, but they did not. During this drying period, it is possible that SP consolidate to form insoluble high molecular weight polyphenols (Piccolo et al. 1999, Hattenschwiler and Vitousek 2000). However, it seems obvious that, along with resin analysis of ARC, resin analysis of SP needs more study to determine its relevance to microbial activity.

CONCLUSION

Inorganic N concentrations and NRN concentrations all varied significantly with season, time since fire, their interaction, and decreased linearly with time since fire for both the winter and spring seasons. By the summer season, these trends had become non-significantly different from each other for season or time since fire. This indicates that it is of paramount importance to make soil biochemical measurements that capture the period of greatest activity, early in the season of this ecosystem. Samples taken during the dry summer months will likely produce uninteresting results of questionable ecological relevance.

There is an increase in precipitation during the months before winter in ponderosa pine-Douglas-Fir ecosystems of western Montana (Nimlos 1986). This moisture undoubtedly causes an increase in N mineralization and it was mistake that this study did not attempt to document the amount of this increase. However, we can speculate that it N levels would have been high on the recently burned sites for NH_4^+ and NO_3^-, and would have exhibited a significantly decreasing trend with time since fire for NH_4^+, NO_3^- and NRN.

This study demonstrated that significant additive effects exist for N mineralization and microbial community dynamics when both time since fire and season are analyzed together. Available N levels taper off with time since fire and season while NRN actually increased with time since fire and season. The fact that NRN is highest in the summer when it does not show a significant trend with time since fire provides more evidence that the microbial community is being inhibited in late secondary succession (MacKenzie et al. *in press, in review*). The use of non-ionic resin capsules to extract soluble or active organic compounds in soil need to be studied in detail to ascertain their relationship to microbial activity and specific ecosystem processes. Season of sampling has also been identified as a uniquely important variable in this environment as moisture limits microbial processes during much of the summer. Summer months may have been erroneously perceived as the period of greatest N mineralization as a result of work performed in higher moisture ecosystems.

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Table 1: Fire chronosequence site despcriptions for 10 sites at 4 locations around western Montana including: time since fire (TSF-sampled in 2002), position on the landscape, pH and texture. All soils were classified as Typic Dystrocryepts (Adapted from Mackezie et al., in review b).

Location	TSF (years)	Slope (°)	Aspect (°)	Elevation (m.)	pH	Texture
Ninemile	122	10.3	137.0	1218.6	4.36	SL
	92	16.8	175.3	1272.2	4.28	L
	2	13.7	187.0	1181.5	4.85	SL
Clearwater	122	11.0	141.3	1452.9	4.72	SL
	45	8.8	163.1	1457.1	4.78	SL
	24	5.0	172.7	1396.8	4.55	SL
Lost Horse	132	11.3	207.0	1393.8	4.22	SL
	87	12.7	143.8	1390.6	4.35	SL
Lake Como	132	15.3	40.7	1678.5	4.27	SL
	78	19.5	106.7	1696.0	4.26	SL

Table 2: Repeated measures ANOVA p-values for seasonal soil biochemical measurements from 10 sites in western Montana with increasing time since fire (TSF). Variables include: ammonium (NH_4^+), nitrate (NO_3^-), ninhydrin reactive nitrogen (NRN), anthrone reactive carbon (ARC) and soluble phenols (SP).

	Repeated Measures ANOVA (p-value)		
Variable	TSF	Season	TSF*Season
NH_4^+	0.059	0.000	0.079
NO_3^-	0.000	0.000	0.008
NRN	0.000	0.000	0.066
ARC	0.004	0.001	0.066
SP	0.018	0.000	0.038

Table 3: Regression analysis including slope, R^2 and p-values for seasonal soil biochemical measurements from 10 sites in western Montana with increasing time since fire. Variables include: ammonium (NH_4^+), nitrate (NO_3^-), ninhydrin reactive nitrogen (NRN), anthrone reactive carbon (ARC) and soluble phenols (SP).

	Season								
	Winter			Spring			Summer		
Variable	Slope	R^2	p-value	Slope	R^2	p-value	Slope	R^2	p-value
NH_4^+	-6.283	0.758	0.005	-4.420	0.879	0.001	0.503	0.051	0.592
NO_3^-	-2.689	0.428	0.081	-2.722	0.403	0.091	-0.190	0.009	0.827
NRN	-3.400	0.535	0.039	-4.873	0.478	0.057	-4.580	0.229	0.237
ARC	-0.025	0.004	0.885	-0.181	0.337	0.132	-0.045	0.33	0.668
SP	-0.001	0.009	0.814	-0.003	0.180	0.295	0.000	0.001	0.847

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Figure 1: Monthly mean temperature (°C) and precipitation (cm) data for westerm Montana (Montana region 01) from November 2001 to November 2002 (NOAA data, 2004).

Figure 2: Contour analysis of seasonal ionic resin capsule data from a chronosequence of sites with increasing time since fire (TSF-years) in western Montana. The contour lines are extrapolations of NH_4^+-N concentration (µg capsule^{-1}) between seasons and TSF.

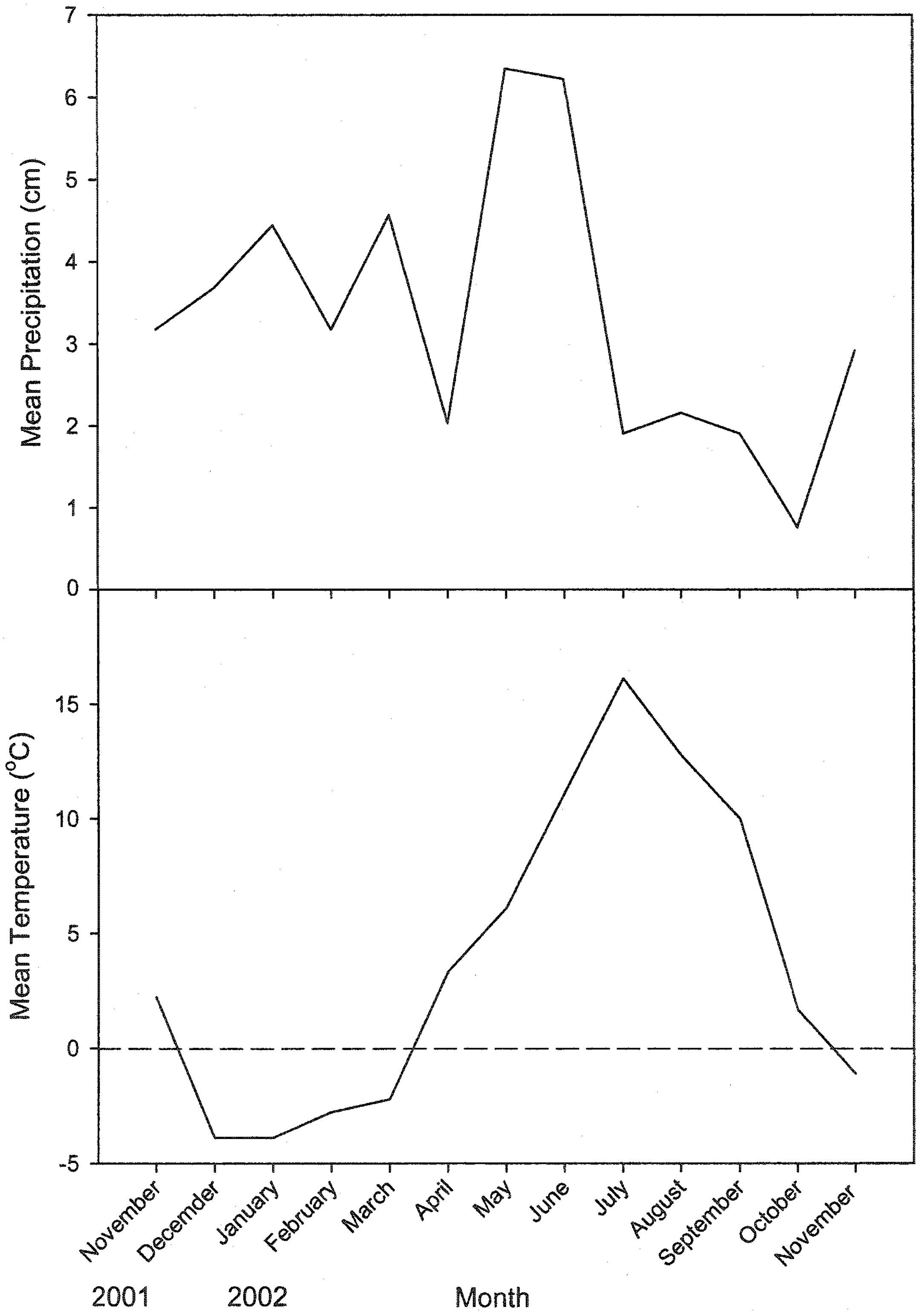
Figure 3: Contour analysis of seasonal ionic resin capsule data from a chronosequence of sites with increasing time since fire (TSF-years) in western Montana. The contour lines are extrapolations of NO_3^--N concentration (µg capsule -1) between seasons and TSF.

Figure 4: Contour analysis of seasonal ionic resin capsule data from a chronosequence of sites with increasing time since fire (TSF-years) in western Montana. The contour lines are extrapolations of ninhydrin reactive N (NRN) concentration (µg capsule^{-1}) between seasons and TSF.

Figure 5: Contour analysis of seasonal non-ionic resin capsule data from a chronosequence of sites with increasing time since fire (TSF-years) in western Montana. The contour lines are extrapolations of anthrone reactive C (ARC) concentration (µg capsule^{-1}) between seasons and TSF.

Figure 6: Contour analysis of seasonal non-ionic resin capsule data from a chronosequence of sites with increasing time since fire (TSF-years) in western Montana.

The contour lines are extrapolations of soluble phenols (SP) concentration (mg capsule -1) between seasons and TSF.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4
3
2
1
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Mean Temperature (°C)
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2001
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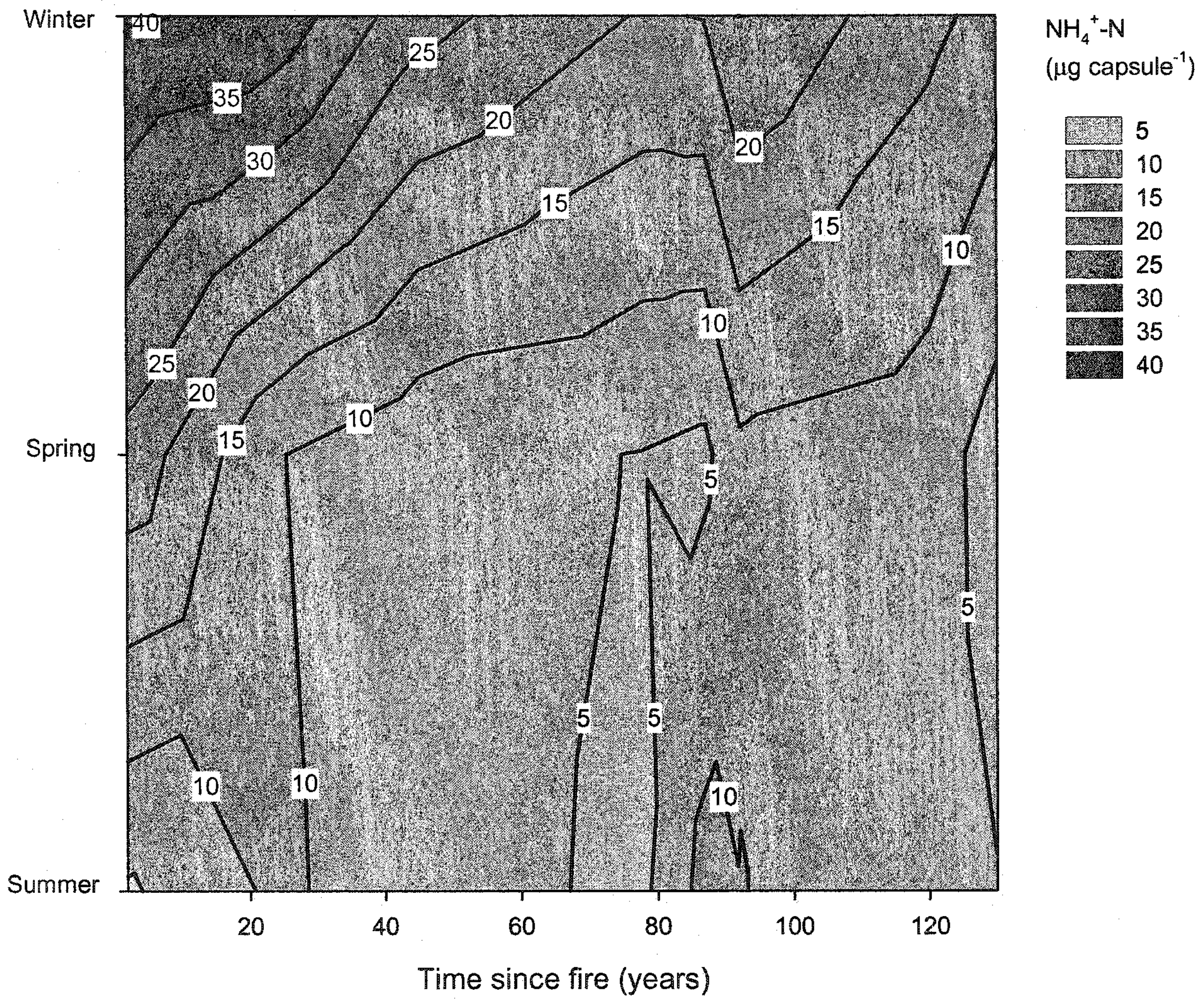
Month


Winter
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Time since fire (years)
NH$_4^+$-N (µg capsule^{-1})
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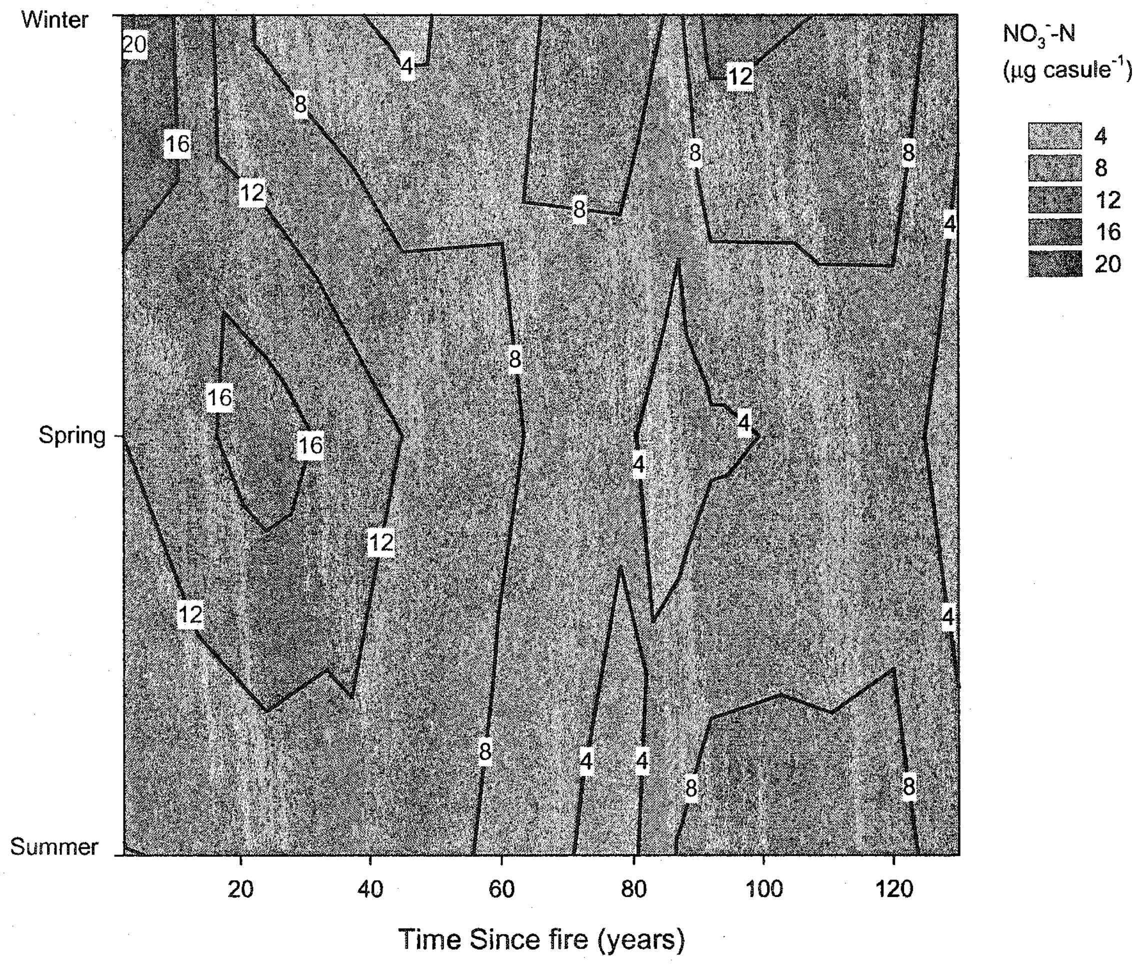
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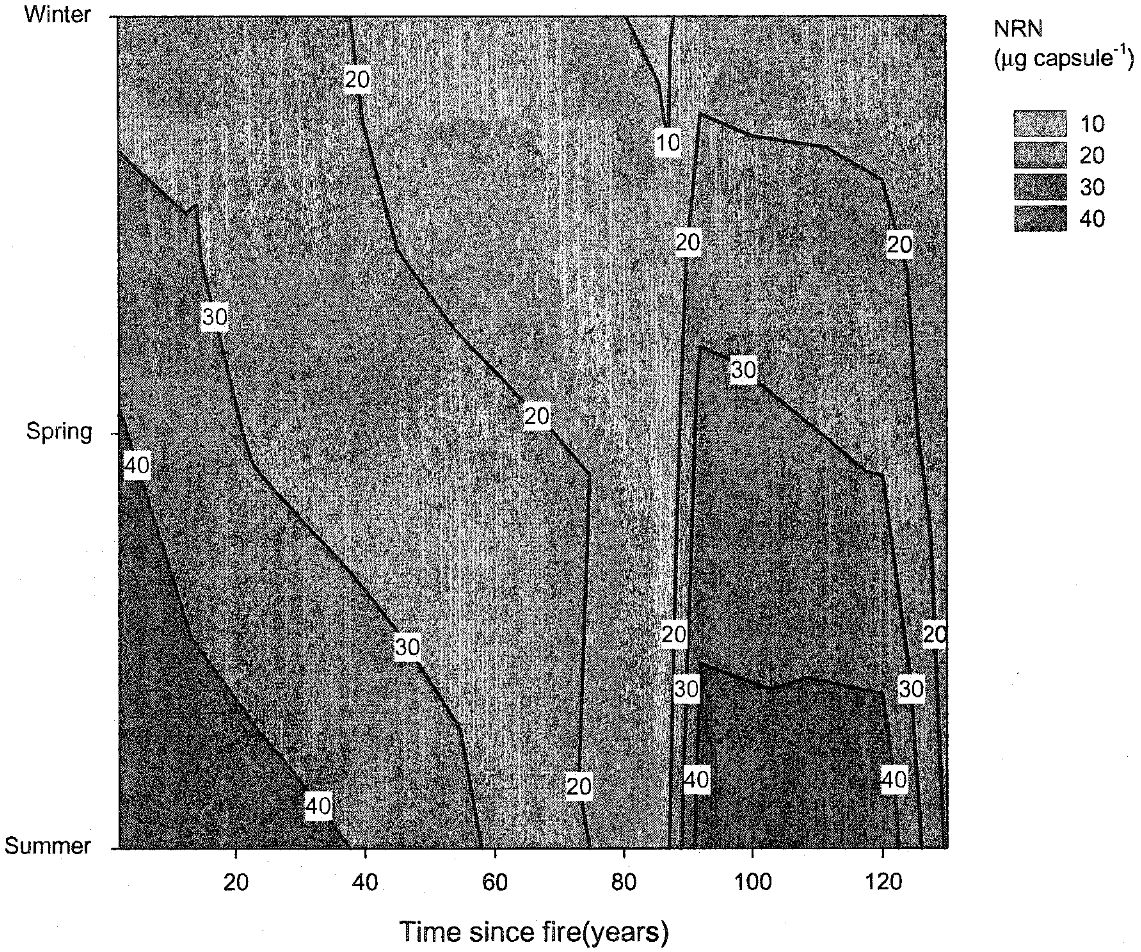
Time Since fire (years)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Time since fire(years)
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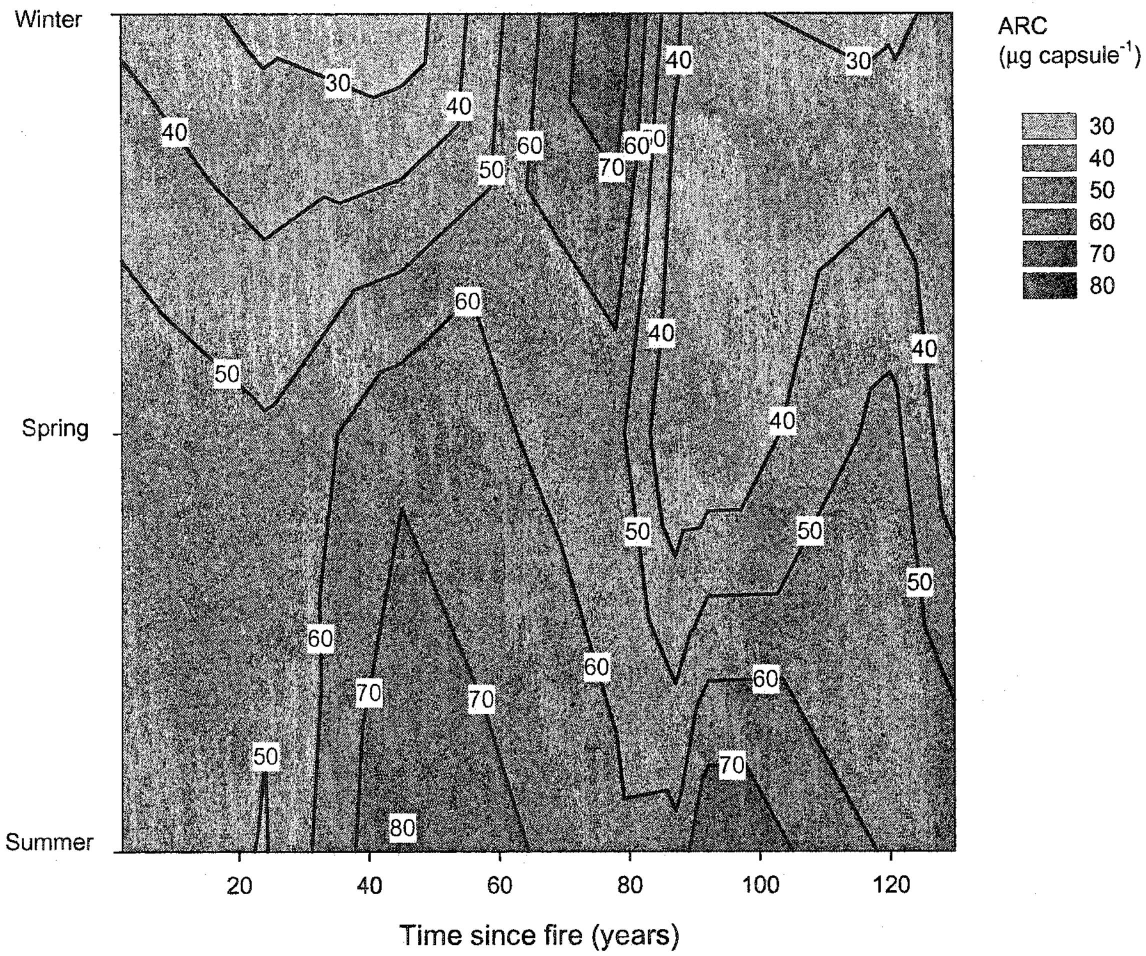
Winter
Spring
Summer
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100
120
Time since fire (years)
ARC
(µg capsule^{-1})
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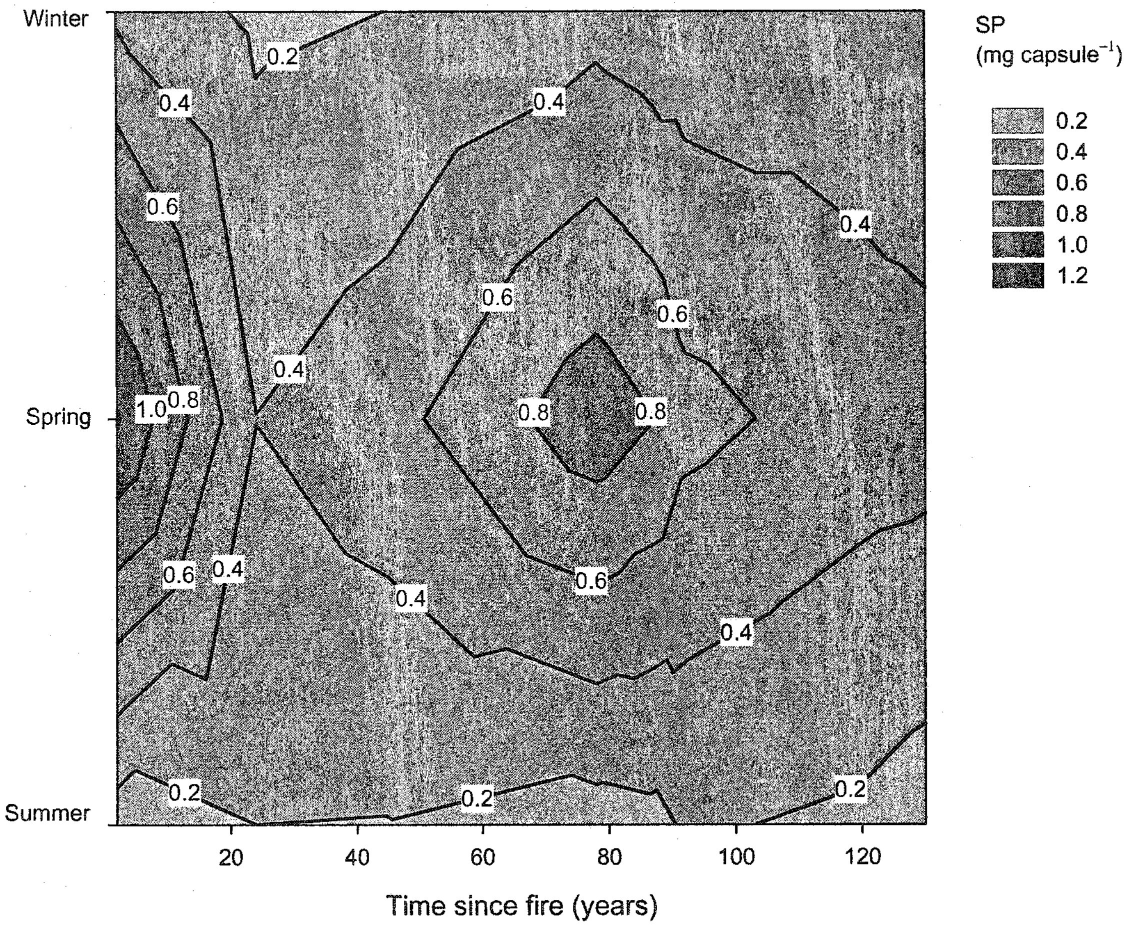
60
70
80


Winter
Spring
Summer
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120
Time since fire (years)
SP
(mg capsule^{-1})
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1.0
1.2
0.2
0.4
0.6
0.8
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0.4
0.4
0.4
0.2
0.2
0.2

Comparison of litter quality and nitrogen mineralization in the understory of Ponderosa pine/Douglas-fir forests

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Abstract: Frequent, low severity fires appear to maintain low elevation ponderosa pine forests of western Montana in an intermediate state of secondary succession. Fire exclusion leads to an increase in the more shade tolerant Douglas-fir in the overstory and to co-dominance among graminoids, forbs and woody shrubs in the understory of these forests. It is thought that some species of woody shrubs produce allelochemicals that can have a significant influence on ecosystem processes in forests, but little evidence exists to support this hypothesis. Charcoal has been shown to sorb inhibitory organic compounds and is a significant component of all pyrogenic ecosystems. Experiments were performed with charcoal treatments and two different species, Elk sedge (*Carex geyeri* Boott) representing graminoids and Kinnikinnick (*Arctostaphylos uva-ursi* (L.) Sprengel-Mjölon) representing woody shrubs. A seed bioassay was performed in which Trembling aspen (*Populus tremuloides* Michx.) seeds were germinated on different concentrations of sedge and shrub leaf extract. Both extracts exhibited allelopathic characteristics at low extract concentration and stimulatory characteristics similar to water at higher concentrations. To test whether either plant type controls N mineralization through substrate quality, a factorial experiment was performed where forest floor microcosms of both sedge and shrub were treated with glycine, as a labile organic N source, and charcoal, both in the greenhouse and in the field. Addition of glycine drastically increased ammonification as measured by ionic resin capsules, for both plant types and indicates substrate limitations. Glycine also stimulated nitrification in the sedge microcosm, but only the addition of charcoal stimulated nitrification in the shrub microcosm. Non-ionic resin capsules were used to measure labile C compounds and demonstrated a significant increase in anthrone reactive C in the presence of shrub litter, glycine and charcoal. *A. uva-ursi* inhibits the nitrifier community and charcoal mitigates this effect for some period of time after fire. The field study did not show the same trends. Glycine stimulated ammonification and nitrification in the litter and mineral soil of both species as shown by resin analysis and a nitrification assay indicating substrate limitations. Overall the results suggest that prolonged fire exclusion shifts the N cycle form being disturbance driven to being driven by specific plant types.

Keywords: Ionic and non-ionic resin, *Arctostaphylos uva-ursi*, ericoid, *Carex geyeri*, nitrification assay, microbial biomass, N cycle, allelopathy

INTRODUCTION

Little is known about factors that affect the nitrogen (N) cycle in low elevation forests of western Montana during succession after fire. Historically, these forests burned with low severity fires on a return interval of 10 to 50 years (Arno et al. 1995, Barrett et al. 1997, Arno and Allison-Bunnell 2002). Charcoal, which is deposited during fire, has been shown to mediate ecosystem function and sub-surface processes, including sorption of organic compounds, for some period of time after fire (Zackrisson et al. 1996, Wardle et al. 1998b). Many studies have shown that fire increases N availability in the short-term (Covington and Sackett 1986, Zackrisson et al. 1996, DeLuca and Zouhar 2000, Newland and DeLuca 2000, Choromanska and DeLuca 2001), however little is known about long-term N availability in the absence of fire. Recent studies have shown that N availability increases in the forest floor and decreases drastically in the mineral soil with increasing time since fire in these systems (MacKenzie et al. *in press, in review*). These studies also indicated an incomplete successional replacement of plants recolonizing the burned over areas. Mixed graminoid species dominate burned sites immediately after fire, but shrubs become co-dominant within 25 years on these sites.

The ability of plants to drive sub-surface processes, including nutrient cycling and microbial community, has recently become more recognized in plant ecology (Bever 1994, Wardle 2002). Allelopathy is the process by which plant secondary metabolites negatively influence other organisms. Many studies have shown how plant-produced chemicals can deter herbivory, interfering with digestion (Wardle et al. 1998a), suppress neighboring plants (Hattenschwiler and Vitousek 2000), act as antibiotics and interfere with nutrient cycling (Lodhi 1977, Souto et al. 2000). There is evidence from other mature forest ecosystems that shrubs of the Ericaeceous family produce significant quantities of allelochemicals and interfere with N cycling (Nilsson et al. 1998). Ericoids are common in western Montana and may be having a similar effect when fire is excluded.

Two possible mechanisms exist for the changes occurring with N cycling and increasing time since fire: 1) different plant types create inhospitable chemical climates with the addition of secondary metabolites (allelochemicals), contained in leaf litter, that reduce microbial activity; 2) the decomposition of woody material produces chemicals that bind proteins and reduce litter quality. In both cases, it is possible that inhibitory chemicals are alleviated by charcoal. Charcoal may act to level the competitive field for nutrient acquisition. Charcoal is the long-term foot-print of fire and acts to enhance nutrient cycling by sorbing organic compounds (Zackrisson et al. 1996, Wardle et al. 1998b). Although it is clear that changes in overstory or understory species composition will influence litter quality or allelopathic potential, this study was designed to specifically address the influence of two dominant understory species that are common in late succession ponderosa pine ecosystems of the inland Northwest.

The purpose of this study was to examine the different sub-surface biochemical conditions created by two different plant types commonly found in the low elevation forests of western Montana, kinnikinnick (*Arctostaphylos uva-ursi* (L.) Sprengel-Mjölon), an ericoid species common in the understory and Elk sedge *(Carex geyeri* Boott), representing graminoids. The specific objectives of this study were: 1) determine whether either species exhibits allelopathic inhibition of seed germination; 2) determine whether inhibition of the microbial community occurs during litter decomposition; and 3) determine the effect of charcoal on litter decomposition. We hypothesize that *A. uva-ursi* may inhibit both seed germination and nitrification as a means of competitive exclusion of graminoids with increasing time since fire.

MATERIALS AND METHODS

Study Site

The Willow Creek fire complex in western Montana was chosen for the collection of plant material, forest floor microcosms and the installation of field plots because it was well catalogued in previous work (see Choromanska and DeLuca 2001). Located between N 46° 17' 37-39" latitude and W 113° 55' 28-59" longitude, the site rests in the Sapphire mountains of the Bitterroot valley. Part of the site experienced a low severity fire in 1996 that consumed large patches of forest floor, killed many of the sub-dominant Douglas-firs, most of the understory species and scarred some of the ponderosa pines. The soil great group is a Lithic Dystrustepts, formed on granitic parent material, with a slope of 33 degrees, elevation of 1590 to 1668 m and a predominantly southern aspect.

Seed Bioassay

In June of 2002 a leaf extract was produced from fresh tissue of each species, *A. uva-ursi* and *C.geyeri*, by adding 100 g of ground tissue to 1 liter of deionized water, shaking for 24 hours and filtering through 0.20 um Millipore filters to remove microbes. Leaf extracts were then diluted to four concentrations for the seed bioassay, 1 %, 5 %, 10 % and 50 % of the original extract, in an attempt to re-create what occurs naturally. The extracts were analyzed for various chemical elements, including the nutrients NH_4^+, NO_3^- and PO_4^{-3}, and a common group of secondary metabolites, phenols. These compounds were measured with the techniques listed in the Soil Chemical Analysis section. Aspen seeds (*Populus tremuloides* Michx.) were used in the bioassay because they germinate quickly, require no seed preparation prior to germination except cold storage (Morgan

1969) and are a local species that may experience the chemical environments produced by each of these understory species. In a plastic Petri-dish, 20 aspen seeds were laid out on a Whatman # 2 filter paper, 2 ml of extract was added to the filter paper and the dish was sealed with parafilm. Five replicates of each concentration, for each species were prepared in this way including five replicates of a control, consisting of deionized water, for a total of 45 dishes. The replicates were placed in a small cabinet germination chamber on a cycle of 12 hours light, 12 hours dark and monitored every two hours for germination. After seven days, germination was terminated and the radicle length was measured.

Greenhouse Experiment

Ten replicates of forest floor microcosms including both organic material and at least 5 cm of mineral soil, were collected intact for both plant types in October 2001. Microcosms were returned to a greenhouse facility and potted in 15 by 30 cm rectangular pots that were 10 cm deep. Ionic and non-ionic resin capsules were installed in these potted samples just below the organic matter/mineral soil interface and left to incubate for two months (Kjønaas 1999, Morse et al. 2000) and watered twice weekly to field capacity. Ionic resins were incased in polyester capsules containing 10 ml (approximately 1g dry weight) of mixed bed ionic resins (PST-2, Unibest, Bozeman, MT). Fishing line and flagging tape were attached to each capsule to aid in recovery. Non-ionic resins were incased in the same polyester capsules (Unibest, Bozeman, MT), but filled with approximately one gram dry weight (about 1100 m^2 of surface area) of XAD-7 resin (Rohm and Hass Inc., Philadelphia, PA). In December 2001, the resin capsules were removed and frozen until they could be analyzed. Analysis of ionic resin capsules included amino-N, NH_4^+ and NO_3^- (Keeney and Nelson 1982), while analysis of non-ionic resins included anthrone reactive carbon (ARC) (Morse et al. 2000) and soluble phenols (SP) (Stern et al. 1996). Both ARC and SP represent measures of labile C and are a surrogate for microbial activity (MacKenzie et al. in press, in review).

In January 2002, the microcosms were split in two 15 by 15 cm samples for a total of 20 replicates per plant type and again, ionic and non-ionic resin capsules were installed just below the organic matter/mineral soil interface. Two treatments were applied to the defend plant litter types with a two by two factorial design and randomly assigned locations on the greenhouse bench. The treatments consisted of applying 50 kg per ha glycine and 1000 kg per ha charcoal to the microcosms, as these represent contents of each material that may be found naturally. To accomplish this, 30 ml of a 0.32 M solution of glycine and 30 ml of a 7.5 % charcoal solution were injected into the organic matter of each sample with a syringe and modified 5 ml pipette tip. Glycine was added as an organic N source that can be easily mineralized and the charcoal was applied at a rate that is commonly found in forests that are pyrogenic in nature (Zackrisson et al. 1996). The charcoal was collected form other ponderosa pine-Douglas-fir sites that had burned in 2000 and therefore should have a high activity level. Replicates were watered twice weekly to field capacity and incubated in the greenhouse for two months. In March 2002, the resin capsules were removed and analyzed for post-treatment biochemistry.

Field Experiment

In March of 2003, a similar experiment was set-up at the study site. Three replicates of each treatment were applied to different patches of both plant types. The same concentration and amount of both glycine and charcoal were applied to an area roughly equivalent to the 15 by 15 cm pots used in the greenhouse experiment. Ionic and non-ionic resins were installed and analyzed in the same manner as before, but were left to incubate *in-situ* for two months rather than one. Therefore, in May of 2003 the resin capsules were removed and frozen until they could be extracted and analyzed. Mineral soil samples were collected from the 0-5 cm depth with a one inch diameter soil probe at the end of the incubation period also. Approximately 8 samples were composited per replicate and analyzed fresh for N availability, microbial biomass and nitrification activity.

Resin Capsule Analysis

Ionic resins were extracted by 3 successive 10 ml rinses and 30 min. of shaking with 2 M KCl (Kjønaas 1999, Morse et al. 2000). After each rinse the KCl was decanted into a centrifuge tube and after all 3 rinses the extractant was spun down at 3000 RPM for 10 min. NH_4^+, NO_3^- and NRN were analyzed as outlined above. Non-ionic resins were extracted with two different extractants in succession. Resin capsules were placed on top of 200 ml French square bottles under a partitioning pump that delivered 0.8 ml of extractant per minute for 35 minutes. The first extractant was distilled water for determination of ARC (Morse et al. 2000) and the second extractant was 50% methanol for analysis of soluble phenol (Stern et al. 1996). Studies have shown that distilled water does not remove phenols while 50 % methanol removes 95 % of the phenols adsorbed (Choromanska and DeLuca 2002).

Soil Chemical Analysis

Oven dried weight was determined by drying a 30 g sub-sample at 105 ° C for 24 h. The difference between the field moist value and oven dried value were used to determine a moisture factor that would allow us to report chemical data on a per gram dry soil basis. Twenty-five grams of air dried sample was extracted with 50 % methanol for 24 h for determining total phenols by the Prussian Blue method (Stern et al. 1996). These samples were filtered with Whateman 42's and the extracts were analyzed at 720 nM against (+)-catechin as the standard.

Extractable NH_4^+ and NO_3^- were removed by shaking 30 g field moist soil in 50 ml of 2 M KCl for 30 min. and filtering with Whatman 42's on a vacuum manifold. The extracts were analyzed for NH_4^+ and NO_3^- on a Technicon II auto-analyzer. We used the salicilate-nitroprusside method for NH_4^+ and the ammonium chloride-Cd reduction method for NO_3^- (Keeney 1982). These extracts were also used to determine the amount of amino N by the Ninhydrin reactive N (NRN) method (DeLuca et al. 1992) for comparison with the fumigation results. The extract was analyzed for NH_4^+on a Technicon II auto-analyzer using the same method as discussed above.

Microbial biomass was measured by the fumigation extraction method (Amato and Ladd 1988). Briefly, 30 g of soil were fumigated with chlorophyll in an air-tight bell jar for 24 hrs. Fumigated samples were extracted in 50 ml of 2 M KCl with the same method as above and extracts are analyzed for Amino N by NRN method (DeLuca et al.

1992). Unfumigated data was subtracted from fumigated data to give organic N concentrations which were multiplied by 3.2 for Biomass N or 22 for biomass C (Amato and Ladd 1988). Microbial respiration was measured by the alkali trap-HCl titration method (Fierer and Schimel 2002). Briefly, 50 g of field moist soil was sieved to 4 mm to remove roots and allowed to respire in a mason jar for 3 days. Scintillation vials with 20 ml of 1 M NaOH were placed in the jars to trap CO_2 evolved. After 3 days, the traps were removed and the solution transferred to 250 ml Erlenmyer flasks containing 30 ml of 2 m BaCl and a few drops of phenathalien. Samples were titrated to a neutral end point with 1 M HCl and the volume recorded. From this data the amount of CO_2 evolved per g dry soil was calculated by multiplying the ml HCl by 22 μg CO_2 per ml HCl.

The rate of nitrification was measured to assess nitrifier activity after treatment by the nitrification aerated slurry method (Hart et al. 1994). One hundred ml of nutrient solution containing NH_4^+, K^+ and PO_4^{-3} were added to 15 g of refrigerated soil in 250 ml Erlenmyer flasks capped by rubber stoppers with holes in the middle to allow for aeration of the slurry. Another 15 g was weighed and oven-dried to calculate a moisture factor that would allow nitrification rates to be reported on a dry weight basis. The flasks were placed on an orbital shaker for 24 hr and 10 ml samples were removed with a 5 ml modified pipette at 2, 3, 23 and 24 hrs. Samples were then filtered on an vacuum manifold with Whatman 42 filter papers and frozen until analyzed for NO_3^- concentration. The rate of nitrification is calculated as the slope of the line produced by plotting concentration by time and are reported as ug nitrate per g per hr.

Statistical Analysis

Analysis of variance (ANOVA) was used to determine if statistical differences existed between different treatments of the seed bioassay, greenhouse and field experiments (Underwood 1997, Wilkinson 1999). In each case we tested the error terms to ensure that the assumptions of heterogeneity, random variance and normal distribution were met, and transformed the data as needed (Underwood 1997, Wilkinson 1999). When ANOVA results indicated a significant difference, Tukey multiple comparison tests (HSD) were performed *ad hoc* to identify pair wise treatments differences. An alpha of 0.05 was accepted in all cases unless otherwise noted. The statistical package used for these analyses was Systat 9.0 (Wilkinson 1999).

RESULTS AND DISCUSSION

Seed Bioassay

Analysis of the chemical constituents of the two leaf extracts revealed little difference in nutrient concentration, but substantial differences for total phenols (Table 1). *A. uva-ursi* extracts contained 20 times more total phenol than *C.geyeri* extracts. This may indicate that *A.uva-ursi* has the ability to inhibit seed germination. Phenolic compounds have been reported as allelopathic inhibitors of seed germination and nitrification in other ecosystems (Zackrisson et al. 1997, Nilsson et al. 1998, Northup et al. 1998, Wardle et al. 1998a, Hattenschwiler and Vitousek 2000, Hierro and Callaway 2003), and were tested to determine if the same mechanism is prevalent in the dry inland Northwest.

The seed bioassay revealed that both *A. uva-ursi* and *C.geyeri* leaf extracts inhibited seed germination significantly when compared to the control (Fig. 1). Different concentrations showed that only the 1 % extract was significantly different for *A. uva-ursi* extracts, while the 1, 5 and 10 % extracts were significantly different for *C.geyeri* extracts (Fig. 1). These results were not expected and show that *A.uva-ursi* is not having a unique inhibitory effect on seed germination, but rather that both plant types inhibit other plants from establishing in their proximity. Low concentrations of leaf extract are what we would most likely find in natural settings and may explain why only low concentrations act as inhibitors of seed germination. At higher concentrations a nutritional factor may be overcome, thereby releasing the seeds from the inhibitory compounds, but this is speculation.

Inhibition of seed germination is a useful competitive tactic for maintaining a foot-hold in a highly diverse successional environment such as a forest. Therefore, it is not surprising to learn that both plant types exhibit some ability to exclude other plant types from germinating in their vicinity. Nilsson et al. (1998) showed that *Empatrum hermaphroditum*, a member of the ericaceous family, produces seasonally variable quantities of a phenolic compound known to inhibit seed germination. The *A.uva-ursi* plant type is also in the ericaeceous family and was chosen as such to examine the effect of one particular species in the woody shrub functional group that had good allelopathic potential. However, there is some evidence that *Carex spp.* also compete intensely with seed germination although it is not clear if it is direct competition for resources or indirect competition via interference (Del Moral et al. 1985).

Greenhouse Experiment

Factorial applications of glycine and charcoal altered N mineralization for the two different plant litter samples as measured by sorption of NH_4^+ and NO_3^- to ionic resin capsules (Fig. 2). Charcoal by itself had no effect on NH_4^+ or NO_3^- concentrations for either plant type. However, the addition of glycine as a labile organic N source stimulated a significant amount of ammonification for *A.uva-ursi*, but not a significant amount of nitrification and had the opposite effect on *C.geyeri* samples. Only in the presence of glycine and charcoal was there significantly more NO_3^- accumulation. *C.geyeri* samples had increased nitrification in the presence of glycine and equal amounts of NO_3^- and NH_4^+ with glycine and charcoal additions. Pre-treatment NO_3^- concentrations were significantly lower for *A.uva-ursi* compared to *C.geyeri* and showed no statistical difference post-treatment (Fig. 3).

These results suggest that *A.uva-ursi* inhibits nitrification which may be a means of competitive nutrient acquisition. It is not clear however, if this is a unique trait of *A.uva-ursi* or the result of general ecosystem lignification due to increasing woody litter. Woody plants generally exhibit slow growth and tolerate low nutrient environments (Lambers et al. 1998). Inhibiting nitrification is potentially a means of reducing N loses by leaching and denitrification (both of which act on NO_3^- predominantly), resulting in an increase in slowly available N. Inhibition of nitrification has been demonstrated in other forest ecosystems characterized by periodic wildfires (Likens et al. 1969, Rice and Pancholy 1972, Lodhi 1977, White 1988). However, other studies have refuted such claims (McCarty and Bremner 1986, 1989) and the mechanism is still under debate. In a similar study, DeLuca et al. (2002) showed that a significant amount of NO_3^- was produced in the presence of charcoal only, in a pyrogenic Boreal system. However, these systems have shrub dominated understories and lack a significant grass component in late secondary succession. The opposite is true in western Montana, where grasses are a distinct part of understory vegetation, are adapted to nutrient pulses (Bardgett et al. 1999) and do not inhibit nitrification given the data above.

Charcoal is a significant component of pyrogenic ecosystems and has been shown to sorb organic compounds (Zackrisson et al. 1996, Wardle et al. 1998b, DeLuca et al. 2002). It represents the long-term 'foot-print' of the wildfire disturbance regime, but has an activity self-life of 100 years in Boreal systems (Zackrisson et al. 1996) and perhaps shorter (10 – 50 years) in the dry inland Northwest (MacKenzie et al. *in press*). During the active phase, charcoal increases nitrification as shown above, however, it is not clear if this is the result of sorbing allelopathic compounds that inhibit microbes, sorbing polyphenolic compounds that may sorb proteins (Hattenschwiler and Vitousek 2000), sorbing hydrophobic compounds notoriously low in N (Piccolo et al. 1999) or by providing microbes a safe site with adequate resources and away from predation. It is interesting to note that in the presence of active charcoal, it is the disturbance regime that drives N mineralization (a top down control). Whereas, in the absence of active charcoal and beyond the natural disturbance interval it seems that it is the plant community that drives N mineralization with the quality of organic inputs (a bottom up control) (Wardle 2002). This shift in ecosystem function is potentially a very important one given the environment of fire exclusion that has existed for the last 100 years with regard to resource extraction and forest management in the dry inland Northwest.

Analysis of different pools of soluble C was performed as an index of microbial activity as an attempt to isolate the inhibitory mechanism. Non-ionic resin extracted phenols (50% MeOH) did not exhibit any significant trends for either plant litter type (data not shown). It should be emphasized that only soluble or weakly sorbed phenols collect on resin capsules and represent the low molecular weight (LMW) category of these compounds (Hattenschwiler and Vitousek 2000). LMW phenols are most likely a food source for various microbes rather than a chemical inhibitor (Côte and Schimel 1996). As such, soluble phenols tell us nothing about treatment effects on the microbial community in this experiment. However, ARC was measured on water extracts from non-ionic resin and showed a significant trend for *A.uva-ursi* samples, but not *C.geyeri* samples (Fig. 4). Anthrone reactive C is thought to represent bioavailable C (DeLuca 1998), and is potentially a surrogate for microbial activity. Since ARC was found to be significantly higher in the glycine and charcoal treatment as compared to the control for *A.uva-ursi* (Fig. 4, a) it is likely that this treatment increased microbial activity which resulted in a significant increase in nitrification. This points to some form of chemical inhibition of the microbial community by *A.uva-ursi*, but still does not tell us if the interference is direct (allelopathic) or indirect (chemical immobilization), where polyphenol protein complexes reduce N availability. It is also not clear if bacteria are using charcoal as a safe site. In our previous work, we identified that the microbial biomass remains constant with increasing time since fire (a surrogate for increasing shrub cover) which might indicate indirect inhibition (MacKenzie et al. *in press, in review*). Results from nitrification assay indicate that the nitrifier community is intact, but not active (unpublished data), again supporting indirect inhibition.

Field Experiment

Results from the analysis of soil samples taken from beneath the two different plant types revealed few similarities with the greenhouse study. Only *A.uva-ursi* NO_3^- concentrations for the glycine treatment were significantly greater than the control for available N (Fig. 5). Microbial biomass C or N was not significantly influenced by any treatment for either plant type (data not shown).

Unlike soluble phenols measured from non-ionic resin extracts, total phenols represent the pool of both soluble and insoluble phenolic compounds. It is the high molecular weight phenols or polyphenols that have been implicated in chemical inhibition (Northup et al. 1995, Northup et al. 1998, Hattenschwiler and Vitousek 2000). Total phenols were measured from soil extracts, but showed no significant difference between treatments for either plant type (Fig. 6). However, it is evident that there is a slight reduction in total phenols for both charcoal treatments for *A.uva-ursi.* This indicates that charcoal is sorbing phenols and may be the reason for microbial release as indicated by the greenhouse experiment.

Charcoal amendments suggested that the nitrifier community was negatively affected by *A.uva-ursi* in the greenhouse experiment, therefore we performed a nitrification assay on soil samples from the field experiment to see if charcoal released nitrifiers. The rate of nitrification increased significantly with glycine treatments for the two different plant types (Fig. 7). The treatments with glycine and glycine plus charcoal produced significantly higher rates of nitrification, while charcoal alone had no significant or additive effects. This indicates that both plant types are substrate limited in this environment which was also indicated by the greenhouse results, but does not show the direct release of nitrifiers by charcoal that was expected. It is surprising that *C.geyeri* had the same rate of nitrification as *A.uva-ursi* given the evidence that graminoids seem to maintain a faster N cycle.

Available N was measured from ionic resin extracts for the field experiment, but these were also not synonymous with the greenhouse results (Fig. 8). Resin capsules retrieved from *A.uva-ursi* micro plots had no significant effect on NH_4^+ concentrations between treatments and NO_3^- concentrations were significantly greater than the control for the glycine treatment only (Fig. 8, a). *C.geyeri* had a significant influence on concentrations of both NH_4^+ and NO_3^- for the combination of glycine and charcoal treatment compared to the control (Fig. 8, b).

Non-ionic resin extracts produced no significant trends for either soluble phenols (data not shown) or ARC (Fig. 9). However the ARC data is presented because there is similarity between the field results and the greenhouse experiment for the A. uva-ursi (Fig. 9, a). These results show a slight increase in ARC concentrations for the glycine/charcoal treatment, which may indicate increased microbial activity. This is similar to the greenhouse results indicating that the microbial community was being released form some form of inhibition. This evidence is not reflected in the available N data possibly due to high turnover in an active system as opposed to decomposing potted samples, as previously mentioned.

The field experiments are in direct contrast to greenhouse trials and previous studies (DeLuca et al. 2002). Nitrogen mineralization appears to be substrate limited for both plant types as shown by the increases in available N and rate of nitrification when glycine was added. However, there is only vague evidence that this limitation is plant mediated in the field, based on total phenol sorption by charcoal and resin sorbed ARC trends. Given these results, there are several caveats with the field experiment that merit discussion. As we saw in an earlier study of this environment, soil grab samples are not representative of seasonal forest soil N dynamics (MacKenzie et al. *in review*). However, they were useful for determining microbial biomass differences and total phenol differences on sites with increasing time since fire, but not here. The rate of application of glycine and charcoal was the same as the greenhouse experiment for comparability, however this was a mistake for charcoal. Charcoal particles were suspended in a colloidal solution with water and probably did not disperse well or evenly at the same rate as was used in the greenhouse. DeLuca et al. (2002) applied some 10 times more charcoal in the field and measured a significant increase in nitrification. Therefore, it seems likely that not enough charcoal was used or insufficient mixing was attained in this experiment to initiate a response and we expect higher rates of nitrification and microbial enzyme activity with applications of more concentrated charcoal solutions in a repeat of this field trial.

CONCLUSION

It seems that both plant types have evolved means of persistence regardless of successional pressures in low elevation forests of the dry inland Northwest. In the case of both *A.uva-ursi* and *C.geyeri*, it seems that chemical inhibition of alternate species seed germination is one mechanism of securing space in the forest understory. This allelopathic interaction was not expected for grasses, but may be a function of *Carex spp.* competitive ability (Del Moral et al. 1985). The allelopathic maintenance of space is the most likely reason that *Carex geyeri* persists with increasing time since fire as shown by MacKenzie et al (*in press*). Very different strategies existed for acquiring available N between these two different plant types, in an environment that is substrate limited. Grasses are known to thrive with pulse oriented, fast N cycles which are numerous in this environment as shown by seasonal data analysis (MacKenzie and DeLuca *in review*). However, *A.uva-ursi* seemed to promote a slower N cycle that was chemically inhibited. This could be an evolved trait for the maintenance of N in an already N limited environment in which woody shrubs tolerate low availability by having slow growth rates. Whether or not these factors are representative of the their respective functional groups needs further investigation. Finally, we were able to produce more evidence for the positive effect of charcoal on N mineralization and lean towards the explanation that charcoal does this by sorbing chemicals that indirectly inhibit microbial decomposition. We also believe that these chemicals are plant mediated based on evidence here and from our previous work.

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Table 1: Nutrient and phenol concentrations ($\mu g\ g^{-1}$) in leaf extracts produced from two different plant types, *Artostaphylos uva-ursi* (Shrub) and *Carex geyeri* (Sedge), found in the understory of ponderosa pine/Douglas-fir forests of western Montana

	Nutrient ($\mu g\ g^{-1}$)			Total Phenol
Plant Type	NH_4^+	NO_3^-	PO_4^{-3}	($\mu g\ g^{-1}$)
1% Sedge	0.49	0.09	1.26	10.7
1% Shrub	0.24	0.10	1.60	200.5

List of Figures

Figure 5: Available N (μg g^{-1}) measured for soil samples from factorial applications of glycine (50 kg ha^{-1}) and charcoal (1000 kg ha^{-1}) to a field site in western Montana. Materials were applied to the litter below two different plant types, *Arctostaphylos uva-ursi* (Shrub, a) and *Carex geyeri* (Sedge, b), found in the understory of ponderosa pine-Douglas-fir forests

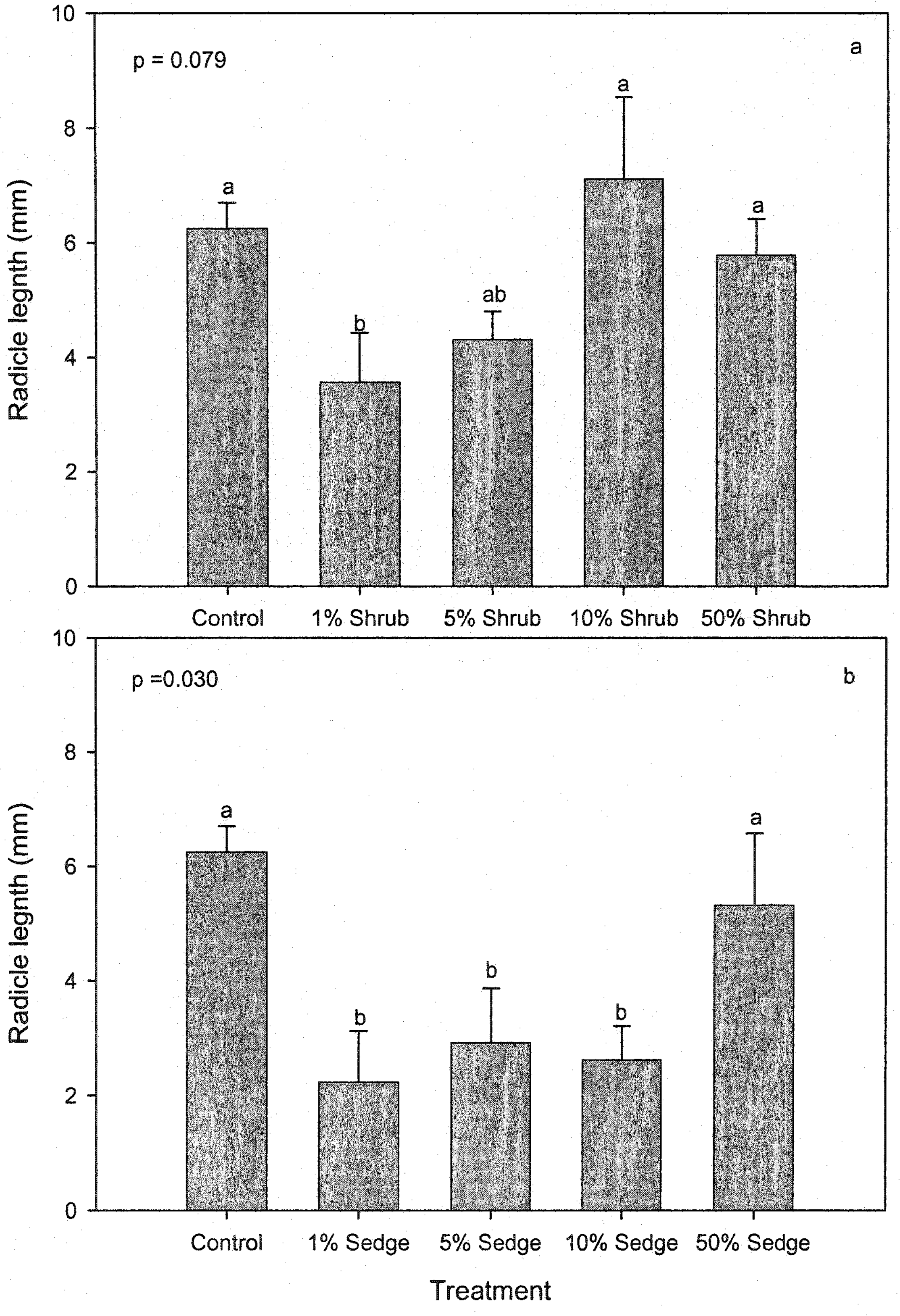
Figure 6: Total phenols (μg g^{-1}) measured for soil samples from factorial applications of glycine (50 kg ha^{-1}) and charcoal (1000 kg ha^{-1}) to a field site in western Montana. Materials were applied to the litter below two different plant types, *Arctostaphylos uva-ursi* (Shrub, a) and *Carex geyeri* (Sedge, b), found in the understory of ponderosa pine-Douglas-fir forests

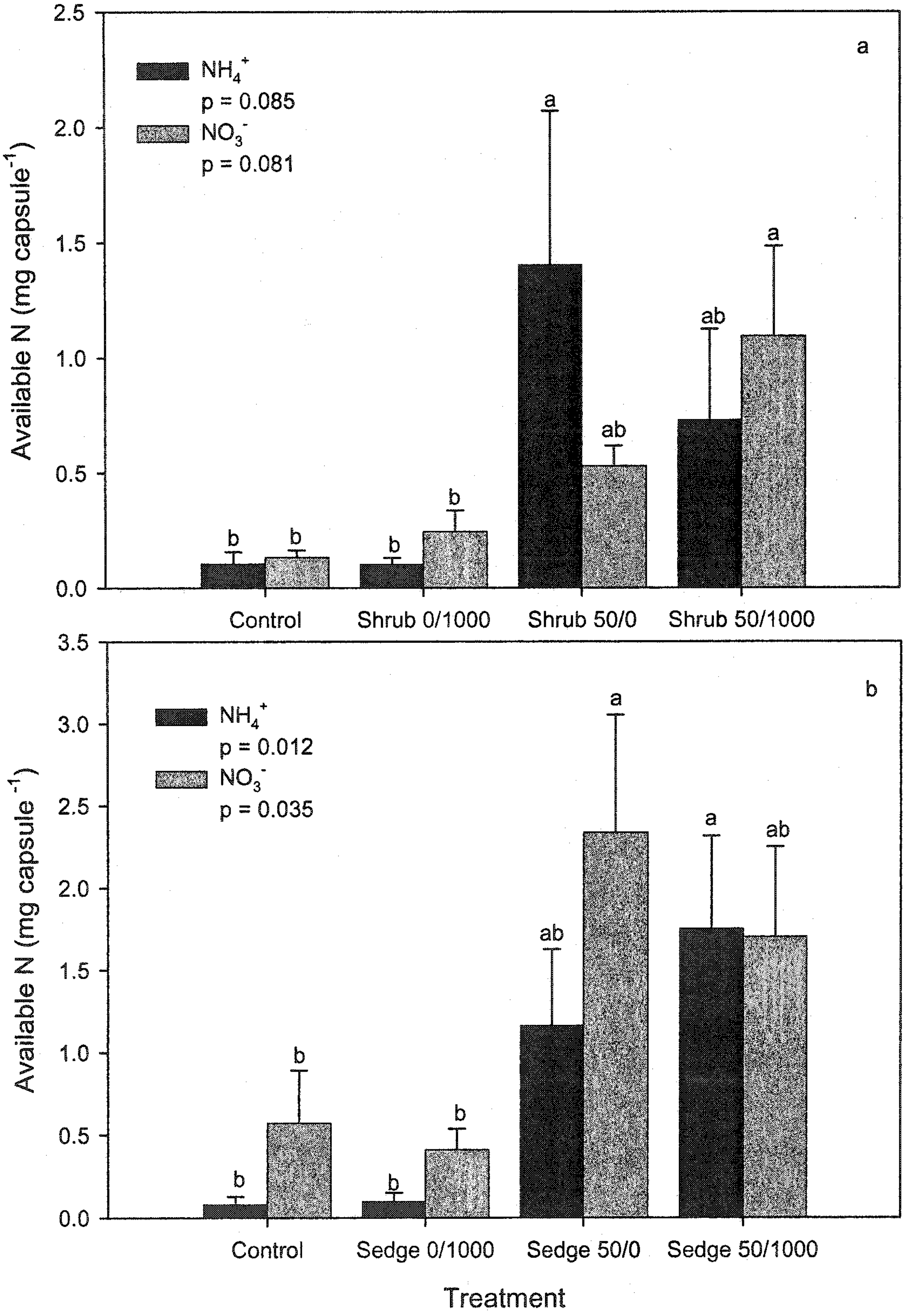
Figure 7: Available N (μg $capsule^{-1}$) measured with ionic resin capsules from factorial applications of glycine (50 kg ha^{-1}) and charcoal (1000 kg ha^{-1}) to a field site in western Montana. Materials were applied to the litter below two different plant types, *Arctostaphylos uva-ursi* (Shrub, a) and *Carex geyeri* (Sedge, b), found in the understory of ponderosa pine-Douglas-fir forests

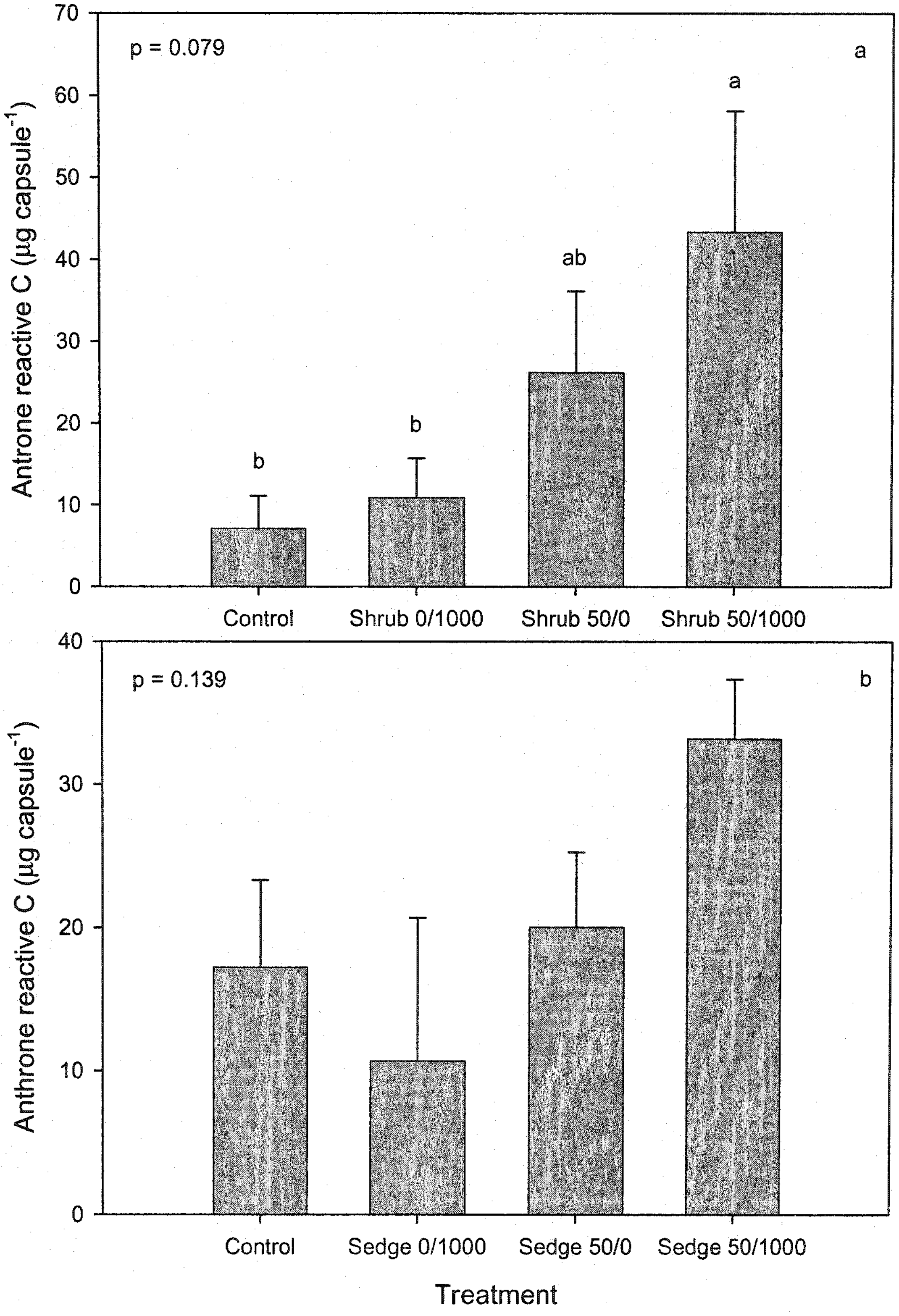
Figure 8: Anthrone reactive C (μg $capsule^{-1}$) measured with ionic resin capsules from factorial applications of glycine (50 kg ha^{-1}) and charcoal (1000 kg ha^{-1}) to a field site in western Montana. Materials were applied to the litter below two different plant types, *Arctostaphylos uva-ursi* (Shrub, a) and *Carex geyeri* (Sedge, b), found in the understory of ponderosa pine-Douglas-fir forests

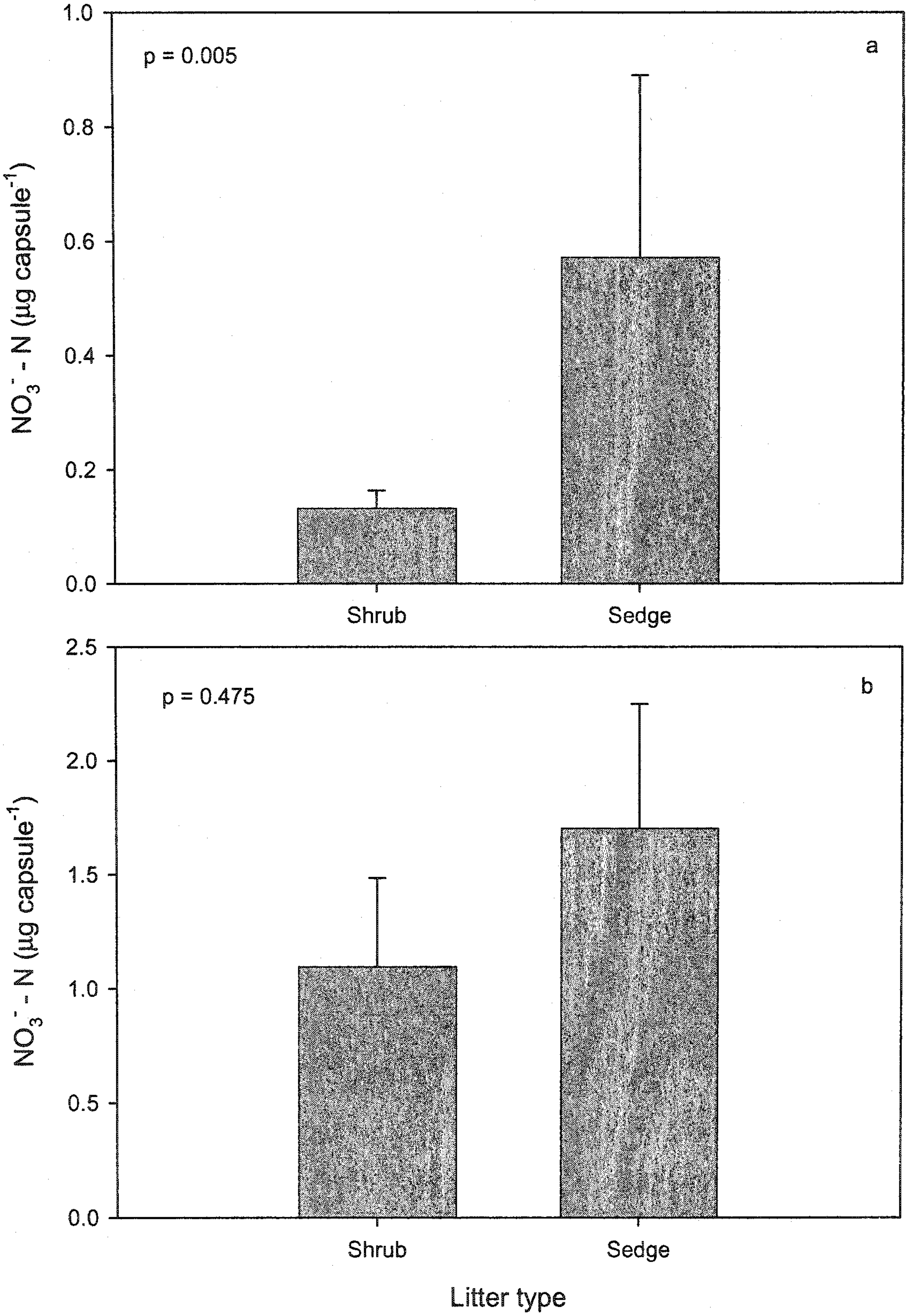
Figure 9: Nitrification rate (μg g^{-1} h^{-1}) measured for soil samples from factorial applications of glycine (50 kg ha^{-1}) and charcoal (1000 kg ha^{-1}) to a field site in western

Montana. Materials were applied to the litter below two different plant types, *Arctostaphylos uva-ursi* (Shrub, a) and *Carex geyeri* (Sedge, b), found in the understory of ponderosa pine-Douglas-fir forests

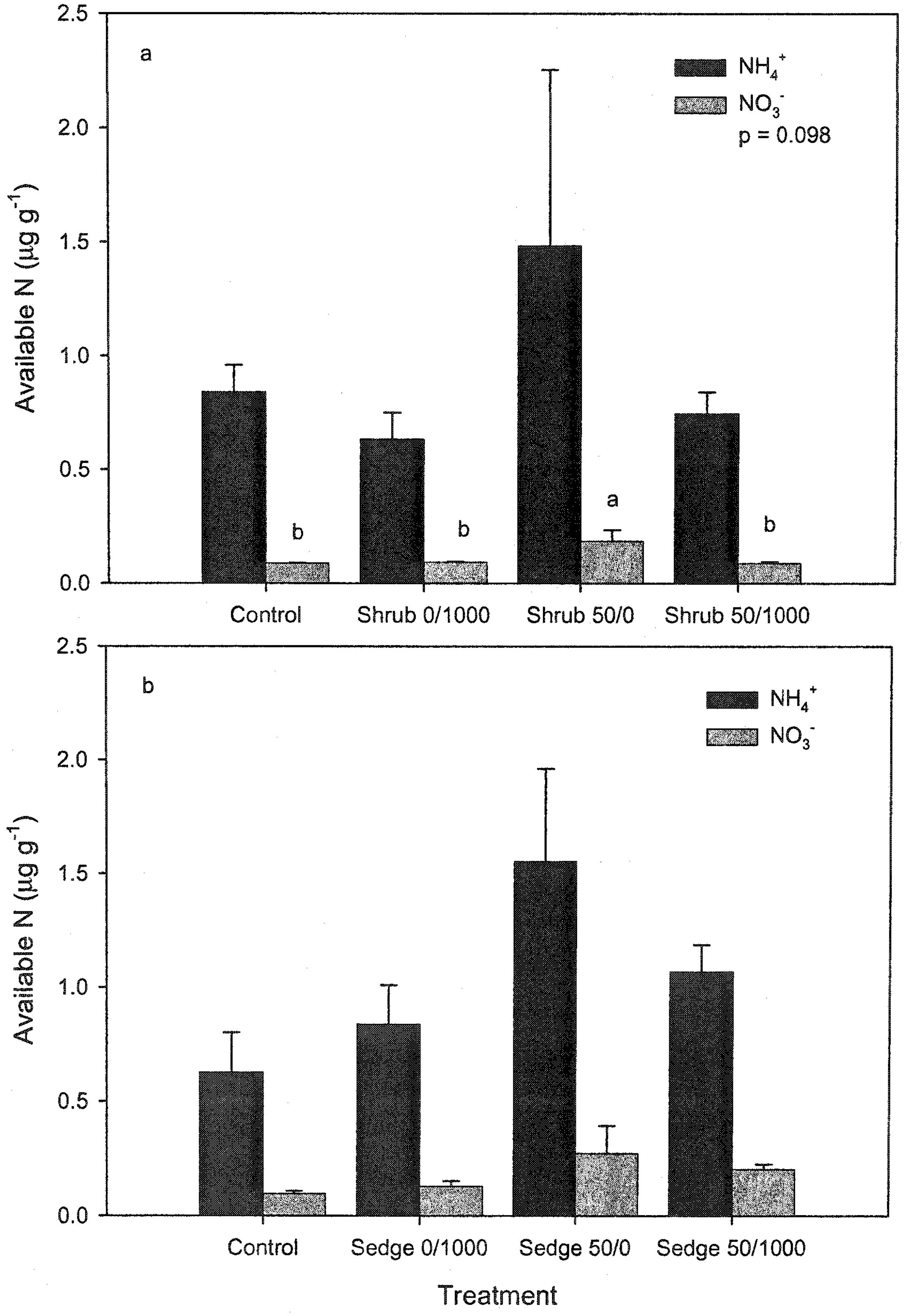

a
p = 0.079
Radicle legnth (mm)
0
2
4
6
8
10
a
b
ab
a
a
Control
1% Shrub
5% Shrub
10% Shrub
50% Shrub
b
p =0.030
Radicle legnth (mm)
0
2
4
6
8
10
a
b
b
b
a
Control
1% Sedge
5% Sedge
10% Sedge
50% Sedge
Treatment

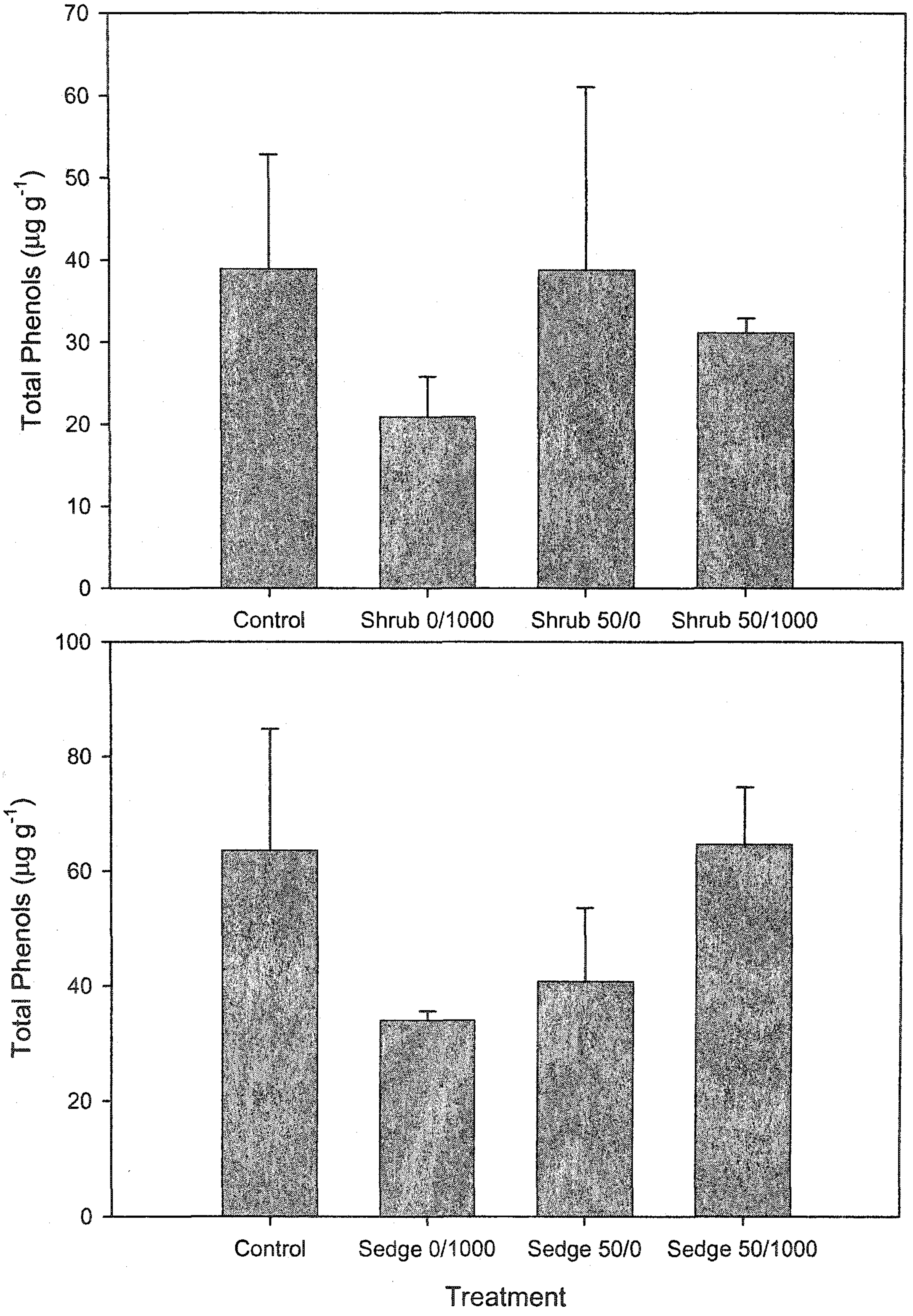

a
NH4+
p = 0.085
NO3-
p = 0.081
Available N (mg capsule-1)
2.5
2.0
1.5
1.0
0.5
0.0
Control
Shrub 0/1000
Shrub 50/0
Shrub 50/1000
b
b
b
b
a
ab
ab
a
b
NH4+
p = 0.012
NO3-
p = 0.035
Available N (mg capsule-1)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Control
Sedge 0/1000
Sedge 50/0
Sedge 50/1000
Treatment
b
b
b
b
ab
a
a
ab

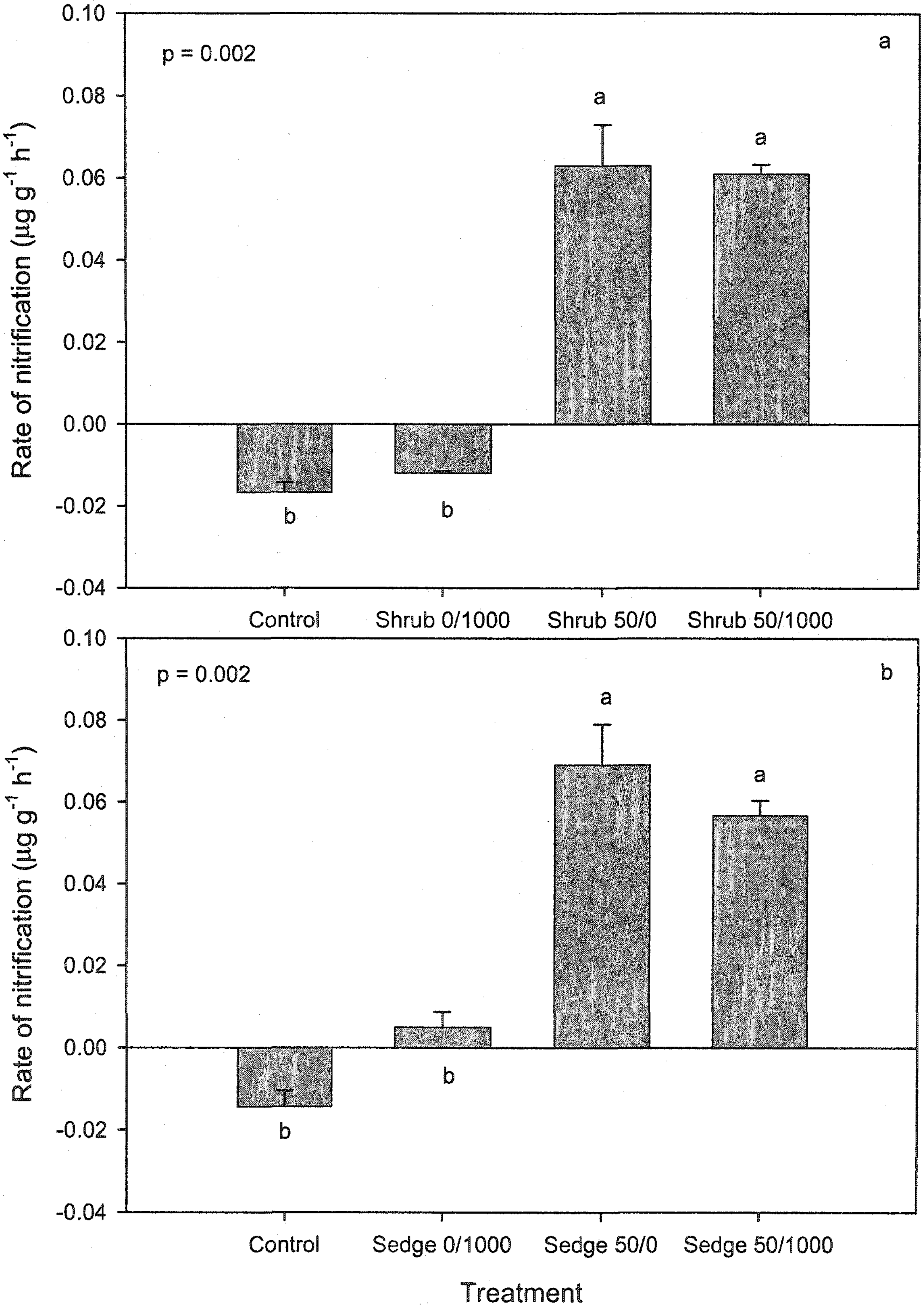

p = 0.079
a
Antrone reactive C (μg capsule^{-1})
70
60
50
40
30
20
10
0
a
ab
b
b
Control
Shrub 0/1000
Shrub 50/0
Shrub 50/1000
p = 0.139
b
Anthrone reactive C (μg capsule^{-1})
40
30
20
10
0
Control
Sedge 0/1000
Sedge 50/0
Sedge 50/1000
Treatment



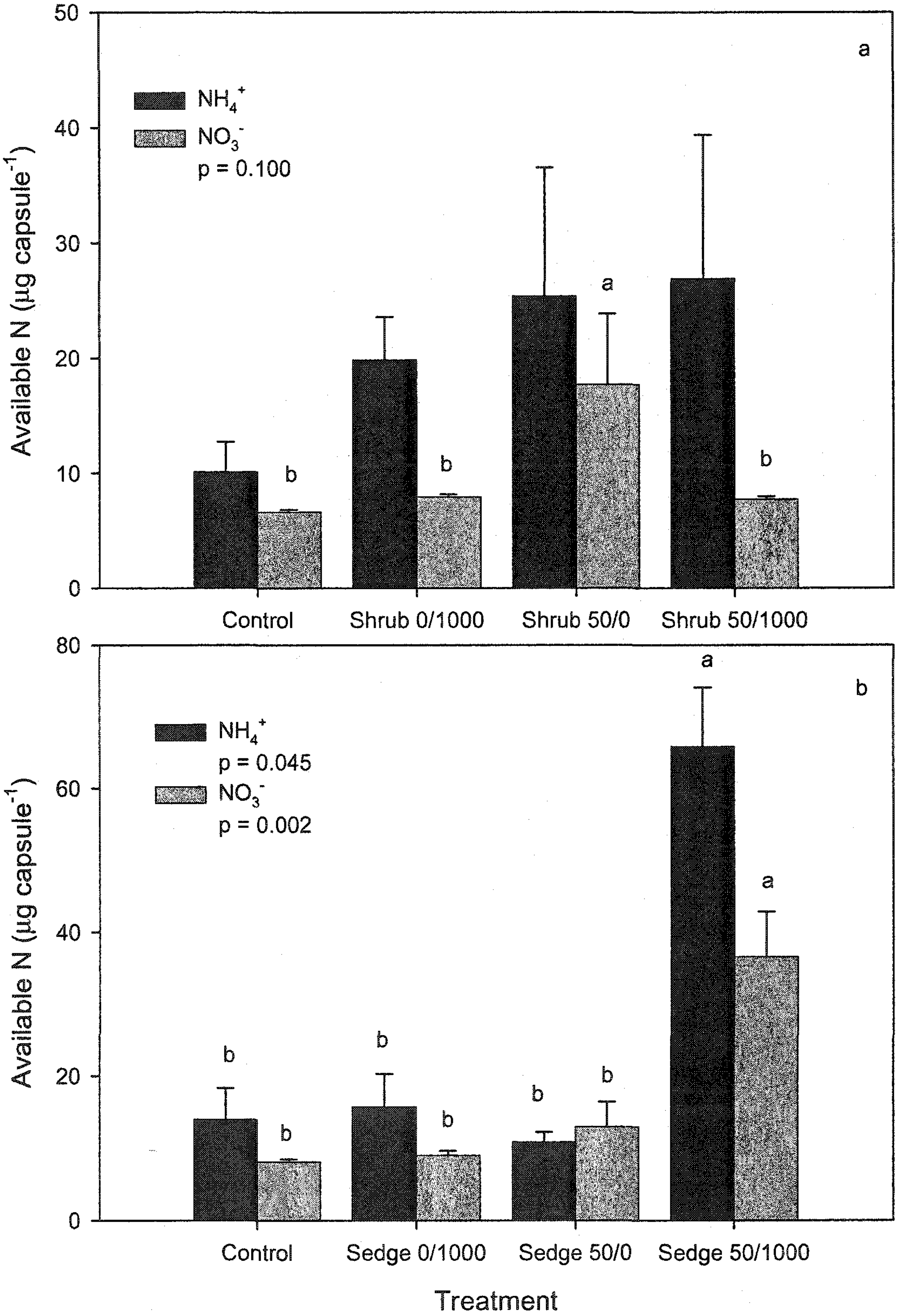
1.0
0.8
0.6
0.4
0.2
0.0
NO_3^- - N (μg capsule^{-1})
p = 0.005
a
Shrub
Sedge
2.5
2.0
1.5
1.0
0.5
0.0
NO_3^- - N (μg capsule^{-1})
p = 0.475
b
Shrub
Sedge
Litter type

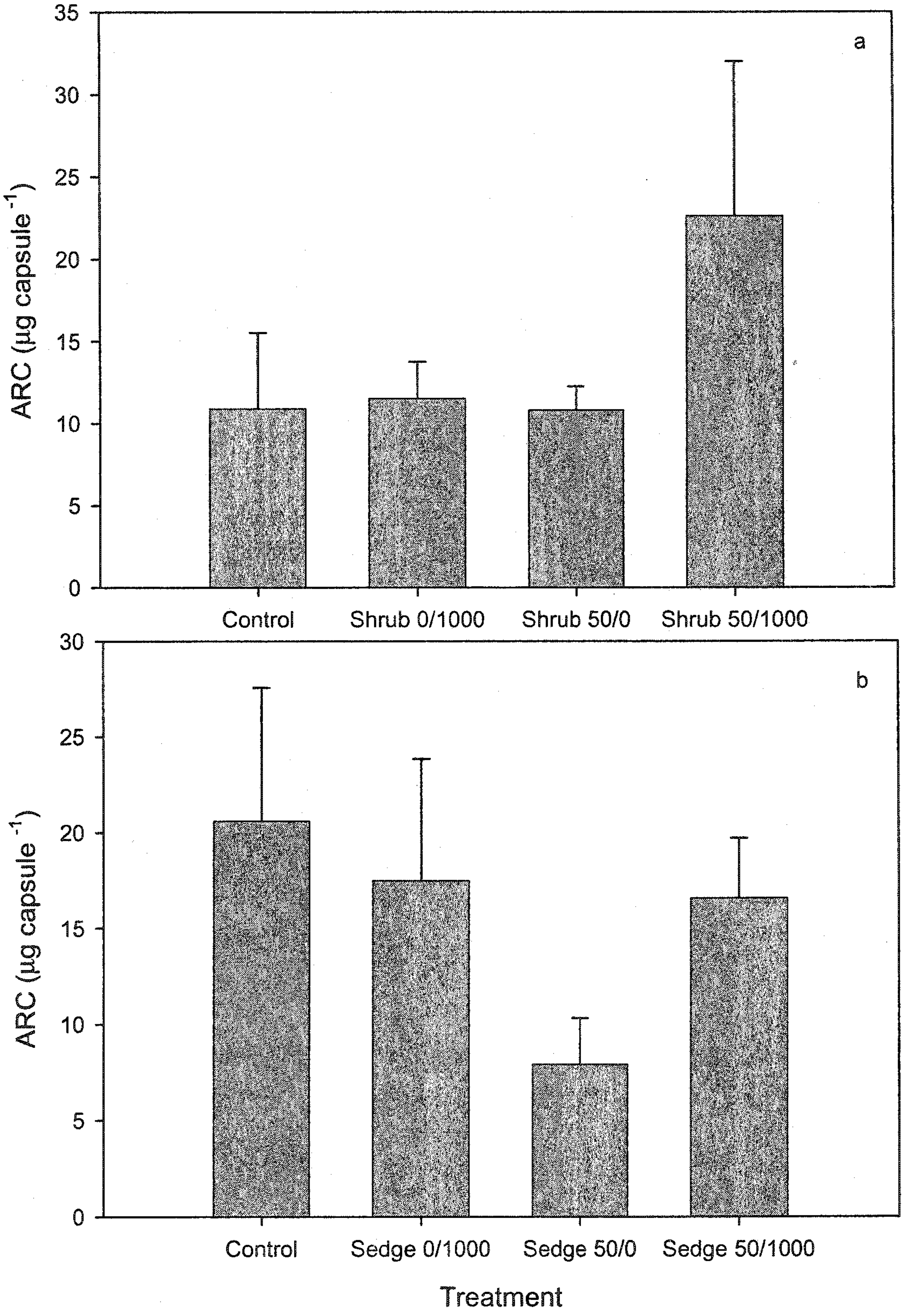

a
2.5
2.0
1.5
1.0
0.5
0.0
Available N (μg g⁻¹)
▇ NH4+
▨ NO3-
p = 0.098
b
b
a
b
Control
Shrub 0/1000
Shrub 50/0
Shrub 50/1000
b
2.5
2.0
1.5
1.0
0.5
0.0
Available N (μg g⁻¹)
▇ NH4+
▨ NO3-
Control
Sedge 0/1000
Sedge 50/0
Sedge 50/1000
Treatment


Total Phenols (µg g^{-1})
70
60
50
40
30
20
10
0
Control
Shrub 0/1000
Shrub 50/0
Shrub 50/1000
Total Phenols (µg g^{-1})
100
80
60
40
20
0
Control
Sedge 0/1000
Sedge 50/0
Sedge 50/1000
Treatment



0.10
0.08
0.06
0.04
0.02
0.00
-0.02
-0.04
Rate of nitrification (µg g⁻¹ h⁻¹)
p = 0.002
a
a
a
b
b
Control
Shrub 0/1000
Shrub 50/0
Shrub 50/1000
0.10
0.08
0.06
0.04
0.02
0.00
-0.02
-0.04
Rate of nitrification (µg g⁻¹ h⁻¹)
p = 0.002
b
a
a
b
b
Control
Sedge 0/1000
Sedge 50/0
Sedge 50/1000
Treatment


50
40
30
20
10
0
a
NH$_4^+$
NO$_3^-$
p = 0.100
Available N (µg capsule^{-1})
a
b
b
b
Control
Shrub 0/1000
Shrub 50/0
Shrub 50/1000
80
60
40
20
0
a
b
NH$_4^+$
p = 0.045
NO$_3^-$
p = 0.002
Available N (µg capsule^{-1})
a
b
b
b
b
b
b
Control
Sedge 0/1000
Sedge 50/0
Sedge 50/1000
Treatment


a
ARC (µg capsule $^{-1}$)
0
5
10
15
20
25
30
35
Control
Shrub 0/1000
Shrub 50/0
Shrub 50/1000
b
ARC (µg capsule $^{-1}$)
0
5
10
15
20
25
30
Control
Sedge 0/1000
Sedge 50/0
Sedge 50/1000
Treatment