

**WILDFIRE AND PRESCRIBED BURNING EFFECTS  
ON NITROGEN DYNAMICS  
IN CENTRAL IDAHO HEADWATER ECOSYSTEMS**

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## ABSTRACT

Wildfire, a common natural disturbance in many biomes, can have profound effects on nitrogen (N) dynamics in both terrestrial and aquatic ecosystems. Therefore, the objective of this study was to improve the understanding of post-fire N dynamics in small watersheds that experienced different fire severities. This was achieved by quantifying and interpreting the N concentrations and N isotopic signatures of soil, understory plants, streamwater and aquatic biota in paired watersheds (burned-unburned) for two to four years after wildfire and spring prescribed burns in a total of eight independent sites.

After wildfires, short-term increases in inorganic N concentrations in mineral soil and streamwater resulted in increased N concentration in terrestrial plants and in-stream moss, providing analogous and important N retention mechanisms in the terrestrial and aquatic ecosystem components. The temporal disconnect between major losses of available N from the soil during snowmelt and the onset of the growing season highlighted the importance of aquatic N retention.

After spring prescribed burns, increases in soil inorganic N concentrations and plant foliar N concentrations occurred as well, however the magnitude and/or duration of increase of available soil N was reduced relative to that after wildfires. In prescription-burned watersheds, there were no effects on the aquatic ecosystem component due to the complete retention of post-fire available N by plants and the soil microbial community within either the burned or downhill unburned areas.

The use of N stable isotopes at natural abundance confirmed the differences between effects of wildfire and spring prescribed burns. A directly fire-induced isotopic signal in soil, in combination with altered soil N transformation processes, resulted in the isotopic enrichment of linked N pools (plants, streamwater, in-stream moss) after wildfires. Only plant foliage was enriched after spring prescribed burns, although to a lesser degree than after wildfire. Thus, isotopic shifts in terrestrial plant foliage or aquatic biota after fire are a useful indicator of the spatial extent, magnitude, and duration of fire effects and the fate of post-fire available N.

The watershed-ecosystem approach applied to first-order coniferous watersheds furthered the understanding of complex interactions between the terrestrial and aquatic components with regard to post-fire N dynamics.

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## CHAPTER 1

### INTRODUCTION

Fire may have profound effects on many properties of ecosystems (DeBano et. al 1998). Effects have generally been studied in terrestrial uplands or streams, resulting in a paucity of data from riparian areas (Dwire and Kauffman 2003) and the interconnections between the various ecosystem components within watersheds. Riparian areas are defined as “three dimensional zones of direct physical and biotic interactions between terrestrial and aquatic ecosystems” (Gregory et al. 1991, p. 540). Riparian areas thus represent an important link between these two ecosystem components. Additionally, relative to their absolute extent, riparian areas are of disproportional importance in the landscape due to supporting a wide array of species, biogeochemical cycles and rates, and due to their role in aquatic functioning (providing shade, organic matter inputs, and terrestrial sediment retention) (Naiman and Decamps 1997). Since riparian areas often differ from upland areas in terms of microclimate, moisture availability, and species composition, the resulting different fuel loads and fuel moisture (Dwire and Kauffman 2003; Agee et al. 2002) may in turn lead to differing fire intensities and severities. In conifer-dominated areas, however, as in this study, differences between upland and riparian areas may be small due to similar topography, microclimate, and vegetation (Dwire and Kauffman 2003). For example, in drier forest types, Olson (2000) found similar fire-return intervals in riparian and upslope areas. Nevertheless, a knowledge gap exists on the ecological role and importance of fire in riparian areas and on the linkages between uplands, riparian areas, and streams in small headwater watersheds.

This lack of knowledge combined with the adverse effects of previous and current management of forested upslope areas (e.g., logging, grazing, roads) on aquatic ecosystems has led to a conservative management approach of riparian areas, i.e., the exclusion of riparian areas from prescribed burning, on many National Forests. This management approach is also prevalent on the Boise and Payette National Forests (Boise National Forest 2003; Payette National Forest 2003) where this study was located. However, during the past century of fire suppression, fuel accumulations in low to mid-elevation forests that were historically characterized by a low to mixed-severity fire regime (Agee 1993), are likely not restricted to upland forests but also occurred in the coniferous riparian areas.

The goal of the original study was therefore to compare the response of upslope and riparian vegetation, soils, and intermittent streams of replicated headwater watersheds that were prescription-burned, including deliberate ignition of the riparian area, to those that were unburned at a single site. Since nitrogen (N) is a critical nutrient and often limiting productivity in both forests (Vitousek and Howarth 1991; Moore et al. 1991; Garrison et al. 2000) and streams (Grimm and Fisher 1986; Thomas et al. 2003; Munn and Meyer 1990) of the western US, and since fire can strongly influence N dynamics, the study focus was on fire effects on N dynamics in riparian areas and streams. More specifically, my objective was to elucidate the fate of N post-fire and the linkages between terrestrial and aquatic ecosystem components. Concomitantly, microbial soil N transformations were to be studied by Akihiro Koyama, also a doctoral student at the University of Idaho Department of Forest Resources. Due to unforeseeable difficulties with timing and insufficient severity of these spring prescribed burns, the original design was altered in that data from several additional study sites with spring prescribed burns in 2004 were collected. This alternative design strengthened conclusions on the observed pattern and quantified processes with regards to the general applicability within the region. Additionally, the same types of data were collected from watersheds burned by wildfires in 2002 and 2003 which enabled the direct comparison between spring prescribed burns and wildfires that had occurred at the same time and within the same geographic area (Appendices A, B, C). In contrast to the original study design, which included collecting both pre-burn data and reference data from unburned watersheds, no pre-burn data was available with the alternative design.

In the small, steep headwater watersheds studied, riparian areas are dominated by conifers and understory vegetation very similar to that of upland areas. Obligate riparian shrubs and herbs were confined to the immediate streamside. Therefore, the riparian area was defined broadly as an about 30 m wide area to either side of the stream, since mature coniferous trees at this distance still provide shade and woody material inputs to the streams and might still benefit from the higher availability of water (Naiman and Decamps 1997). The riparian area defined for this study lies within the area excluded from ignition during prescribed burning (Boise National Forest 2003; Payette National Forest 2003). Data was collected from important ecosystem N pools within the riparian area and streams (mineral soil, foliage of obligate riparian and non-obligate riparian ('upland') vegetation, streamwater, in-stream biota) for two to four post-fire years.

In the following chapter of this dissertation (Chapter 2) I explore the linkages between soils and plants, soils and streamwater, and streamwater and aquatic biota after wildfires. This was approached by repeatedly measuring inorganic N concentrations in soils and streamwater throughout three growing seasons post-fire, and by measuring N concentrations of terrestrial plant foliage and aquatic biota as potential N retention mechanisms. This chapter also includes assessments of fire severity via

a remote sensing index (dNBR), modeled total N export from watersheds, and the correlation between dNBR and N export or streamwater nitrate concentrations as indicators of the degree of ecological change post-fire. Chapter 2 thus provides a baseline for comparisons with spring prescribed burns, that were of much lower severity, in Chapter 3. Chapter 4 takes a different approach to study N dynamics by examining the fate of N and mechanisms of post-fire N cycling through the use of  $^{15}\text{N}$  stable isotopes at natural abundance and plant physiological measurements. In nature, minute differences exist in the ratio of two stable forms of N,  $^{14}\text{N}$  and  $^{15}\text{N}$ , in different N pools. These differences are expressed as per mil ( $\delta^{15}\text{N}$ ) deviations from the standard atmospheric  $\text{N}_2$  (Högberg 1997). The use of N stable isotopes allowed the tracing of a fire-induced isotopic signal in the soil through the ecosystem. Combined with N concentration measurements of Chapters 2 and 3, Chapter 4 provides deeper insights in N cycling post-fire *per se* and in the differences between fire effects after wildfires and after spring prescribed burns. A method used in Chapter 4 (diffusion method) is assessed for its suitability for low concentrations of ammonium and nitrate in KCl soil extracts for  $^{15}\text{N}$  analysis at natural abundance in Appendix D.

Chapters 2-4 in this dissertation are formatted as manuscripts to be submitted for publication with Dr. Kathleen Kavanagh and Akihiro Koyama as co-authors. Chapters 2, 3, and 4 are intended for submission to the peer-reviewed journals *Ecosystems*, *Forest Ecology and Management*, and *Biogeochemistry*, respectively. When cross-referencing to other chapters within the text, chapters will be referred to as K. Stephan, K. L. Kavanagh, and A. Koyama Unpublished Manuscript a, b, or c, respectively. The manuscript in Appendix D is currently in review and will be referred to as K. Stephan and K. L. Kavanagh Unpublished Manuscript.

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## CHAPTER 2

### FATE OF NITROGEN IN WATERSHED ECOSYSTEMS AFTER WILDFIRE IN CENTRAL IDAHO: A MULTI-YEAR REPLICATED STUDY INTEGRATING TERRESTRIAL AND AQUATIC ECOSYSTEM COMPONENTS

#### ABSTRACT

In this study we link soil and stream water nitrogen (N) concentrations and the cascading effects on N concentrations in understory plants and aquatic biota following wildfire. Soil ammonium ( $\text{NH}_4^+$ ) concentrations increased about ten-fold and nitrate ( $\text{NO}_3^-$ ) concentrations increased from below detection limit to  $9.4 \pm 5.4 \text{ mg NO}_3^- \text{-N kg}^{-1}$  ( $P < 0.05$ ) in burned relative to unburned watersheds in the first post-fire year (PFY 1). Streamwater  $\text{NO}_3^-$  concentrations were about two orders of magnitude higher ( $P < 0.05$ ) in burned than in unburned watersheds during spring runoff without a decreasing trend over the three-year study period. Increased soil and streamwater inorganic N concentrations post-fire were not temporally coupled. Increased soil N represented the net effect of microbial and plant activity over the growing season, whereas the streamwater N was due to flushing of winter and early spring mineralization products before the onset of the growing season. The increases in available N post-fire led to significantly increased ( $P < 0.05$ ) foliar N concentrations in all terrestrial upland species (0.8 % absolute difference between burned and unburned watersheds in PFY 1) and in-stream moss (0.9 % absolute difference across all PFYs) for the same durations as increases were observed in soil and streamwater. Higher foliar N concentrations in terrestrial plants and in-stream moss represent analogous and important N retention mechanisms. The simultaneous study of many components of watershed ecosystems revealed the importance of complex interactions between biotic, abiotic and hydrological factors influencing post-fire N retention and loss after wildfire.

## INTRODUCTION

Wildfire, a common natural disturbance in many biomes, may have profound effects on nitrogen (N) dynamics in both terrestrial and aquatic ecosystems. Since N often limits primary productivity in forests (Vitousek and Howarth 1991; Moore et al. 1991; Garrison et al. 2000) and streams (Grimm and Fisher 1986; Thomas et al. 2003; Munn and Meyer 1990) of the western US, and N can be combusted during fire (Raison et al. 1985), it is desirable to know the fate of the residual N after fire and to better understand post-fire N cycling. Despite the wealth of literature on post-fire effects on N cycling in individual fire sites, the general applicability of these findings has traditionally been assessed by review papers (Raison 1979; Neary et al. 1999; Smithwick et al. 2005) and meta-analysis (Wan et al. 2001) rather than by replicated studies. Moreover, these individual-site studies generally focus on one or two ecosystem components which are mostly confined to either the terrestrial or aquatic part of the ecosystem (e.g., soil: Covington and Sackett 1992; Chorover et al. 1994; plants: MacLean and Wein 1977b; Harris and Covington 1983; streamwater chemistry: Bailey and Schindler 1991; Hauer and Spencer 1998; soil and plants: Grogan et al. 2000; Christensen 1977; soil and streamwater: McGoll and Grigal 1977; Richter et al. 1982).

N cycling is complex even without the influence of severe disturbances. In western coniferous forests, gross microbial ammonium ( $\text{NH}_4^+$ ) and nitrate ( $\text{NO}_3^-$ ) production rates can be high but are matched by an equally high microbial N immobilization (uptake) (Carmosinia et al. 2002; Stark and Hart 1997). This explains the commonly observed low *net* ammonification/nitrification rates (gross inorganic N production minus microbial N uptake). Low net mineralization (ammonification + nitrification) coupled with plant N uptake results in low in situ extractable soil inorganic N during the growing season. As a consequence of these small inorganic N pools in the soil, little inorganic N is available for leaching, especially under conditions of low  $\text{N}_2$  fixation (Compton et al. 2003) and low atmospheric N inputs (Perakis and Hedin 2002; Binkley et al. 2004).

The lack of significant leaching of inorganic N from the terrestrial ecosystem results in low inorganic N concentrations in associated streams. The major forms of N exported from such watersheds are dissolved and particulate organic N (Hedin et al. 1995; Vanderbilt et al. 2003) that is largely recalcitrant to decomposition (Qualls and Haines 1992). In contrast, inorganic N in stream water will have undergone numerous complete cycles between sediment adsorption and re-suspension, and biotic uptake and release on its way downstream ('nutrient spiraling', Webster and Patten 1979; Newbold et al. 1981).

Nutrient uptake and transformations occur rapidly in shallow headwater streams due to the high ratio of biologically active and absorptive surface area relative to water volume (Alexander et al.

2000; Peterson et al. 2001).  $\text{NH}_4^+$  and  $\text{NO}_3^-$  are assimilated by autotrophic (algae, bryophytes) and heterotrophic (bacteria, fungi) organisms at about the same rate per stream area; however  $\text{NO}_3^-$  molecules travel further than  $\text{NH}_4^+$  molecules prior to uptake in direct proportion to  $\text{NO}_3^-:\text{NH}_4^+$  ratios (Peterson et al. 2001). Eventually, N is lost from the watershed ecosystem through hydrologic export or in-stream denitrification (Mulholland et al. 2004).

With fire, the most conspicuous effect on terrestrial N cycling is the combustion of vegetation and forest floor and the release (oxidation and volatilization) of the organic and inorganic N contained within (Raison et al. 1985). Even though the absolute N loss to the atmosphere can be substantial (e.g., Grier 1975), short-term post-fire increases of net mineralization rates (White 1986a; Kaye and Hart 1998; DeLuca and Zouhar 2000; Choromanska and DeLuca 2001; Gundale et al. 2005; A. Koyama, K.L. Kavanagh and K. Stephan Unpublished Manuscript) and thus higher inorganic soil N concentrations (Wan et al. 2001) are commonly observed relative to unburned soils.

Higher post-fire soil  $\text{NH}_4^+$  concentrations have been attributed to reduced plant uptake and higher gross ammonification rates (i.e., increased decomposition rates; Schoch and Binkley 1986; Kaye and Hart 1998). The latter is assumed to be a result of the generally warmer and moister soils in the absence of vegetation cover (Raison 1979; Woodmansee and Wallach 1981; Silva et al. 2002; A. Koyama, K. L. Kavanagh, and K. Stephan Unpublished Manuscript; J. A. Hubbart, T. E. Link, J.D. Marshall et al., Unpublished Manuscript). Increased  $\text{NO}_3^-$  concentrations are usually attributed to increased gross nitrification rates, following increased substrate availability and increased pH that enables nitrification in previously acidic soils. However the roles of substrate availability and pH have been questioned (DeLuca et al. 2006; Likens et al. 1969). Given the tight coupling of microbial  $\text{NO}_3^-$  production and consumption, the reduced availability of plant-released carbon (C) (fueling heterotrophic ammonifiers) and the availability of  $\text{NH}_4^+$  (inhibiting  $\text{NO}_3^-$  uptake by ammonifiers) are likely decreasing microbial  $\text{NO}_3^-$  assimilation post-fire (Hart et al. 1994; Stark and Hart 1997; Kaye and Hart 1998; Bradley et al. 2000; Bradley 2001; A. Koyama, K.L. Kavanagh and K. Stephan Unpublished Manuscript). However, there is evidence that gross nitrification post-fire is also stimulated, potentially due to the role of charcoal in removing phenolic compounds (DeLuca et al. 2006) or due to directly combusting inhibitory compounds (White 1986b).

After loss of vegetation by fire, the understory recovers via sprouting and from seed (Miller 2000). Commonly observed are higher post-fire understory biomass and higher foliar N concentrations (Harris and Covington 1983; MacLean and Wein 1977a) which pose important N retention mechanisms in addition to microbial immobilization (Weston and Attiwill 1996). Vitousek and Matson (1984) showed that in the first growing season after vegetation harvest, microbial N uptake played an even greater role in N retention than plant N uptake.

Nevertheless, N losses from the soil can occur post-fire. This is reflected in elevated stream  $\text{NO}_3^-$  concentrations occurring for several years post-fire (Hauer and Spencer 1998; Tiedeman et al. 1978; Williams and Melack 1997). Similar observations have been made following other watershed-scale disturbances to soil or vegetation (soil freezing: Mitchell et al. 1996; clear-cuts: Pardo et al. 1995; ice storm-induced forest tree crown damage: Bernhardt et al. 2003).

Increased streamwater  $\text{NO}_3^-$  levels in streams of both disturbed and undisturbed (Coats and Goldman 2001; Likens et al. 1995) watersheds coincide with spring runoff in areas with considerable snow accumulation. In undisturbed watersheds, this has been attributed to flushing of  $\text{NO}_3^-$  contained in snowpack (Baron et al. 1994; Bowman 1992) and from winter build-up in soil (Rascher et al. 1987) resulting from microbial activity in soils insulated by snow (Brooks et al. 1996; Kielland et al. 2006). Knowledge of in-stream processing of  $\text{NO}_3^-$  entering in pulses post-disturbance is scant but there is evidence of considerable potential for at least short-term retention of N in streams (Bernhardt et al. 2003).

The historically separate consideration of the terrestrial and aquatic landscape components has prevented progress in understanding complex relationships and feedbacks between the land and surface waters (Grimm et al. 2003). Thus, much could be learned from a holistic watershed-ecosystem approach in terms of N cycling in mature forested watersheds and after disturbances such as fire. Therefore, in this study we explore the linkages between soil and stream water N concentrations and the cascading effects on N concentrations in understory plants and aquatic biota, respectively. We accomplish this by simultaneously studying these ecosystem components in replicated paired (burned and unburned) watersheds over the course of three growing seasons post-fire.

## **METHODS**

### **Study Sites**

The four wildfire sites (Hall, Canyon Creek, South Fork, Danskin Creek) are located on the Boise and Payette National Forests in the Salmon River Mountains and West Mountains of central Idaho, USA ( $44^{\circ}05' - 44^{\circ}57' \text{N}$ ,  $115^{\circ}12' - 116^{\circ}21' \text{W}$ ). The regional climate is characterized by warm dry summers and cool, moist winters. Depending on site elevation (1400-2350 m) mean annual air temperature ranges from 0.1 to 5.4 °C and mean annual precipitation, falling mainly as snow, ranges from 720 to 1060 mm. The geology underlying the study area is either Idaho Batholith Granitics (Canyon Creek, South Fork, Danskin Creek sites) or Columbia River Basalt (Hall site). Watershed

areas are small (mean 96 ha, range 10-480 ha) and drained by either first-order perennial or intermittent streams with a westerly aspect. Stream channels are confined by relatively steep hill slopes (15-41°) and fringed by only a narrow strip ( $\leq 1$  m width) of obligate riparian shrubs and forbs. The overstory on all sites is characterized by a mix of mature Douglas-fir (*Pseudotsuga menziesii*), that is ca. 90 y old at the Hall, Canyon Creek and South Fork sites, and mature ponderosa pine (*Pinus ponderosa*). At the highest elevation site (Canyon Creek), ponderosa pine is replaced by subalpine fir (*Abies lasiocarpa*). Due to the low population density and absence of large industries in the region, atmospheric N inputs are very low ( $1.4 \text{ kg N ha}^{-1} \text{ y}^{-1}$  wet deposition in 2005) (NADP 2006).

Three of the wildfire sites (Canyon Creek, South Fork, Hall) burned in August of 2003, one site (Danskin Creek) burned in late July of 2002. The area burned by each fire ranged from 80 to 2700 ha consisting of a mix of crown fire, surface fire, and unburned area within the fire perimeter.

### **Sampling Design**

At each site, samples of soil, upland and obligate riparian plant foliage, and aquatic biota (moss, biofilm) were collected from each of several plots within the riparian area of one burned watershed and one nearby unburned reference watershed outside the fire perimeter. Riparian area was broadly defined as the slope distance of one site-potential tree height of ca. 35 m (Boise National Forest 2003; Payette National Forest 2003; Naiman and Decamps 1997). Streamwater was collected from the mouths of the streams draining these watersheds and from generally one additional burned and unburned watershed per site. At Danskin Creek, there were two additional burned and unburned watersheds for streamwater sampling. The burned watersheds chosen had not been affected by fire retardant (contains inorganic N) applied during the fire containment effort. This had been verified with Forest Service personnel and it was also verified in the field by the absence of red dye contained in fire retardant. None of the watersheds had been burned by stand-replacing fires for about a century as indicated by the tree ages.

For collecting soil and upland plant foliage, four upland plots were established in the lower portion of each watershed. Plots were located at least 100 m upstream from an access road, crossing the stream near its mouth. Two plots were located on the north and south aspects, respectively, ca. 5 to 25 m upslope from the stream bank, and with the two plots per aspect being ca. 100 m apart. Upland plots had about a 10 m radius. Plots in burned watersheds were placed where overstory had been killed and forest floor had been consumed. Obligate riparian plant foliage and aquatic biota samples were collected from two riparian plots, each corresponding to a stream reach of ca. 20 m length, per watershed. These riparian/aquatic plots were downslope from the upland plots, at the intersections of upland plots from opposing aspects. Samples were collected in three growing seasons

(2003-2005 for Danskin Creek site and 2004-2006 for other three sites) following the fires (abbreviated PFY, post-fire year).

### **Soil**

Mineral soil samples were collected in June, July and August of 2004, in May, August and October of 2005, and in June of 2006. One sample per plot was taken; it was a composite of several cores (five cores with 1.9 cm diameter or four cores with 5 cm diameter) taken from 0 to 10 cm depth. The soil samples were transported to the laboratory on ice and passed through a 4-mm sieve. Fresh soil was extracted with 2 M KCl while shaking for 1 h. Soil to extractant ratio was 1 : 2.7 or 1 : 2 because soil inorganic N concentrations were very low. Soil extracts were filtered through Whatman No. 42 filters and extracts were stored frozen until analysis. Gravimetric soil moisture was determined from a subsample of sieved fresh soil by assessing water loss after drying for 48 h at 105 °C. On two sample dates (July and August 2004) soils were extracted in the field. Fresh soil was added to specimen cups with a KCl solution of a known weight. In the laboratory, specimen cups with soil and KCl were re-weighed to obtain the amount of fresh soil added. After filtering the extract as described above, remaining soil slurry was passed through a 4-mm sieve. The dry weight of material larger than 4 mm was subtracted from the fresh soil weight in the cup in order to correctly calculate N concentration for soil < 4 mm. Gravimetric soil moisture was determined as above.

Soil extracts were analyzed for inorganic N concentrations by continuous flow colorimetry in the Department of Plant, Soil, and Entomological Sciences, University of Idaho, Moscow (Lachat QuikChem AE Automated Ion Analyzer [Zellweger Analytics Inc., Milwaukee, Wisconsin, USA], QuikChem® methods 12-107-06-2-A for  $\text{NH}_3$  and 12-107-04-1-B for  $\text{NO}_3^- + \text{NO}_2^-$ ), in the School of Biological Sciences, Washington State University, Pullman (Alpkem Autoanalyzer FS 3000 [OI Analytical, College Station, Texas, USA], methods P/N 002053 for  $\text{NH}_3$  and P/N A001559 for  $\text{NO}_3^- + \text{NO}_2^-$ ), or in the Marine Science Laboratory, University of California Santa Barbara (Lachat QuikChem 8000 [Zellweger Analytics Inc., Milwaukee, Wisconsin, USA], QuikChem® methods 31-107-06-5-A for  $\text{NH}_3$  and 31-107-04-1-A for  $\text{NO}_3^- + \text{NO}_2^-$ ).

### **Understory Vegetation**

Foliage was collected from four common upland species in upland plots and four obligate riparian species in riparian plots in either late July or mid August (except in 2004, obligate riparian foliage was collected in early June). Some 'species' are actually composites of closely related species or genera when a single species did not occur on all sites. Within a single site, however, the species was consistent between burned and unburned treatments. Upland species are the shrubs birchleaf spiraea

(*Spiraea betulifolia*), common or mountain snowberry (*Symphoricarpos albus* or *S. oreophilus*), mallow ninebark (*Physocarpus malvaceus*), and elk or northwestern sedge (*Carex geyeri* or *C. concinoides*). Obligate riparian species include the shrubs redosier dogwood (*Cornus stolonifera*), bramble (*Rubus spp.*) or current/gooseberry (*Ribes spp.*), and the forbs small enchanter's nightshade (*Circaea alpina*), and fragrant bedstraw (*Galium triflorum*). Each foliage sample per plot is a composite of one leaf (shrubs and *Carex*) or all leaves (forbs) from three to six different, randomly chosen individuals per species. Foliage was dried at 70 °C for 24 h (all 2003 foliage, 2004 obligate riparian foliage) or freeze-dried (2004 upland foliage, all 2005 and 2006 foliage).

Dried foliage was ground to a fine powder with a ball mill, packed into a tin capsule and analyzed for total N with continuous-flow direct combustion isotope ratio mass spectrometry (IRMS) following combustion in an elemental analyzer. Analyses of upland and obligate riparian foliage collected in 2004, 2005, and 2006 were carried out by the laboratory of R. Lee at the School of Biological Sciences at Washington State University (Isoprime [Micromass Ltd. Manchester, UK] coupled to an EuroEA 3000 elemental analyzer [EuroVector S.p.A., Milan, Italy]). Foliage samples collected at Danskin Creek in 2003 were analyzed at the University of Idaho Stable Isotope Laboratory (ISIL) (Finnigan Delta Plus [Finnigan MAT, Bremen, Germany] coupled to a Carlo Erba NC 2500 elemental analyzer [CE Instruments, Milan, Italy]). Analytical precision for N concentration was < 0.1‰ (standard deviation) between replicates of actual sample material. Duplicate samples run at the two laboratories had standard deviations of  $\leq 0.7\%$  (mean 0.2 %, n = 7) for leaves. Variability in leaf N concentrations due to the identity of the analytical laboratory could be confounded in seasonal variation but would not affect treatments differences.

### **Streamwater**

Streamwater was collected in July 2003 (Danskin Creek only), and approximately monthly from June through August of 2004, April through July 2005, and March through August in 2006. The initial spring sampling was limited by snowmelt on these remote sites. Water samples were filtered through Gelman A/E glassfiber filters (1  $\mu\text{m}$  pore size) in the field, transported on ice to the laboratory and frozen until analysis.  $\text{NO}_3^-$  and  $\text{NH}_4^+$  concentrations were determined colorimetrically (Lachat Quickchem 8000 [Lachat Instruments, Milwaukee, Wisconsin, USA], method 31-107-06-5-A for  $\text{NH}_3$ , method 31-107-04-1-A for  $\text{NO}_3^- + \text{NO}_2^-$ ) in the Marine Science Laboratory of the University of California, Santa Barbara. Total dissolved nitrogen was analyzed as  $\text{NO}_3^-$ -N by spectrophotometry (Beckman Coulter DU®640 [Beckman Instruments, Inc., Fullerton, California, USA]) following high temperature persulphate digestion (based on Qualls 1989). Dissolved organic nitrogen (DON) was calculated as total dissolved nitrogen (TDN) minus  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N.

We estimated total annual streamwater N export from watersheds in 2005 (calendar year) by adding the products of daily streamwater N concentration and discharge. N concentrations on each day in between actual sampling occasions were estimated by interpolation between concentrations on the preceding and succeeding sampling occasions. Because no sampling occurred in fall and winter we assumed the generally low N concentrations at base flow at the last sampling occasion to prevail through October and then we interpolated to the next spring sampling. The resulting temporal pattern of  $\text{NO}_3^-$  concentrations mimicked that of other streams with snowmelt-dominated flow regimes (e.g., Hubbard Brook Experimental Forest in New Hampshire (Likens et al. 1995)), i.e., constant low levels in summer, an increase starting in late fall which culminates in spring and a decline towards summer. Streamwater discharge in our study watersheds was not directly measured but estimated by subtracting evapotranspiration from precipitation. Evapotranspiration rates were obtained from long-term datasets of discharge from gauged large watersheds (that encompassed the watersheds of this study) and corresponding precipitation published in Hortness and Berenbrocks (2001). A long-term average precipitation estimate at the mean elevation of each watershed under study was obtained from PRISM models (Daly et al. 1994; Scheele et al. 2001) (data available at <http://forest.moscowfsl.wsu.edu/cgi-bin/fswepp/rc/rockclim.pl>). The resulting annual discharge per watershed was then assumed to have the same daily discharge pattern as Johnson Creek, a nearby though larger watershed for which a 2005 hydrograph was available (USGS 2006). Daily discharge modeled for our watersheds was then multiplied with daily N concentration. Results were summarized as annual N export per hectare watershed area.

### **Moss and Biofilm**

Moss was collected from streams in May or June. Collected mosses (comprising various species, e.g., *Bryum* and *Schistidium* species) were not strictly aquatic but grew on rocks that were submerged during spring run-off. Each moss sample represents a composite of moss collected from three randomly chosen rocks per plot. The moss was rinsed on site to remove as much sediment as possible. In the laboratory, moss was freeze-dried and ca. 1 cm long actively growing tips were picked and finely ground.

Biofilm (epilithon) was collected in June or July by scraping with a wire brush an area of ca. 20  $\text{cm}^2$  from each of three rocks (2004 samples) or from one to two 120- $\text{cm}^2$  clay tiles per plot that had been placed one month prior (2005 and 2006 samples). The slurry was collected in a plastic bowl and transferred into 50-ml plastic centrifuge tubes. In the laboratory, samples were frozen, freeze-dried, and subsequently finely ground. Moss and biofilm samples were analyzed for total N as described above. Samples collected in 2004 and 2005 were analyzed at ISIL, samples collected in 2006 were

analyzed at the Laboratory for Biotechnology and Bioanalysis Stable Isotope Core at Washington State University (Delta PlusXP [ThermoFinnigan, Bremen, Germany] coupled to an ECS 4010 elemental analyzer [Costech Analytical, Valencia, California, USA]). Duplicate samples run at the two laboratories had standard deviations of  $< 0.1\%$  ( $n = 2$ ) for moss and biofilm, respectively. A subsample of material was ashed for 5 h at  $500\text{ }^{\circ}\text{C}$  in order to determine ash free dry mass (AFDM).

All sampled materials were stored on ice in a cooler during the collection period and transport to the laboratory. Due to the remoteness of the field sites, all sample processing in the laboratory commenced one to five days after field collection.

### **Fire Severity**

Fire severity, defined as the effect of fire on the ecosystem (Ryan and Noste 1985), was assessed from satellite imagery (Landsat) before and after fires for all watersheds from where streamwater had been collected. We used the delta Normalized Burn Ratio (dNBR), calculated as  $\text{NBR}_{\text{postfire}} - \text{NBR}_{\text{prefire}}$ , as an index of fire severity. NBR values, in turn, are calculated as the difference between near-infrared (NIR) and middle-infrared (MIR) reflectance divided by their sum (Key and Benson 2006; Brewer et al. 2005). High NBR values are indicative of surfaces with high water content (i.e., high NIR reflectance of green vegetation or moist soil) whereas low NBR values indicate dry surfaces (i.e., high MIR reflectance dry or charred biomass and dry exposed soil). In most of our study sites, tree canopy was the dominant surface cover so that dNBR would mainly reflect post-fire overstory mortality rather than effects on the ground. At the Hall site, however, about half of the watershed area was covered by herbaceous vegetation. At this site, dNBR would represent a composite of the changes in the overstory as well as on the ground. Landsat imagery was obtained from an archive established by Beck and Gessler (2007). Post-fire Landsat imagery was from ca. one month after the fires. In order to minimize non-fire related differences, pre- and post-fire imagery were matched phenologically, i.e., for the three 2003 wildfires, pre- and post-fire imagery was from September 2002 and 2003, respectively, and for the 2002 Danskin Creek wildfire pre- and post-fire imagery was from August 2001 and September 2002, respectively. Additionally, dNBR values of burned watersheds were standardized by subtracting the average dNBR value of the two (three in Danskin Creek) corresponding unburned watersheds. This accounted for non-fire related differences in pre- and post-fire NBR. Delta NBR values ranging from 100 to 1300 are typical of burned forested areas (Key and Benson 2006). Severities were classified as enhanced regrowth, unburned, low, low-moderate, moderate-high, and high for dNBR ranges of -500 to -101, -100 to +100, 100 to 259, 260 to 439, 440 to 659, and 660 to 1300, respectively.

Increases in streamwater  $\text{NO}_3^-$  concentrations and total  $\text{NO}_3^-$  export (as indicators of post-fire

watershed N cycling) were regressed against dNBR values in order to test the ecological relevance of dNBR.

### **Statistical Analysis**

The study design is comparable to a block design (site = block, watershed = plot). At each watershed or site  $\times$  treatment combination we collected several subsamples (i.e., two riparian/aquatic or four upland sampling plots per watershed, or two streams per site  $\times$  treatment combination). These subsamples were averaged for each watershed before statistical analysis.

Data were subjected to analyses of variance (ANOVA) with linear mixed-effect models in SAS (SAS 9.1, SAS Institute Inc., Cary, NC, USA) using the 'proc mixed' statement. The main objective of the statistical analysis was to test for the absence of a difference between burned and unburned watersheds (i.e., treatment effect). We also assessed the effect of time since fire on N concentrations and the interactions of treatment and time since fire. Mixed-effects models were used because they allowed addressing the often unbalanced data and modeling the correlation structure induced by the nested design (watersheds within sites) and repeated sampling in time.

Model assumptions were checked graphically prior to analysis in R (open-source statistical language, Version 1.9.1, The R Development Core Team 2004) and data were transformed (log or power) if necessary. Analysis results of soil  $\text{NO}_3^-$  concentrations often were negative. Therefore, we added the smallest possible constant (0.9) to all values so that the necessary log-transformation could be performed.

In our mixed-effect models, site was specified as random effect ('random' statement). That is, study sites are a random sample from a population of sites or, as with this study, selection of sites was deterministic but the effect of sites on the outcome was of stochastic nature (Schabenberger and Pierce 2002). In consequence, inference drawn from this study is not limited to the sites studied, but applies to similar wildfires in mid-elevation headwater watersheds within the central Idaho region. In addition to site, the watersheds nested within each site were included as random effects if permitted by the data structure. This allowed random interactions between site and treatment, i.e., the magnitude of the burn effect could vary between sites and/or the two watersheds within each site could differ from each other due to, e.g., slight variation in elevation, slope, or (pre-fire) soil characteristics. As a consequence, only those treatment effects that were sufficiently strong in all sites were detected.

Serial correlation between N concentrations of samples collected through time was assumed and accounted for with repeated measures ('repeated' statement). Adjustments to the random effects structure and serial correlation were made (i.e., one random effect and/or the serial correlation was dropped) if the data structure did not support having all components in the model. Pairwise

comparisons for a given sample date were carried out in SAS using the Satterthwaite function to approximate degrees of freedom. In the results, model P-values and/or P-values of pairwise comparisons are presented. Means and standard errors presented in graphs and tables are based on the untransformed raw data.

## RESULTS

### Soil

Soil  $\text{NH}_4^+$  and  $\text{NO}_3^-$  concentrations were higher in burned than unburned watersheds but for different durations.  $\text{NH}_4^+$  concentrations tended to be higher in burned than unburned watersheds across all sample dates ( $P = 0.07$ ), varied by sample date ( $P = 0.003$ ), and the seasonal patterns of soil  $\text{NH}_4^+$  concentrations varied significantly between the burned and unburned watersheds (treatment  $\times$  time interaction  $P = 0.03$ ). Soil  $\text{NH}_4^+$  concentrations were  $6.8 \pm 5.6$  (SD)  $\text{mg NH}_4^+\text{-N kg}^{-1}$  and  $44.2 \pm 29.1$   $\text{mg NH}_4^+\text{-N kg}^{-1}$  when averaged across the three 2003 wildfires and the three sample dates of the first PFY (PFY 1) for unburned and burned watersheds, respectively. The soil  $\text{NH}_4^+$  concentrations were significantly higher in the burned compared to unburned watersheds during the first growing season but not thereafter (Figure 1a). Even though  $\text{NH}_4^+$  concentrations of burned watersheds did not differ significantly from one another in PFY 1 ( $P > 0.1$ ) there was a decreasing trend over the season whereas in the unburned watersheds there was no consistent pattern (Figure 1a). Standard errors of the burned watersheds (Figure 1a) are relatively large, reflecting the variability between sites. Canyon Creek had consistently the highest soil  $\text{NH}_4^+$  concentrations among the burned watersheds; in PFY 1, average concentrations across the three sample dates were  $74.2 \pm 31.7$  (SD)  $\text{mg NH}_4^+\text{-N kg}^{-1}$ .

Fire affected soil  $\text{NO}_3^-$  concentrations in different ways compared to  $\text{NH}_4^+$  concentrations.  $\text{NO}_3^-$  concentrations were mostly below the detection limit in unburned watersheds. In burned watersheds,  $\text{NO}_3^-$  was detected and significantly higher than in unburned watersheds ( $P = 0.01$ ) but concentrations were then generally five to ten times lower than soil  $\text{NH}_4^+$  concentrations (Figure 1). Average soil  $\text{NO}_3^-$  concentrations in burned watersheds of the three 2003 wildfires in PFY 1 were  $9.4 \pm 8.6$  (SD)  $\text{mg NO}_3^-\text{-N kg}^{-1}$ . The magnitude of increase in burned watersheds varied by sample date (treatment  $\times$  time interaction  $P = 0.004$ ). Pairwise comparisons per sample date showed that soil  $\text{NO}_3^-$  concentrations were significantly increased ( $P < 0.05$ ) in burned relative to unburned watersheds throughout PFY 1, May of PFY 2, and June of PFY 3 (Figure 1b). The large error bars for burned watersheds were caused by relatively low concentrations at South Fork in PFY 1 ( $1.2 \pm 0.6$  (SD)  $\text{mg NO}_3^-\text{-N kg}^{-1}$ ), and relatively high concentrations at Canyon Creek in PFY 2 ( $3.4 \pm 2.4$   $\text{mg NO}_3^-\text{-N}$

kg<sup>-1</sup>) and PFY 3 (10.6 mg NO<sub>3</sub><sup>-</sup>-N kg<sup>-1</sup>).

### Understory Vegetation

Burning resulted in a large but short-term increase in foliar N concentrations. In unburned watersheds, foliar N concentrations varied by species ranging from 1.3 to 1.9 % for upland species and 2.2 to 2.9 % for obligate riparian species (Table 1). With upland species, there was a significant treatment × PFY interaction ( $P < 0.0001$ ), species effect ( $P = 0.001$ ), and PFY effect ( $P < 0.0001$ ). N concentrations increased with similar magnitude across species in burned relative to unburned watersheds (i.e., species × treatment interaction was statistically not significant). In PFY 1, the absolute difference between burned and unburned watersheds was 0.8 % ( $P = 0.001$ ) translating into a relative difference of 57 %. In PFY 2, this difference persisted as a trend and at smaller magnitude (absolute difference 0.2 %,  $P = 0.08$ ) for all species except ninebark (no difference); by PFY 3 no difference could be detected for any upland species (Table 1, Figure 2).

Burning had no effect on foliar N concentrations of obligate riparian species in any of the three post-fire years ( $P = 0.61$ ) but foliar N concentration varied by species ( $P = 0.002$ ) and PFY ( $P < 0.0001$ ) (Table 1, Figure 2). The latter was likely due to sampling in early June, late July and mid August in PFY 1, 2 and 3 respectively.

### Streamwater

Fire caused a large increase in streamwater NO<sub>3</sub><sup>-</sup> concentrations. Streamwater NO<sub>3</sub><sup>-</sup> concentrations were significantly increased in burned relative to unburned sites (each site value represents the average of two watersheds) throughout the three post-fire seasons ( $P = 0.02$ ) (Figure 3). Streamwater NO<sub>3</sub><sup>-</sup> concentrations averaged  $34.8 \pm 57.1$  (SD)  $\mu\text{g NO}_3^- \text{-N L}^{-1}$  (range 0.3-260  $\mu\text{g NO}_3^- \text{-N L}^{-1}$ ) and  $292 \pm 321$   $\mu\text{g NO}_3^- \text{-N L}^{-1}$  (range 5.9-1560  $\mu\text{g NO}_3^- \text{-N L}^{-1}$ ) across all individual streams and sample dates in unburned and burned watersheds, respectively. Amongst the burned sites, streamwater NO<sub>3</sub><sup>-</sup> concentrations tended to be highest at the South Fork site, e.g., maximum concentration of 1560  $\mu\text{g NO}_3^- \text{-N L}^{-1}$  occurred in the spring of PFY 2. NO<sub>3</sub><sup>-</sup> concentrations also varied significantly in time ( $P = 0.005$ ) in both burned and unburned watersheds. That is, they were highest in spring and low in summer (Figure 3). There was no attenuation of peak NO<sub>3</sub><sup>-</sup> concentrations between PFY 2 and 3; peak concentrations of PFY 1 had likely occurred before the first sampling.

Streamwater NH<sub>4</sub><sup>+</sup> concentrations were  $10.0 \pm 6.8$  (SD)  $\mu\text{g NH}_4^+ \text{-N L}^{-1}$  (simple average across all individual streams and sample dates), ranging between 1.8 and 57.4  $\mu\text{g NH}_4^+ \text{-N L}^{-1}$ . Concentrations did not differ between burned and unburned sites and they did not vary in any systematic fashion within given sampling seasons. Across all unburned watersheds and sample dates the NO<sub>3</sub><sup>-</sup> : NH<sub>4</sub><sup>+</sup>

ratio was  $4.7 \pm 8.1$  (SD).

Concentrations of DON were variable between individual streams and sample dates (mean  $92 \pm 68$  (SD)  $\mu\text{g N l}^{-1}$ , range 1.4-632  $\mu\text{g N l}^{-1}$ ). DON did not differ between burned and unburned watersheds, or between sample dates. At one site, however, DON concentrations in both burned and unburned watersheds showed a seasonal pattern similar to that of  $\text{NO}_3^-$  in PFY 2 and 3 (i.e., high in spring and low in summer) (data not shown). DON comprised most of the TDN in unburned watersheds ( $69 \pm 23$  (SD) %) (simple average across all individual streams and sample dates), range 6-97 %); DON in burned watersheds comprised only ca. 22 % of TDN early in the season and increased to ca. 54 % until August caused by decreasing  $\text{NO}_3^-$  concentrations.

N exported from watersheds in 2005 via streams as modeled are presented in Figure 4. Total N export from unburned watersheds is low ( $\leq 1 \text{ kg N ha}^{-1} \text{ y}^{-1}$ ) and a large proportion (ca. 63 %) is exported as DON. After wildfires, total N export increased due to the increased streamwater  $\text{NO}_3^-$  concentrations. This also rendered  $\text{NO}_3^-$  the dominant form of N exported post-fire. Most of the annual TDN export (ca. 80 %) occurred during spring runoff (March-June).

### **Moss and Biofilm**

Burning resulted in a large and sustained increase in moss N concentrations. New moss foliage had N concentrations of  $2.2 \pm 0.6$  % per AFDM and  $3.1 \pm 0.3$  % per AFDM (mean  $\pm 1$  SD across three PFYs and four sites) in unburned and burned watersheds, respectively. Moss N concentrations were significantly increased across all three PFYs ( $P = 0.02$ ) and moss N concentrations of neither burned nor unburned watersheds changed in time ( $P = 0.6$ ). N concentrations in biofilm were highly variable (range 1.9-10.5 % N in AFDM) and did not differ systematically between burned and unburned watersheds.

### **Fire Severity**

The change in surface reflectance between pre- and post-fire spectral images varied between the sites and between burned and unburned watersheds. The standardized dNBR of burned watersheds containing the sampling plots and of burned watersheds for the additional streamwater sample(s), respectively, were 563 and 462 at Canyon Creek, 263 and 294 at South Fork, 125 and 266 at Hall, and 200, 233, and 136 at Danskin Creek (x-axis of Figure 5). These dNBR values translate into moderate-high, moderate, low, and low severities at the four sites, respectively. The proportions of each watershed burned in each severity class are presented in Table 2.

When regressing these standardized dNBR values (and using  $\text{dNBR} = 0$  for each unburned watershed) against streamwater  $\text{NO}_3^-$  concentrations, regression slopes were statistically significant ( $P$

$\leq 0.05$ ) for seven out of thirteen streamwater sample dates (May and June of PFY 1; June of PFY 2; April, May, June, August of PFY 3). Coefficients of determination ( $r^2$ ) ranged from 0.29 to 0.87. Examples of two sample dates when dNBR and  $\text{NO}_3^-$  concentrations were strongly and weakly correlated, respectively, are shown in Figure 5. Our estimated total  $\text{NO}_3^-$  export in 2005 was also correlated with standardized dNBR values (Figure 5).

## DISCUSSION

In our study, inorganic N concentrations increased in all ecosystem components (except obligate riparian foliage and biofilm) after wildfire. This is despite a net loss of (mainly organic) N due to combustion highlighting the importance to differentiate between available (inorganic) and unavailable (organic) N. The post-fire increases in available N in soil and streamwater were attenuated by increased uptake by terrestrial vegetation and moss in streams, respectively. Thus, terrestrial understory vegetation and in-streams moss represent analog means of retaining N within their respective ecosystem components. In the following we will briefly discuss the mechanisms of increased N concentrations in each ecosystem component and then highlight the linkages between the components.

### Soil and Streamwater

Post-fire soil inorganic N concentrations were strongly increased during the first growing season compared to the low N concentrations in unburned watersheds that are typical for N-limited ecosystems of the region (Stark and Hart 1997; Vitousek et al. 1982). The post-fire temporal patterns in soil inorganic N concentrations we observed agree with those described in the literature: immediate but short-term increases in soil  $\text{NH}_4^+$  and somewhat delayed but more persistent increases in soil  $\text{NO}_3^-$  concentrations (Wan et al. 2001).

The observed post-fire changes in soil inorganic N concentrations represent the net effect of gross microbial N production, microbial N immobilization, and plant N uptake. After fire, plant cover in the first PFY was very low and, thus, reduced N uptake by vegetation was likely an important factor of observed higher soil inorganic N concentrations. Because of the importance of microbial immobilization for N retention (Vitousek and Matson 1984; Zak et al. 1990), reduced microbial immobilization post-fire due to the lack of labile C inputs via fresh plant litter or root exudates (Hart et al. 1994; Kaye and Hart 1998; Bradley et al. 2000; Bradley 2001) likely also contributed to the increased soil inorganic N concentrations. In fact, A. Koyama, K. L. Kavanagh and K. Stephan

(Unpublished Manuscript) showed that in the watersheds of this study microbial N-immobilization of both  $\text{NH}_4^+$  and  $\text{NO}_3^-$  was lower in burned than in unburned watersheds in PFY 2.

Soil inorganic N concentrations in burned watersheds were similar to those of unburned watersheds during the growing seasons of PFY 2 and 3, reflecting both increased plant uptake by recovering understory vegetation and likely increased microbial immobilization due to increased C supply from vegetation compared to PFY 1. This highlights an important synergy between plant and microbial activity in N retention after disturbance.

Despite a relative short-term fire effect on soil inorganic N concentrations, increased streamwater  $\text{NO}_3^-$  concentrations persisted in PFY 2 and 3 in burned relative to unburned watersheds. This indicates that observations on  $\text{NO}_3^-$  concentrations in soil and streamwater during the study season do not represent a direct cause and effect relationship. Furthermore, even though in PFY 1 high soil inorganic N concentrations coincided with high streamwater  $\text{NO}_3^-$  concentrations in burned watersheds across sites, there was no correlation between soil and streamwater  $\text{NO}_3^-$  concentrations among burned watersheds (data not shown). For example, even though soils at the South Fork burned watershed consistently had low soil inorganic N concentrations, this watershed had the highest streamwater  $\text{NO}_3^-$  concentrations relative to the other sites; the opposite was the case at Canyon Creek. At this scale it appears that increased post-fire rates of  $\text{NO}_3^-$  leaching in spring might actually lead to lower soil  $\text{NO}_3^-$  concentrations during the subsequent growing season. Unfortunately, we had too few data to explore this relationship more fully.

Increased streamwater  $\text{NO}_3^-$  concentrations in early spring also tended to occur in our unburned watersheds (Figure 3) as is commonly observed in areas where most precipitation falls as snow (e.g., Coats and Goldman 2001; Williams et al. 1993; Baron 1992; Likens and Bormann 1995). This phenomenon has been attributed to a pulsed release of snowpack-entrained N (i.e., wet + dry deposition) during early snowmelt (Williams and Melack 1991; Baron et al. 1994; Bowman 1992) and to flushing of winter-buildup of mineralized N in forest floor or soil. The latter is the result of microbial activity in soils insulated by snow (Rascher et al. 1987; Brooks et al. 1996; Kielland et al. 2006) and in warming soils after snowmelt (Zak and Pregitzer 1990; Groffman et al. 1993). The magnitude of the spring N flush from soils will thus depend on the balance between inputs via snowpack N and mineralization under snow and after snow melt on one hand and microbial immobilization (Groffman et al. 1993) on the other. Plant uptake will have a minor role in N retention during that time because most N is exported from the soil before plants represent a significant sink. Therefore, post-fire increased streamwater  $\text{NO}_3^-$  concentrations during spring runoff (April/May) in burned relative to unburned watersheds represent flushing of winter and/or early spring build-up in the soil, whereas the concentrations in the top 10 cm of mineral soil (measured between May and

October) represent the net effect of microbial and plant activity during the growing season. That is, we sampled the soil after the  $\text{NO}_3^-$  from winter build-up had already been flushed and thus we missed this important aspect of watershed N cycling.

Our data indicate that fire exacerbates the conditions that lead to spring  $\text{NO}_3^-$  flushing in unburned watersheds (Figure 3). To be more explicit, the large increase in spring streamwater  $\text{NO}_3^-$  concentrations in burned relative to unburned watersheds are consistent with lower microbial N immobilization during winter and early spring. This low microbial N immobilization can result from more severe C limitations in burned than in unburned watersheds. Litter inputs from regenerating understory in the fall of PFY 1 probably were relatively small since most of the foliage was consumed in the fire and had a lower C:N ratio (data not shown) compared to unburned conditions. Our study period was not long enough for a persistent litter layer (and thus, winter C source) to reestablish, which may have caused the lack of decreasing streamwater  $\text{NO}_3^-$  concentrations by PFY 3. Despite recovering understory vegetation, high streamwater  $\text{NO}_3^-$  concentrations might have also been related to tree mortality since live tree roots are an important source of labile soil C (Jones et al. 2004).

Similar seasonal patterns, magnitudes, and durations of  $\text{NO}_3^-$  concentration increases have also been observed in other wildfire areas (Hauer and Spencer 1998; Tiedemann et al. 1978; Bayley and Schindler 1991) or after canopy disturbances (e.g., Bernhardt et al. 2003). Hauer and Spencer (1998) showed a return of post-fire increased streamwater  $\text{NO}_3^-$  concentrations to reference levels five years after a severe fire in Glacier National Park, Montana, USA. Even though  $\text{NO}_3^-$  concentrations in streamwater strongly increased after fire, post-fire  $\text{NO}_3^-$  concentrations were still lower than in undisturbed streams of the northeastern United States that are impacted by high atmospheric N deposition (Perakis and Hedin 2002). The low streamwater  $\text{NO}_3^-$  concentrations in the unburned watersheds of our study thus signify the relatively pristine nature of these watersheds, although streamwater  $\text{NO}_3^-$  concentration is also confounded with vegetation type (i.e., coniferous in our study vs. deciduous in the northeastern US) (Binkley et al. 2004).

### **N Retention by Terrestrial Plants and In-Stream Moss**

Despite the large flush of available N from the soil in early spring, upland plants were nevertheless able to take advantage of the increased availability of soil N during the growing season immediately after the fire. The strong increase in foliar N concentrations of the first and the return to N concentrations similar to those in unburned watersheds by the second growing season post-fire across all four upland species coincided with the temporal pattern of soil  $\text{NH}_4^+$  and  $\text{NO}_3^-$  concentrations. Since N uptake by plants increases linearly with increased soil N availability under most natural conditions (Ullrich 1992; Evans 2001), the high soil inorganic N concentrations likely

caused high foliar N concentrations in the first PFY. Further indications for increased nutrient uptake by post-fire vegetation are the vigorous regrowth of understory vegetation as documented by larger leaves (DeByle et al. 1989), and/or individuals (Morgan and Neuenschwander 1988), or increased standing crop (Harris and Covington 1983). Similar observations with respect to foliar N concentrations and/or understory biomass have been made after forest fertilization with inorganic N (Tamm 1990; Riegel et al. 1991 (elk sedge); Prescott et al. 1993 (common snowberry); Vanderschaaf 1999). Soil inorganic N concentrations are not necessarily a good indicator of N availability to plants as it is rather a consequence of microbial and plant N uptake. Therefore, despite decreased soil inorganic N concentrations in burned watersheds in PFY 2 and 3, potentially sustained high post-fire N availability (MacKenzie et al. 2006) to plants, might have been reflected in higher plant biomass rather than increased foliar N concentration in PFY 2 and 3.

Resprouting plants will initially also have a high root:leaf ratio that might explain the higher increases in foliar N concentration post-fire than observed by fertilization alone (Chapin and Van Cleve 1981). However, regrowing shrub foliage after clipping shoots in spring in unburned sites did not result in higher foliar N concentrations in summer (K. Stephan Unpublished Data). Therefore, an initially increased root:leaf ratio post-fire is unlikely to contribute significantly to increased post-fire foliar N concentrations. In addition to higher post-fire availability of nutrients, the higher light and water availability in the absence of overstory also will stimulate understory response (Nabuurs 1996; Riegel et al. 1995). Higher foliar N concentrations post-fire have also been attributed to tissue age (Christensen 1977) and a reduction in fiber (structural C) content (Chapin and Van Cleve 1981). Tissue age might be a factor with our evergreen sedges, but not with deciduous shrub foliage. If a reduction in structural C content rather than an increase in N per unit dry mass had caused the increased foliar N concentration, the increase in percent N should balance the decrease in percent C. However, C concentrations in leaves did not differ between burned and unburned sites in PFY1 (data not shown).

In sum, post-fire N retention in understory plants is reflected in initially higher N concentrations and possibly a subsequently higher post-fire understory plant biomass. The magnitude of post-fire N retention by understory plants likely depends on the depth of lethal soil heating. If deep soil heating during the fire had killed all belowground meristems for vegetative regeneration and local seed sources, post-fire N leaching losses from the terrestrial part of the ecosystem might have been higher than in this study, as recovery of vegetation would have been slower following recolonization from off-site seeds.

The parallels between the response of in-stream moss and terrestrial upland vegetation are intriguing. Moss N concentrations were increased in burned relative to unburned watersheds during

the entire study period- the same duration as increased streamwater  $\text{NO}_3^-$  concentrations. The importance of in-stream N processing has been highlighted by the Lotic Intersite Nitrogen eXperiment (LINX) (e.g., Mulholland et al. 2000; Peterson et al. 2001). Using tracer- $^{15}\text{NH}_4^+$  and  $^{15}\text{NO}_3^-$  additions, important sinks for  $\text{NH}_4^+$  and  $\text{NO}_3^-$  have been identified and uptake and release rates have been quantified. In Mack Creek, a third order stream in the Oregon Cascades and the closest LINX site to our study area, Ashkenas et al. (2004) showed that aquatic bryophytes and biofilm on large wood were the largest sinks for the  $^{15}\text{NH}_4^+$  tracer signal. At the same site, D. J. Sobota, S. L. Johnson, L. R. Ashkenas et al. (Unpublished Manuscript) identified allochthonous organic matter as having the highest biomass-specific uptake of the  $^{15}\text{NO}_3^-$ -tracer. However bryophytes also retained 10 to 50 % of the added  $^{15}\text{NO}_3^-$  (D. J. Sobota Personal Communication 2007). Thus, it is likely that the higher moss N concentrations in our study resulted from the increased uptake of abundant streamwater  $\text{NO}_3^-$ . This is supported by a companion study (K. Stephan, K. L. Kavanagh, and A. Koyama Unpublished Manuscript c) where we traced a fire-induced isotopic signal in soil to the in-stream moss. Furthermore,  $\text{NO}_3^-$  uptake by moss would likely have been stimulated by increased light availability and streamwater temperatures (Mulholland et al. 2006) post-fire. In early spring, coinciding with high streamwater  $\text{NO}_3^-$  concentrations, shading by dense obligate deciduous riparian plants is relatively small even in unburned watersheds, so that increased  $\text{NO}_3^-$  uptake by moss in streams of burned watersheds likely reflected increased post-fire  $\text{NO}_3^-$  rather than increased light availability. After leaf emergence, however, continued higher light availability to streams of burned watersheds might have contributed to higher moss  $\text{NO}_3^-$  concentrations. Therefore, increased light availability to streams post-fire would represent a positive feedback mechanism enhancing moss  $\text{NO}_3^-$  uptake. Whether increased streamwater  $\text{NO}_3^-$  concentrations or increased light availability is the primary cause of increased moss N concentrations in burned watersheds, moss, like terrestrial vegetation, represents at least a temporary retention mechanism for post-fire streamwater N. Other aquatic uptake components likely do so as well (Bernhardt et al. 2003) even though we did not detect a response in biofilm. This might have been due to the relatively small sample size for such a heterogeneous material.

Unlike, e.g., Hauer and Spencer (1998) or Williams and Melack (1997), we did not detect increased streamwater  $\text{NH}_4^+$  levels post-fire. We cannot exclude the possibility that post-fire  $\text{NH}_4^+$  leaching into streams was not detected due to rapid uptake by streamwater biota.

Foliage of obligate riparian plants did not have higher N concentrations in burned relative to unburned watersheds. This might be explained by a lower fire severity due to the presumably moister soil adjacent to the streams. Additionally, a possible burn effect might have been obscured by riparian plots of two sites being directly burned, but at the other two sites plots were not directly burned but

they were adjacent to burned slopes. The lack of post-fire foliar increases in N concentration was nevertheless surprising given the large  $\text{NO}_3^-$  pulses entering the streams in all sites. Either the riparian fringe was bypassed hydrologically (Cirimo and McDonnell 1997) or the obligate riparian plants did take up post-fire generated N but this was not reflected by their foliar N concentrations. Evidence for the latter is provided in a companion study using stable  $^{15}\text{N}$  isotopes (K. Stephan, K. L. Kavanagh, and A. Koyama Unpublished Manuscript c). The relatively high N availability in the moist soil adjacent to streams in general (Garten 1993) enables the presence of species with higher N demand as reflected by generally high foliar N concentrations ( $\approx 2.5\%$  compared to  $\approx 1.6\%$  of upland species) in unburned areas. Therefore, obligate riparian plants may not have responded to additional post-fire N, or alternatively, higher post-fire N availability might have been immediately translated into higher leaf biomass. The role of post-fire N retention by obligate riparian vegetation of first-order watersheds deserves further study.

Despite the potentially important roles of terrestrial and in-stream N retention, dissolved N export from streams of burned watersheds exceeded that of unburned watersheds. Even though our N export estimates are very crude (i.e., few actual N concentration measurements, discharge estimated from long-term averages of precipitation and evapotranspiration) values for unburned watersheds matched those reported from an old-growth Douglas-fir forest in Oregon (Vanderbilt et al. 2003). In burned watersheds, it appears that post-fire hydrologic losses ( $< 10 \text{ kg N ha}^{-1} \text{ y}^{-1}$ ) are orders of magnitude smaller than N loss during combustion (range  $10\text{-}1000 \text{ kg N ha}^{-1}$ ; Fisher and Binkley 2000). Assuming that total streamwater  $\text{NO}_3^-$  export equals four times the level estimated in 2005 (Figure 4) (and thus mimicking the total of five years with concentrations tapering off per Hauer and Spencer 1998), inputs due to atmospheric N deposition of  $1.4 \text{ kg ha}^{-1} \text{ y}^{-1}$  (wet deposition) (NADP 2006), and N inputs via free living N-fixers of  $1.2 \text{ kg ha}^{-1} \text{ y}^{-1}$  post-fire (Wei and Kimmins 1998), we can calculate the time it would take to replace the N lost via streamwater. The increased hydrologic losses caused by fire (beyond 'background' losses of  $0.7 \text{ kg ha}^{-1} \text{ y}^{-1}$  in unburned watersheds) would be replaced in 11 y at the South Fork site, in 4 y at the Canyon Creek site, and in 1.5 y at the Hall site. At Danskin Creek, post-fire hydrologic N export did not differ from that of unburned streams (Figure 4). These estimates are only rough, as there are errors associated with estimates of N export and N inputs; furthermore dry deposition and symbiotic N fixation were not considered due to the lack of data. Symbiotic N fixation might be of local importance due to the occurrence of snowbrush ceanothus (*Ceanothus velutinus*). Depending on the amount of aboveground biomass burned, the replacement of N lost during combustion might require much longer (decades to centuries) than the replacement of hydrologic N losses. However, much of the N combusted would not have been readily available for biotic uptake while the loss of  $\text{NO}_3^-$  in streamwater represents loss from the watershed of

a readily available labile N-form.

### **Fire Severity**

Given the importance of a fast and broad-scale assessment of the impact of wildfires on vegetation and soil, remote sensing indices have become a commonly used tool for scientists and managers. One criticism of indices of surface reflectance is that their ecological significance is unclear (Lentile et al. 2006). Our results demonstrated that dNBR was correlated with streamwater  $\text{NO}_3^-$  concentrations, an integrator of watershed N cycling, thus confirming the potential ecological relevance of this index. The relationship between dNBR and stream water  $\text{NO}_3^-$  concentrations was strongest when using peak  $\text{NO}_3^-$  concentrations in the spring. However, on the dates when correlations were not statistically significant, streamwater  $\text{NO}_3^-$  concentrations at burned watersheds of the South Fork site were especially high, even though the dNBR values were highest at the Canyon Creek site. This pattern was also apparent in total  $\text{NO}_3^-$  export (Figure 5). This apparent disagreement exemplifies that the relationship between dNBR and streamwater  $\text{NO}_3^-$  concentration deserves further study. Potentially important factors influencing this relationship might be soil type (McColl and Grigal 1977) and the spatial configuration of the burned areas within watersheds. Nevertheless, the approach is promising and useful. Linking dNBR and watershed  $\text{NO}_3^-$  loss could provide a first approximation of post-fire N loss from a large number of watersheds or from remote areas when streamwater sampling is not feasible.

### **CONCLUSIONS**

Wildfires strongly influenced N dynamics both locally and across the entire watershed throughout the study period. Local effects were highlighted by the interactions of soils and plants, whereas watershed-scale effects were reflected in  $\text{NO}_3^-$  export via streams. Despite the temporal disconnect between major N loss during snowmelt and the onset of the growing season, terrestrial plants nevertheless provided an important N retention mechanism for post-fire available soil N. Moss represented the analogous retention mechanism in streams. Given the potential importance of aquatic N retention, streamwater biochemistry is an integrator of both terrestrial and aquatic processes. Whether and to what degree streams dampen or enhance the terrestrial signal is yet unknown.

Excessive N losses from N-limited systems are undesirable. Since N loss via combustion, and secondarily via streamwater N export, are a function of area burned and degree of organic matter consumption, the best strategy for avoiding detrimental effects of fire for watershed N cycling is

maintaining the ability of these systems to retain nutrients. Post-fire management for N retention (i.e., grass seeding) may be unnecessary as resprouting terrestrial plants and potentially in-stream biota are able to respond to pulsed, short-term high N availability following disturbances.

In sum, the simultaneous study of many components of watershed ecosystems elucidated the importance of complex interactions between biotic, abiotic, and hydrological factors influencing post-fire N retention and loss. Due to replication across independent wildfires, the patterns and processes described in this study apply to other mid-elevation headwater watersheds of central Idaho Rocky Mountain conifer forests. Further studies from fires at higher elevations, watersheds with higher proportions burned, and/or higher severity burns are needed to assess whether our findings are more widely applicable.

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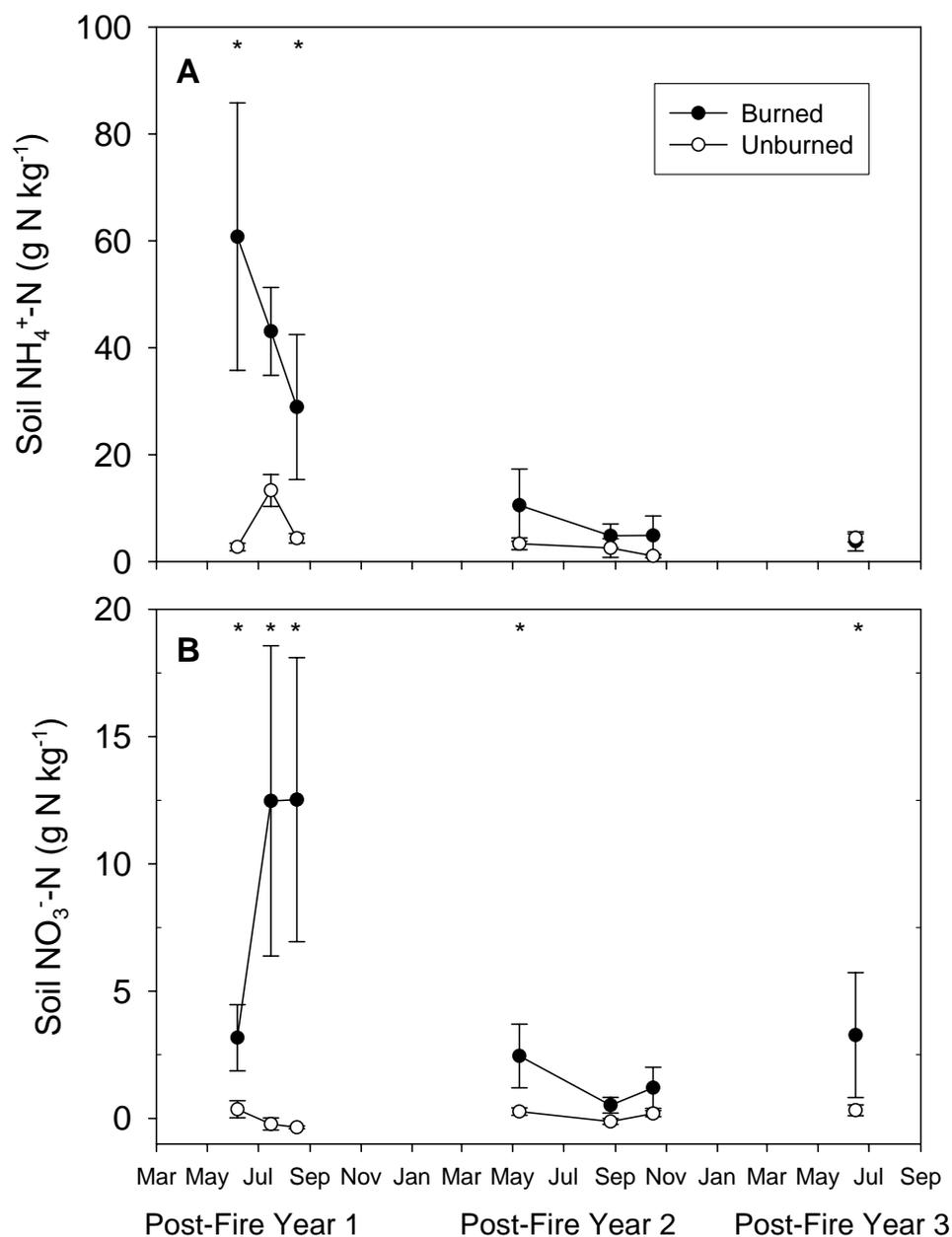
**Table 1.** Foliar N concentrations (%) in burned (B) and unburned (U) watersheds for each species in each post-fire year (PFY). One SE is given in parentheses (n = 4 sites). An asterisk or circle indicates a statistically significant treatment effect (\* P ≤ 0.05, ° P ≤ 0.10) within a given species and PFY. Superscript letters indicate P < 0.05 for comparison between PFYs of burned watersheds for each upland species. There was no treatment effect in obligate riparian species. Values from unburned watersheds did not differ between years in most cases, except for *G. triflorum* and *Rubus/Ribes* (PFY 1 differed from PFY 2 and 3).

|                                  | PFY 1     |                         | PFY 2      |                         | PFY 3      |                        |
|----------------------------------|-----------|-------------------------|------------|-------------------------|------------|------------------------|
|                                  | U         | B                       | U          | B                       | U          | B                      |
| <b>Upland Species</b>            |           |                         |            |                         |            |                        |
| <i>Carex spp.</i>                | 1.2 (0.1) | 2.0 (0.3)* <sup>a</sup> | 1.3 (0.1)  | 1.6 (0.1) <sup>b</sup>  | 1.2 (0.1)  | 1.1(0.1) <sup>c</sup>  |
| <i>P. malvaceus</i>              | 1.7 (0.1) | 2.5 (0.2)* <sup>a</sup> | 2.1 (0.2)  | 2.0 (0.1) <sup>ab</sup> | 1.9 (0.5)  | 1.4 (0.2) <sup>b</sup> |
| <i>Symphoricarpos spp.</i>       | 1.7 (0.2) | 2.4 (0.3)* <sup>a</sup> | 1.8 (0.3)  | 1.9 (0.2) <sup>b</sup>  | 1.7 (0.3)  | 1.6 (0.2) <sup>b</sup> |
| <i>S. betulifolia</i>            | 1.5 (0.1) | 2.6 (0.3)* <sup>a</sup> | 1.7 (0.1)  | 2.2 (0.2) <sup>ob</sup> | 1.6 (0.2)  | 1.5 (0.1) <sup>c</sup> |
| <b>Obligate Riparian Species</b> |           |                         |            |                         |            |                        |
| <i>G. triflorum</i>              | 3.3 (0.5) | 3.3 (0.6)               | 2.5 (0.2)  | 2.7 (0.4)               | 2.1 (0.1)  | 1.9 (0.3)              |
| <i>C. alpina</i>                 | 2.8 (0.4) | 3.0 (0.2)               | 2.6 (0.3)  | 2.9 (0.3)               | 2.6 (0.04) | 3.1 (0.01)             |
| <i>C. stolonifera</i>            | 2.5 (0.3) | 2.4 (0.3)               | 2.2 (0.2)  | 2.4 (0.2)               | 2.0 (0.2)  | 1.8 (0.1)              |
| <i>Rubus/Ribes</i>               | 3.4 (0.5) | 3.3 (0.4)               | 2.3 (0.04) | 2.7 (0.4)               | 2.1 (0.2)  | 2.1 (0.3)              |

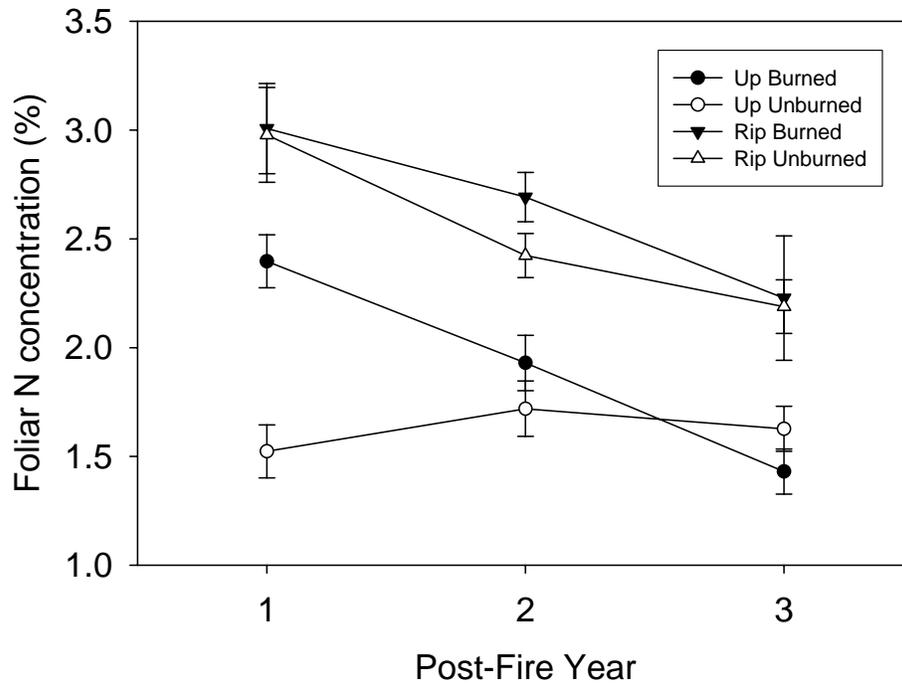
**Table 2.** Proportions of watersheds (%) burned at different severities. B1 denotes the burned watersheds containing the sampling plots, B2 and B3 denote the burned watersheds for additional streamwater sampling. Shifts in the delta Normalized Burn Ratio (dNBR) in unburned watersheds (U) (average across  $n = 4$  sites and one standard deviation in parenthesis) reflect the temporal variability from the pre-burn to the post-burn image.

| dNBR range   | Severity          | U       | Canyon Cr. |    | South Fork |    | Hall |    | Danskin Cr. |    |    |
|--------------|-------------------|---------|------------|----|------------|----|------|----|-------------|----|----|
|              |                   |         | B1         | B2 | B1         | B2 | B1   | B2 | B1          | B2 | B3 |
| -500 - -101  | enhanced regrowth | 26 (19) | 1          | 1  | 2          | 2  | 30   | 21 | 2           | 1  | 1  |
| -100 - +99   | no burn           | 55 (13) | 4          | 5  | 20         | 17 | 44   | 28 | 26          | 33 | 21 |
| +100 - +269  | low               | 15 (7)  | 11         | 12 | 30         | 30 | 14   | 22 | 36          | 44 | 38 |
| +270 - +439  | low-moderate      | 3 (2)   | 15         | 26 | 29         | 26 | 6    | 11 | 23          | 19 | 22 |
| +440 - +659  | moderate-high     | 1 (1)   | 20         | 29 | 15         | 18 | 3    | 10 | 11          | 4  | 13 |
| +660 - +1300 | high              | 0 (0)   | 49         | 27 | 4          | 7  | 3    | 9  | 2           | 0  | 4  |

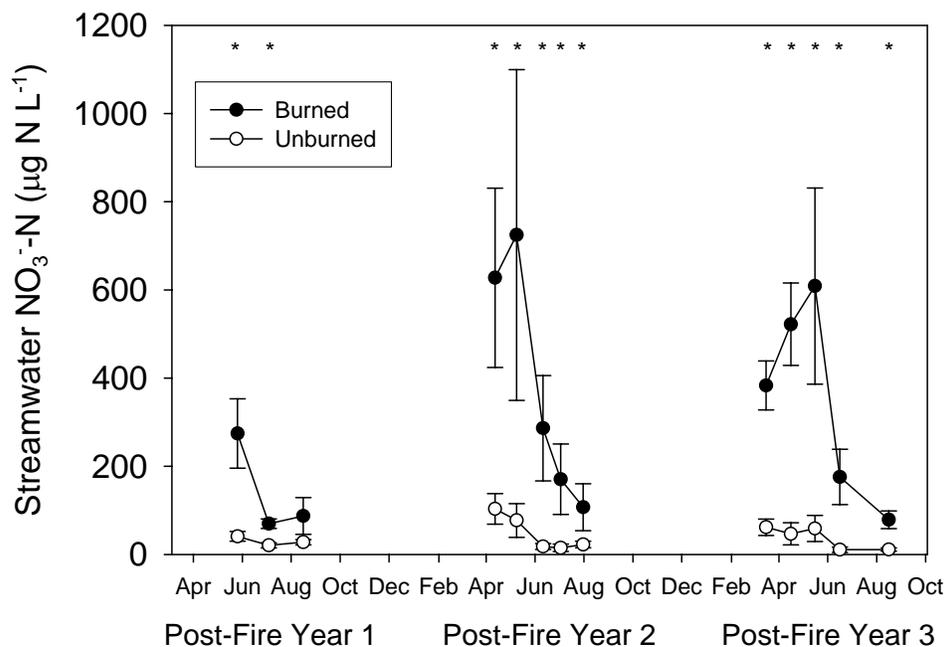
**Figure 1.** Soil  $\text{NH}_4^+$  (a) and  $\text{NO}_3^-$  (b) concentrations in burned (B) and unburned (U) watersheds in three post-fire years. Symbols above sample dates indicate statistically significant differences between treatments after pairwise comparisons (\*  $P \leq 0.05$ ; °  $P \leq 0.1$ ). Note that  $\text{NO}_3^-$  concentrations from unburned watersheds were below detection limit (negative values) at some dates. Error bars (1 SE) represent the variability between sites.



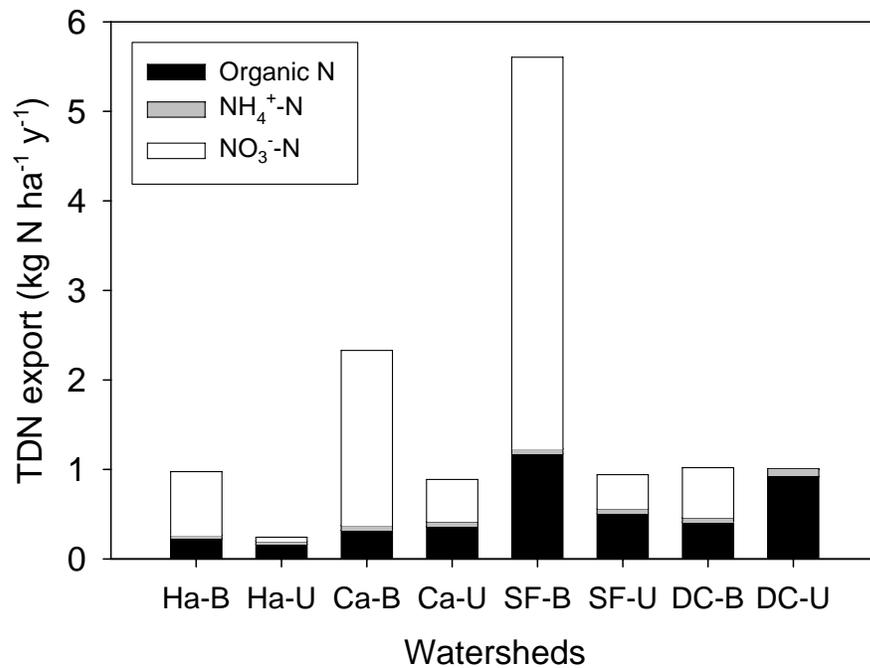
**Figure 2.** Foliar N concentrations across species ( $n = 4$ ) in upland (Up) and riparian (Rip) plots of burned (B) and unburned (U) watersheds in three post-fire years. Error bars (1 SE) represent the variability between species.



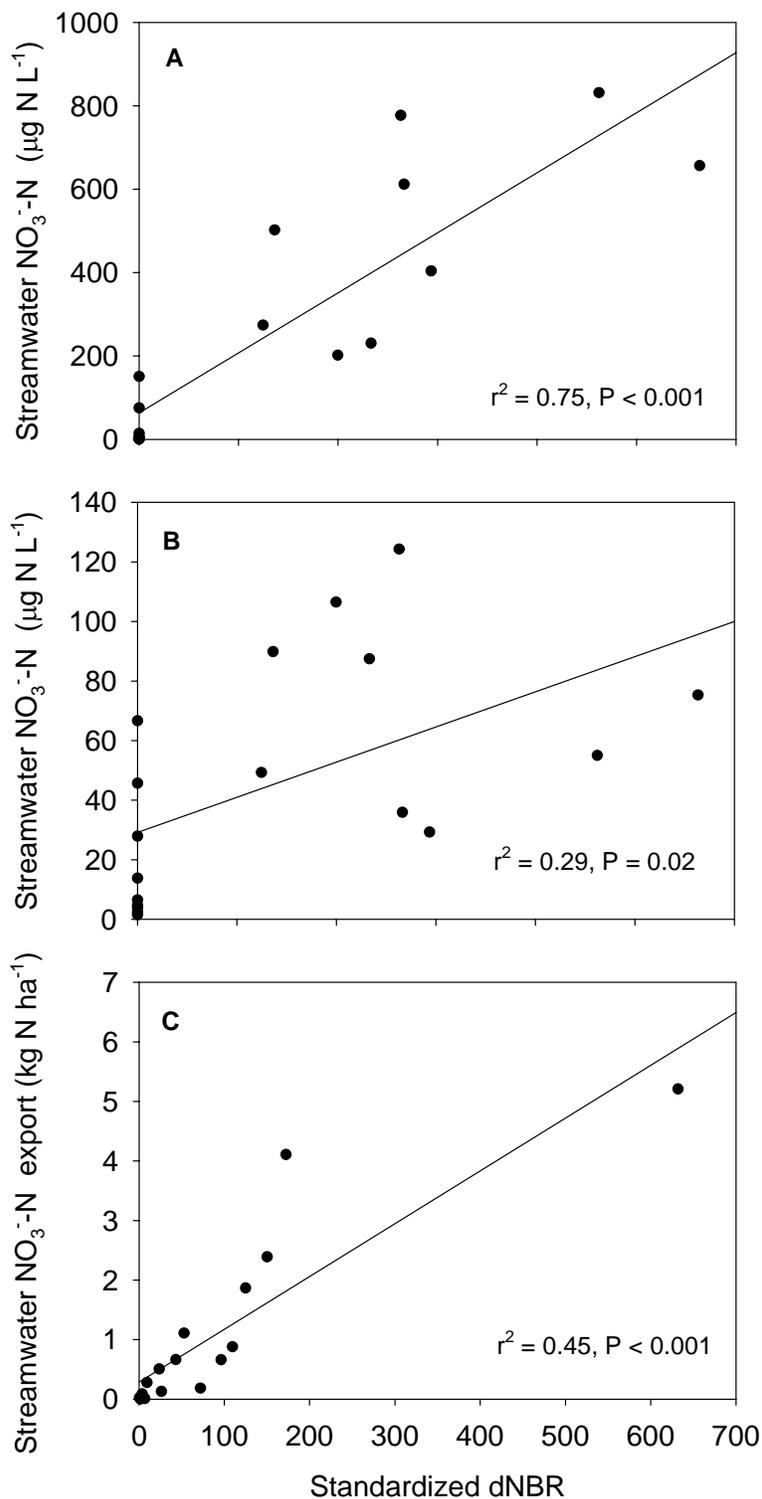
**Figure 3.** Streamwater  $\text{NO}_3^-$  concentrations in burned (B) and unburned (U) sites. Symbols above sample dates indicate statistically significant differences between treatments after pairwise comparisons (\*  $P \leq 0.05$ , °  $P \leq 0.1$ ). Error bars (1 SE) represent the variability between sites. Data points shown do not include Danskin Creek because data was not available for six of the sample dates.



**Figure 4.** Total dissolved N export from watersheds via streams in 2005, the second post-fire year for all sites except Danskin Creek (third post-fire year). Each column represents the average for the two (three at Danskin Creek) watersheds per site and treatment (B, burned; U, unburned). Ha, Hall; Ca, Canyon Creek; SF, South Fork; DC, Danskin Creek.



**Figure 5.** Correlation of the delta Normalized Burn Ratio (dNBR) and streamwater  $\text{NO}_3^-$  concentrations on two sample dates, a) April 2006 and b) July 2004, and c) N export in 2005, respectively. Delta NBR of each burned watershed was standardized by subtracting the average dNBR of the two unburned watersheds from the same site.



## CHAPTER 3

### EFFECTS OF SPRING PRESCRIBED BURNING ON WATERSHED NITROGEN DYNAMICS OF CENTRAL IDAHO HEADWATER AREAS

#### ABSTRACT

Spring prescribed burning is a common tool for fuel reduction. But how do spring prescribed burns affect watershed-scale nitrogen (N) cycling? We simultaneously measured N concentrations in soil, understory plant foliage, stream water, and in-stream biota in headwater watersheds of replicated low-severity spring prescribed burns and one more severe spring test burn for two post-fire years. We also compared the results of this study to the results of our companion study on mixed-severity wildfires. We found that soil ammonium ( $\text{NH}_4^+$ ) and nitrate ( $\text{NO}_3^-$ ) concentrations were significantly increased in burned relative to unburned watersheds about one month and three to four months, respectively, after spring prescribed burning ( $P < 0.05$ ). The magnitude of increase was lower than that observed at the same time but nine to twelve months after wildfire. Interestingly, the magnitude of the post-fire increase in soil  $\text{NH}_4^+$  and  $\text{NO}_3^-$  was similar between the spring test burn site and wildfires ( $P > 0.05$ ) indicating a correlation between fire severity and soil inorganic N concentrations post-fire. Plants resprouting after spring burns retained post-fire available soil N, as indicated by higher foliar N concentrations, in a similar way as observed after wildfires. In stark contrast to wildfires,  $\text{NO}_3^-$  concentrations in streamwater were not increased after spring prescribed burning, indicating complete retention in the terrestrial ecosystem component. Thus, spring prescribed burns did not provide the stream ecosystem with potentially important nutrient pulses. The localized and short-term effects on terrestrial N dynamics after low-severity spring prescribed burns indicate that managers should target higher fire severities at both the plot and watershed scale in order to stimulate N cycling and to reduce fuel loads more substantially.

## INTRODUCTION

Fire, a common natural disturbance in many biomes, is known to have profound effects on nitrogen (N) dynamics in both terrestrial and aquatic ecosystems. It can result in a considerable net N loss to the atmosphere through combustion (Grier 1975; Murphy et al. 2006), but short-term increases in plant available N in the soil are also commonly found (Wan et al. 2001). These, in turn, can give rise to increased N content in understory plants (Harris and Covington 1983; MacLean and Wein 1977a) but also to further losses via leaching of N from the terrestrial component of watersheds (Murphy et al. 2006; Williams and Melack 1997; Chorover et al. 1994). Given that N is limiting forest productivity in the western United States (Moore et al. 1991), understanding short and long-term effects of fire on N dynamics is critical for sustainable management.

The occurrence of larger and more severe fires in some ponderosa pine and mixed-conifer forests over the last two decades can, to some extent, be attributed to fuel load accumulations after almost a century of fire suppression (Agee 1993; Westerling et al. 2006). Prescribed burning is commonly used by managers to reduce down woody material, and/or ladder fuels, while retaining most of the overstory. Prescribed burns often are carried out in spring, when temperatures are lower, humidity is higher, and fuels are moister than during the typical summer wildfire season in the Pacific Northwest. As a consequence of burning objectives and conditions, spring prescribed burns generally have a smaller effect on the ecosystem, i.e., are less severe (Ryan and Noste 1985) than wildfires. Lower overstory mortality and less forest floor consumption and soil heating (DeBano et al. 1998) with prescribed burning might result in changes of post-fire N dynamics that differ from those observed after wildfires. In addition to differing severities of spring prescribed burns and wildfires, the season of burning might also affect post-fire N cycling.

While studies of post-fire effects on N cycling have been conducted previously, they generally have been carried out in single sites and were confined to only one or two components of either the terrestrial or aquatic part of the ecosystem (e.g., soil: Covington and Sackett 1992; Chorover et al. 1994; plants: MacLean and Wein 1977b; Harris and Covington 1983; streamwater chemistry: Bayley and Schindler 1991; Hauer and Spencer 1998; soil and plants: Grogan et al. 2000; Christensen 1977; soil and streamwater: McGoll and Grigal 1977; Richter et al. 1982). This single site and single component approach limits understanding of N dynamics at the watershed scale (Grimm et al. 2003) making generalizations across larger geographic areas difficult due to the lack of replicated observations. Managers, however, make decisions at watershed and regional

scales.

The objective of this study was to characterize the short-term effects of replicated spring prescribed burns on N concentration in several key ecosystem components of headwater watersheds. We simultaneously measured N concentrations in soil, foliage of obligate riparian and upland understory plants, stream water, and in-stream biota for two growing seasons post-fire. We compare the results of this study to the results of the companion study on wildfires (K. Stephan, K. L. Kavanagh, and A. Koyama Unpublished Manuscript a) carried out at the same time and in the same geographic area as the prescribed burns. Based on our findings we discuss the implications for N retention and ecosystem productivity, and provide management recommendations for spring prescribed burning.

## METHODS

### Study Sites

Three spring prescribed burn sites (Danksin Creek, Sixbit, Parks-Eiguren) are located on the Boise and Payette National Forests in the Salmon River Mountains of central Idaho, USA (44°5' - 44°57'N, 115°12'-116°21'W). The regional climate is characterized by warm dry summers and cool, moist winters. Depending on site elevation (1400-2000 m), mean annual air temperature ranges from 2.3 to 4.3 °C and mean annual precipitation, falling mainly as snow, ranges from 680 to 950 mm. The geology underlying the study area is Idaho Batholith Granitics (Danksin Creek, Sixbit, Parks-Eiguren sites). Watershed areas are small (mean 60 ha, range 10-220 ha) and drained by either intermittent or perennial first-order streams that have a southerly or westerly aspect. Stream channels are confined by relatively steep hill slopes (15-41°) and fringed by only a narrow strip ( $\leq 1$  m width) of obligate riparian shrubs and forbs. The overstory is characterized by mature Douglas-fir (*Pseudotsuga menziesii*) and ponderosa pine (*Pinus ponderosa*).

The prescribed burns were carried out in April and May of 2004 by the US Forest Service. In the watersheds chosen for our study, we used the remote sensing index Delta Normalized Burn Ratio (dNBR) (Key and Benson 2006; Lentile et al. 2006) as an indicator of the magnitude of ecological change (also referred to as fire severity) (Ryan and Noste 1985). Delta NBR in burned watersheds was very similar to those of unburned watersheds indicating a lack of overstory mortality. On the ground, there were relatively small patches (5 to 100 m<sup>2</sup>) of charred or consumed understory and forest floor (Figure 1). Such burned patches also occurred immediately next to the streams. Based on ocular estimates, less than one third of the total watershed area was

burned in each site with some variation between sites.

We included data from one additional site (Squaw Creek) from within the region, located in the West Mountains (on Columbia River Basalt) on the Boise National Forest. Squaw Creek was a small test burn (1 ha) prior to an intended 220 ha prescribed burn (Mill Creek project) in May 2004. In contrast to the three other sites, it displayed crown scorching and the forest floor was consumed more completely resulting in the cancellation of the prescribed burn.

### **Sampling Design**

At each site, samples of soil, upland and obligate riparian plant foliage, and aquatic biota (moss, biofilm) were collected from each of several plots within the riparian area of one burned watershed and one nearby similar unburned watershed outside the fire perimeter. Riparian area was broadly defined as the slope distance of one site-potential tree height of ca. 35 m (Boise National Forest 2003; Payette National Forest 2003; Naiman and Decamps 1997). Streamwater was collected from the mouths of the streams draining these watersheds and from one additional burned and unburned watershed per site. For collection of soil and upland plant foliage, four upland plots were established in the lower portion of each watershed. Plots were located at least 100 m upstream from an access road, crossing the stream near its mouth. Two plots were placed on each aspect, ca. 5 to 25 m upslope from the stream bank, and with the two plots per aspect being ca 100 m apart. Upland plots had about a 10 m radius. Plots in burned watersheds were placed in well burned areas. Obligate riparian plant foliage and aquatic biota samples were collected from two plots, each corresponding to a stream reach of ca. 20 m length, per watershed. These riparian/aquatic plots were downslope from the upland plots, at the intersections of upland plots from opposing aspects. Samples were collected in the two growing seasons (2004 and 2005) following the spring burns.

Because of the small proportion of watershed area burned (ca 2.5 %) by the test burn in the Squaw Creek site, unburned reference plots were established ca. 100 m downstream from the test-burn area. Obligate riparian, streamwater and aquatic samples from this site were not collected, the former because the species collected from the other sites were not present and the latter because the intermittent stream dried up soon after the burn.

### **Soil**

Mineral soil samples were collected in June, July and August of 2004, and in May, August and October of 2005. One sample per plot was taken; it comprised several cores (five cores with 1.9 cm diameter or four cores with 5 cm diameter) taken from 0 to 10 cm depth. The soil samples

were transported to the laboratory on ice and passed through a 4-mm sieve. Fresh soil was extracted with 2 M KCl while shaking for 1 h. Soil to extractant ratio was 1 : 2.7 or 1 : 2 because soil inorganic N concentrations were very low. Soil extracts were filtered through Whatman No. 42 filters and extracts were stored frozen till analysis. Gravimetric soil moisture was determined from a subsample of sieved fresh soil by assessing water loss after drying for 48 h at 105 °C. On two sample dates (July and August 2004) soils were extracted in the field. Fresh soil was added to specimen cups with KCl of known weight. In the laboratory, specimen cups with soil were re-weighed to obtain the amount of fresh soil added. After filtering the extract as described above, remaining soil slurry was passed through a 4-mm sieve. The dry weight of material larger than 4 mm was subtracted from the fresh soil weight in the cup in order to correctly calculate N concentration for soil < 4 mm. Gravimetric soil moisture was determined as above.

Soil extracts were analyzed for inorganic N concentrations by continuous flow colorimetry in the Department of Plant, Soil and Entomological Sciences, University of Idaho, Moscow (Lachat QuikChem AE Automated Ion Analyzer [Zellweger Analytics Inc., Milwaukee, Wisconsin, USA], QuikChem® methods 12-107-06-2-A for NH<sub>3</sub> and 12-107-04-1-B for NO<sub>3</sub><sup>-</sup> + NO<sub>2</sub><sup>-</sup>), in the School of Biological Sciences, Washington State University, Pullman (Alpkem Autoanalyzer FS 3000 [OI Analytical, College Station, Texas, USA], methods P/N 002053 for NH<sub>3</sub> and P/N A001559 for NO<sub>3</sub><sup>-</sup> + NO<sub>2</sub><sup>-</sup>), or in the Marine Science Laboratory, University of California Santa Barbara (Lachat QuikChem 8000 [Zellweger Analytics Inc., Milwaukee, Wisconsin, USA], QuikChem® methods 31-107-06-5-A for NH<sub>3</sub> and 31-107-04-1-A for NO<sub>3</sub><sup>-</sup> + NO<sub>2</sub><sup>-</sup>).

### **Understory Vegetation**

Foliage was collected from four common upland species in upland plots and four obligate riparian species in riparian plots in either late July or mid August (except in 2004, riparian foliage was collected in June). Some ‘species’ are actually composites of closely related species or genera when a single species did not occur on all sites. Within a single site, however, the species was consistent between burned and unburned treatments. Upland species are the shrubs birchleaf spiraea (*Spiraea betulifolia*), common or mountain snowberry (*Symphoricarpos albus* or *S. oreophilus*), mallow ninebark (*Physocarpus malvaceus*), and elk or northwestern sedge (*Carex geyeri* or *C. concinnoides*). Obligate riparian species include the shrubs redosier dogwood (*Cornus stolonifera*), bramble (*Rubus spp.*) or current/gooseberry (*Ribes spp.*), and the forbs small enchanter’s nightshade (*Circaea alpina*), and fragrant bedstraw (*Galium triflorum*). Each foliage sample per plot is a composite of one leaf (shrubs and *Carex*) or all leaves (forbs) from three to six different, randomly chosen individuals per species. Foliage was dried at 70 °C for 24

h (2004 obligate riparian foliage) or freeze-dried (2004 upland foliage, all 2005 foliage).

Dried foliage was ground to a fine powder with a ball mill, packed into a tin capsule and analyzed for total N with continuous-flow direct combustion isotope ratio mass spectrometry (IRMS) following combustion in an elemental analyzer. Analyses of upland and obligate riparian foliage were carried out by the laboratory of R. Lee at the School of Biological Sciences at Washington State University (Isoprime [Micromass Ltd. Manchester, UK] coupled to an EuroEA 3000 elemental analyzer [EuroVector S.p.A., Milan, Italy]). Analytical precision for N concentration was < 0.1% (standard deviation) between replicates of actual sample material.

### **Streamwater**

Streamwater was collected in June, July, and August of 2004, in April, June, and July of 2005, and in June of 2006. The initial spring sampling was limited by snowmelt on these remote sites. Water samples were filtered through Gelman A/E glassfiber filters (1  $\mu\text{m}$  pore size) in the field, transported on ice to the laboratory and frozen until analysis.  $\text{NO}_3^-$  and  $\text{NH}_4^+$  concentrations were determined colorimetrically (Lachat Quickchem 8000 [Lachat Instruments, Milwaukee, Wisconsin, USA], method 31-107-06-5-A for  $\text{NH}_3$ , method 31-107-04-1-A for  $\text{NO}_3^- + \text{NO}_2^-$ ) in the Marine Science Laboratory of the University of California, Santa Barbara. Total dissolved nitrogen was analyzed as  $\text{NO}_3^-$ -N by spectrophotometry (Beckman Coulter DU@640 [Beckman Instruments, Inc., Fullerton, California, USA]) following high temperature persulphate digestion (based on Qualls 1989). Dissolved organic nitrogen (DON) was calculated as total dissolved nitrogen minus  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N.

### **Moss and Biofilm**

Moss was collected from streams in May or June. Collected mosses (comprising various species, e.g., *Bryum* and *Schistidium* species) were not strictly aquatic but were submerged during spring run-off. Each moss sample represents a composite of moss collected from three randomly chosen rocks per plot. The moss was rinsed on site to remove as much sediment as possible. In the laboratory, moss was freeze-dried and ca. 1 cm long actively growing tips were picked and finely ground.

Biofilm (epilithon) was collected in June or July by scraping with a wire brush an area of ca. 20  $\text{cm}^2$  from each of three rocks (2004 samples) or from one to two 120- $\text{cm}^2$  clay tiles per plot that had been placed one month prior (2005 samples). The slurry was collected in a plastic bowl and transferred into 50-ml plastic centrifuge tubes. In the laboratory, samples were frozen, freeze dried, and subsequently finely ground. Samples were analyzed for total N as described above at

the University of Idaho Stable Isotope Laboratory (ISIL) (Finnigan Delta Plus [Finnigan MAT, Bremen, Germany] coupled to a Carlo Erba NC 2500 elemental analyzer [CE Instruments, Milan, Italy]). Analytical precision for N concentration was < 0.1% (standard deviation) between replicates of actual sample material. A subsample of material was ashed for 5 h at 500 °C in order to determine ash free dry mass (AFDM).

All sampled materials were stored on ice in a cooler during the collection period and transport to the laboratory. Due to the remoteness of field sites all sample processing in the laboratory commenced one to five days after field collection.

### **Statistical Analysis**

The study design is comparable to a block design (site = block, watershed = plot). Prior to statistical analysis, values of soil, vegetation, and in-stream biota subsamples taken at the two riparian/aquatic or four upland sampling plots (=subplots) within watersheds were averaged; and values of streamwater N concentrations were averaged across the two streams per site × treatment combination.

Data were subjected to analyses of variance (ANOVA) with linear mixed-effect models in SAS (SAS 9.1, SAS Institute Inc., Cary, NC, USA) using the ‘proc mixed’ statement. The objectives of the statistical analysis were to test for the absence of a) a difference between burned and unburned watersheds (i.e., treatment effect), b) an effect of time (sample date) on N concentrations and the interactions of treatment and time, and c) differences in the magnitude of the treatment effect between prescribed burns and wildfires. Mixed-effects models were used because they allowed addressing the often unbalanced data and modeling the correlation structure induced by the nested design (watersheds within sites) and repeated sampling in time.

Model assumptions were checked graphically prior to analysis in R (open-source statistical language, Version 1.9.1, The R Development Core Team 2004) and data were transformed (log or power) if necessary. Analysis results of soil  $\text{NO}_3^-$  concentrations often were negative. Therefore, we added the smallest possible constant (0.9) to all values so that the necessary log-transformation could be performed.

In our mixed-effect models, site was specified as random effect (‘random’ statement). That is, study sites are a random sample from a population of sites or, as with this study, selection of sites was deterministic but the effect of sites on the outcome was of stochastic nature (Schabenberger & Pierce 2002). In consequence, inference drawn from this study is not limited to the very sites studied, but applies to similar spring prescribed burns in mid-elevation headwater watersheds within the central Idaho region. In addition to site, the watersheds nested within each site were

included as random effects. This allowed random interactions between site and treatment, i.e., the magnitude of the burn effect could vary between sites and/or the two watersheds within each site could differ from each other due to, e.g., slight variation in elevation, slope, or (pre-fire) soil characteristics. As a consequence, only those treatment effects that were sufficiently strong in all sites were detected.

Serial correlation between N concentrations of soil and streamwater samples collected through time was assumed and accounted for with repeated measures ('repeated' statement). Adjustments to the random effects structure and serial correlation were made (i.e., one random effect and/or the serial correlation was dropped) if the data structure did not support having all components in the model. Pairwise comparisons for a given sample date were carried out in SAS using the Satterthwaite function to approximate degrees of freedom. In the results, model P-values and/or P-values of pairwise comparisons are presented. Means and standard errors presented in graphs and tables are based on the untransformed raw data.

## RESULTS

### Soil

Soil  $\text{NH}_4^+$  and  $\text{NO}_3^-$  concentrations increased in the growing season immediately following the three prescribed burns (first post-fire year, PFY 1). With  $\text{NH}_4^+$  concentrations, there was an overall significant burn effect ( $P = 0.03$ ), sample date effect ( $P = 0.002$ ), and interaction effect ( $P = 0.006$ ). Pairwise comparisons per sample date showed that soil  $\text{NH}_4^+$  concentrations were higher in burned than unburned watersheds only in May of 2004 ( $P < 0.0001$ ), one to two months after the prescribed burns, but not thereafter (Figure 2). The soil  $\text{NH}_4^+$  concentrations in the burned area of the Squaw Creek test burn site were overall significantly higher than those in the burned watersheds of the three prescribed burn sites ( $P = 0.02$ ) (Figure 2a). With pairwise comparisons for each sample date, these differences were only significant in July of PFY 1 ( $P = 0.06$ ) but the sample size was low ( $n = 1$  for test burn and  $n = 3$  for prescribed burn sites).

$\text{NO}_3^-$  concentrations were mostly below detection limit in unburned watersheds but could be detected in burned watersheds. As with  $\text{NH}_4^+$  concentrations, there was an overall significant treatment effect ( $P = 0.04$ ), sample date effect ( $P = 0.002$ ), and interaction effect ( $P = 0.004$ ) with the three prescribed burns. Pairwise comparisons showed that  $\text{NO}_3^-$  concentrations were significantly higher in July ( $P = 0.0002$ ) and August ( $P < 0.0001$ ) after the prescribed burns, but not immediately after the burn or in PFY 2 (Figure 2b). Soil  $\text{NO}_3^-$  concentrations from within the

Squaw Creek test burn area tended to be overall higher than in the burned watersheds of prescribed burn sites ( $P = 0.07$ ). Pairwise comparisons per sample date showed that concentrations in the test burn were significantly higher than those in the burned watersheds of the three prescribed burn sites in July and August of PFY 1, respectively ( $P < 0.0001$ ) (Figure 2b).

### **Understory Vegetation**

Foliar N concentrations of upslope species were increased in burned relative to unburned watersheds but those of obligate riparian species were not (Table 1, Figure 3a). In upland species, there was a significant treatment  $\times$  PFY interaction ( $P < 0.0001$ ). Thus, N concentrations in burned watersheds were only higher in the same year of the burn (PFY 1), but not in PFY 2. In PFY 1, the magnitude of response differed by species; elksedge foliar N concentrations were on average 0.3 % (absolute value) higher in burned than in unburned watersheds, whereas the absolute difference was 1.0 % for the other three upland species. The absolute differences in foliar N concentrations between burned and unburned watersheds translate into relative differences of 26 % and 56 % for sedge and the other species, respectively. N concentration differences of upland species between the burned and unburned areas at Squaw Creek were not different from those at the three prescribed burns and are therefore included in the values in Table 1. In obligate riparian species, neither treatment, species, sample date, nor any interaction was a significant ( $P > 0.05$ ) predictor of foliar N concentrations (Table 1, Figure 3b).

### **Streamwater**

Streamwater concentrations of  $\text{NO}_3^-$ ,  $\text{NH}_4^+$ , and DON did not differ statistically between burned and unburned watersheds when data from all seven sample dates was analyzed together. Nitrate concentrations averaged across all individual streams and all sample dates were  $12.2 \pm 20.2$  (SD)  $\mu\text{g NO}_3^- \text{-N L}^{-1}$ . Pairwise comparisons of treatments at individual sample dates showed that in April of PFY 2  $\text{NO}_3^-$  concentrations at burned sites ( $30.2 \pm 15.3$  (SE)  $\mu\text{g NO}_3^- \text{-N L}^{-1}$ ) were higher than those of unburned sites ( $4.6 \pm 3.2$   $\mu\text{g NO}_3^- \text{-N L}^{-1}$ ) ( $P = 0.02$  using log-transformed data) (Figure 4). Streamwater  $\text{NH}_4^+$  and DON concentrations averaged across all individual streams and all sample dates were  $11.2 \pm 8.4$  (SD)  $\mu\text{g NH}_4^+ \text{-N L}^{-1}$  and  $106.1 \pm 95.6$  (SD)  $\mu\text{g N L}^{-1}$ , respectively.

### **Moss and Biofilm**

N concentrations of in-stream moss and biofilm did not differ between burned and unburned

watersheds. Moss N concentrations across all watersheds and both years were  $2.0 \pm 0.5$  (SD) % per AFDM. Biofilm N concentrations were  $3.6 \pm 0.8$  (SD) % per AFDM in PFY 1. Biofilm data on N concentrations was not available in PFY 2 (not enough material to analyze for AFDM).

## DISCUSSION

After spring prescribed burns, N concentrations increased for a short time in terrestrial but not in aquatic ecosystem components. Despite the loss of N in forest floor and understory vegetation by combustion (Raison 1979; Johnson et al. 2004), concentrations of inorganic N in the soil increased in the growing season immediately after burning. This highlights the importance of differentiating between N bound in organic matter (i.e., unavailable N) and readily available inorganic N. Increases in soil inorganic N are a common occurrence post-fire (Wan et. al 2001); they are generally interpreted as the net effect of gross microbial N production, microbial N immobilization, and plant N uptake (A. Koyama, K. L. Kavanagh, K. Stephan Unpublished Manuscript). By the second year post-fire, there was no apparent difference on our sites in N concentrations between burned and unburned soils, possibly due to increased microbial and plant N uptake after one year of recovery. It should be noted that post-fire increased N mineralization observed in burned patches, will overestimate post-fire N mineralization for the watershed as a whole since a large proportion of the burned watersheds were actually not impacted by burning.

The readily available inorganic soil N post-burn was sequestered by resprouting upland understory vegetation as indicated by higher N concentrations in foliage (26 % to 66 % relative increase over unburned foliage). Similar increases in N concentrations in understory foliage after have been reported by Harris and Covington (1983) after fall prescribed burning in an Arizona ponderosa pine forest. The lower magnitude in response of the evergreen sedge's foliar N concentration relative to that of the three deciduous upland shrub species post-fire might be explained by species-specific uptake rates (Aerts and Chapin 2000) or by collecting foliage that represented a mix of surviving and resprouting leaves. In the latter case, post-fire available soil N might have been diluted in pre-existing biomass whereas shrub foliage entirely originated from resprouting after the burn. The decrease of foliar N concentrations of upland plants in burned watersheds to levels observed in unburned watersheds by PFY 2 indicates that initially abundant post-fire inorganic N had been diluted in growth. Thus, rapid N uptake by resprouting understory vegetation and the potential for subsequent increased biomass production (Harris and Covington 1983) represent important N retention mechanisms. Furthermore, the burned areas we studied

were surrounded by an unburned matrix. If N had moved downslope it could have been retained by understory plants starting to grow at the time of burning. Additionally, surviving overstory trees within and around burned patches will also sequester post-fire available N. A possible burn effect on foliar N concentrations in obligate riparian plants might have been obscured because not all of the riparian plots had been burned directly. Alternatively, any post-fire increases in N supply to obligate riparian plants might be difficult to detect due to the relatively high N availability (Garten 1993) even under unburned conditions as reflected by high foliar N concentrations relative to upland species (Table 1).

The retention of post-burn available soil N, particularly the mobile  $\text{NO}_3^-$ , within the terrestrial ecosystem was also reflected in the lack of increased streamwater N concentrations that, in turn, explain the lack of response by in-stream moss and biofilm in prescription-burned watersheds. Given the small proportion of the watersheds burned at low severity, this result was not surprising. The statistically significant difference in streamwater  $\text{NO}_3^-$  concentrations between burned and unburned watersheds in April of PFY 2 was quite small and might not be biologically significant. A similar lack in streamwater chemistry change after a wildfire in May in Minnesota has also been attributed to uptake by vegetation (McColl and Grigal 1977). The overall low concentrations of streamwater  $\text{NO}_3^-$  reflect the pristine nature of these headwater streams (Perakis and Hedin 2002).

We had the unique opportunity to study the effects of wildfire on watershed N dynamics within the same geographic area at the same time using identical sampling protocol (K. Stephan, K. L. Kavanagh, and A. Koyama Unpublished Manuscript a). Wildfire severity in watersheds (i.e., overstory mortality), assessed via dNBR, ranged from low to moderate-high for four wildfires studied. Forest floor and understory vegetation generally was consumed completely in burned areas. Direct comparison of the magnitude of N concentration changes in ecosystem compartments post-fire revealed similarities between spring prescribed burns and the higher-severity wildfires in some respects and differences in others. The magnitude of increase in soil  $\text{NH}_4^+$  concentrations (burned minus unburned) was overall higher in wildfire-burned watersheds than in prescription-burned watersheds ( $P = 0.01$ ). Pairwise comparisons per sample date showed that concentration in prescription-burned watersheds were statistically significantly lower than in wildfire burned watersheds only in July of PFY 1 ( $P = 0.04$ ). Soil  $\text{NH}_4^+$  concentrations at the test burn site did not differ from those of wildfire-burned watersheds ( $P = 0.75$ ) (Figure 2). Pyrolysis (heat decomposition of organic N) might have contributed to the post-burn increases in soil  $\text{NH}_4^+$  concentrations in prescription-burned sites and in the test burn site in May of PFY 1 (Kovacic et al. 1986), whereas increased concentrations later on or in wildfire-burned watersheds were likely

a consequence of mainly biological processes (microbial mineralization versus microbial and plant uptake) (A. Koyama, K. L. Kavanagh, and K. Stephan Unpublished Manuscript). Little is known about the contribution of physicochemical versus biological processes to increased soil  $\text{NH}_4^+$  concentrations immediately post-fire.

Similarly to  $\text{NH}_4^+$  concentrations post-fire, the magnitude of increase in soil  $\text{NO}_3^-$  concentrations in prescription-burned watersheds was overall significantly lower than that in wildfire burned sites ( $P < 0.04$ ). Pairwise comparisons per sample date showed that concentration in prescription-burned watersheds were lower than in wildfire-burned watersheds in PFY 1 (in May  $P = 0.06$ , July  $P < 0.0001$ , August  $P = 0.001$ ) and May of PFY 2 ( $P = 0.04$ ). However, the magnitude of post-fire soil  $\text{NO}_3^-$  increase in the test burn site was similar ( $P = 0.68$ ) to those of wildfire sites in PFY 1 (Figure 2b). The different responses after the low-severity spring prescribed burns and wildfires, but the similar responses of the more severe spring test burn and wildfires, indicate that the magnitude of post-fire increases in both soil  $\text{NH}_4^+$  and  $\text{NO}_3^-$  concentrations are a function of severity rather than burning season. This relationship between post-fire soil inorganic N concentrations and severity has also been observed by Gundale et al. (2005), Weston and Attiwill (1990), and Covington and Sackett (1992).

Potential mechanisms explaining higher soil inorganic N concentrations with higher fire severities are higher degrees of a) stimulation of mineralization due to moister and warmer soil (Schoch and Binkley 1986; MacKenzie et al. 2006; Bissett and Parkinson 1980) correlating with degree of vegetation and forest floor removal, b) removal of phenolic compounds that might otherwise diminish net mineralization and nitrification (MacKenzie et al. 2006; Gundale et al. 2005), and c) tree mortality leading to reduced N uptake by trees but also exacerbating microbial C limitation resulting in reduced microbial N immobilization (Stark and Hart 1997; Hart et al. 1994; Bradley et al. 2000; Bradley 2001; A. Koyama, K. L. Kavanagh, K. Stephan Unpublished Manuscript). It should be noted that burning season and fire severity are confounded in our study (with the exception of the test burn). Since plant physiology and phenology (Miller 2000) as well as microbial composition and biomass (Lipson et al. 1999; Schadt et al. 2003) depend on season, we cannot exclude the possibility that, at equal fire severity, season of burning will influence post-fire N cycling. Altered N cycling after spring prescribed burns, relative to summer/fall prescribed burns or wildfires, might become apparent after repeated burning in spring. This aspect of spring prescribed burning clearly deserves further research.

Interestingly, understory plants in burned watersheds of both spring prescribed burn and wildfire sites had very similar foliar N concentrations ( $P = 0.75$ ) in PFY 1 (Figure 4), indicating that soil extractable  $\text{NH}_4^+$  and  $\text{NO}_3^-$  concentrations are not a good indicator for the amount of N

taken up by plants but rather the consequence of plant uptake and net microbial mineralization.

Since spring burning coincided with the onset of budbreak of understory shrubs, and top-killed plants will resprout with some delay, foliage from plants resprouting after prescribed burns was somewhat younger than foliage in unburned watersheds at the time of collection. This might have contributed to higher foliar N concentrations in prescription-burned relative to unburned watersheds since younger foliage has higher foliar N concentrations than older foliage (Christensen 1977). However, an effect of foliage age might have dissipated by the time of foliage collection in August at the end of active growth.

The most drastic difference between spring prescribed burns and wildfires was the effect on streamwater  $\text{NO}_3^-$  concentrations (Figure 4). Streamwater  $\text{NO}_3^-$  concentrations were significantly lower in prescription-burned sites than in wildfire-burned sites on all sample dates except August of PFY 1 and 2. High  $\text{NO}_3^-$  concentrations in streams of watersheds burned by wildfire relative to unburned watersheds were attributed to reduced microbial N immobilization in winter and spring (K. Stephan, K. L. Kavanagh, and A. Koyama Unpublished Manuscript a). Reduced microbial N immobilization in burned soil might be caused by a carbon (C) limitation (Stark and Hart 1997), given relatively small amounts of labile C from forest floor or root exudates initially available in wildfire sites (A. Koyama, K. L. Kavanagh, K. Stephan Unpublished Manuscript) where most vegetation has been killed and forest floor is completely consumed. Low microbial N demand will result in inorganic N accumulation in the soil resulting in a nitrate flush from the soil with subsurface flow during snowmelt (K. Stephan, K. L. Kavanagh, and A. Koyama Unpublished Manuscript a). No nitrate flushing was observed after our spring prescribed burns. This is not surprising given the small proportion of watershed area burned, the incomplete removal of forest floor and surviving overstory in burned patches, all contributing to N retention via non C-limited microbial activity, in early spring, prior to vigorous plant N uptake. Not all of the  $\text{NO}_3^-$  that entered the streams after wildfires was exported from the watersheds via stream flow. We showed in our companion study (K. Stephan, K. L. Kavanagh, and A. Koyama Unpublished Manuscript a) that in-stream moss retained some of the post-wildfire abundant streamwater  $\text{NO}_3^-$  via increased foliar concentrations. This is potentially important for stimulating future aquatic N cycling. No such effect could be observed after spring prescribed burns as apparently none of the post-fire available soil N had entered the stream. Thus, spring prescribed burns did not affect stream N cycling. Similar results have been reported after winter and summer prescribed burning of only one fifth of the watershed area in a loblolly pine forest in South Carolina (Richter et al. 1982). However, prescribed burns can affect streamwater chemistry in a similar way as wildfires (Williams and Melack 1997) if they burn a larger proportion of the watershed and if they burn

more severely.

Even though the objective of prescribed burning is to lower fuel loads rather than emulating wildfire effects on enhancing post-fire N availability, we argue that if watershed-scale N cycling is largely unaffected by spring prescribed burning, then a significant fuel reduction cannot have occurred. Higher severity prescribed burning would be beneficial for N availability, and thus productivity, in the short term and long term, potentially due to the input of charcoal to the soil enhancing N cycling (MacKenzie et al. 2006; Zackrisson et al. 1996), as well as reducing future hazard of uncharacteristic stand-replacing wildfires.

## CONCLUSIONS

Watershed-scale understanding of N cycling can support scientifically sound management of headwater areas. Effects of low-severity spring prescribed burns were localized, and tended to be of shorter duration and/or lower magnitude than wildfire effects. Thus, the degree of above-ground biomass and forest floor consumption by fire will likely determine the short and long-term changes in N cycling processes. Whether mechanism of terrestrial post-fire N cycling are altered proportionally to fire severity or whether changes are non-linear is in need of further study (Hart et al. 2005).

Fire severity will also affect the amount of nutrients entering streams. The low severity spring prescribed burns did not provide the stream ecosystem with potentially important nutrient pulses. Whereas chronic N inputs from atmospheric or other pollution will lead to detrimental effects on water quality and aquatic organisms, short-term inputs of post-fire N delivered during snowmelt may be critical for the productivity of often N-limited aquatic ecosystems while not impairing water quality. Therefore, from a watershed N cycling perspective, and also to achieve a higher fuel load reduction, managers should target higher fire severities over larger proportions of the watersheds than the spring prescribed burns described in this study. This might be accomplished best by fall prescribed burns. Since streamwater chemistry is an integrator of watershed processes, streamwater  $\text{NO}_3^-$  concentrations post-fire may be a useful tool for gauging watershed-scale fire severity. Finally, larger areas treated with prescribed fire will also be necessary to be effective on a landscape scale.

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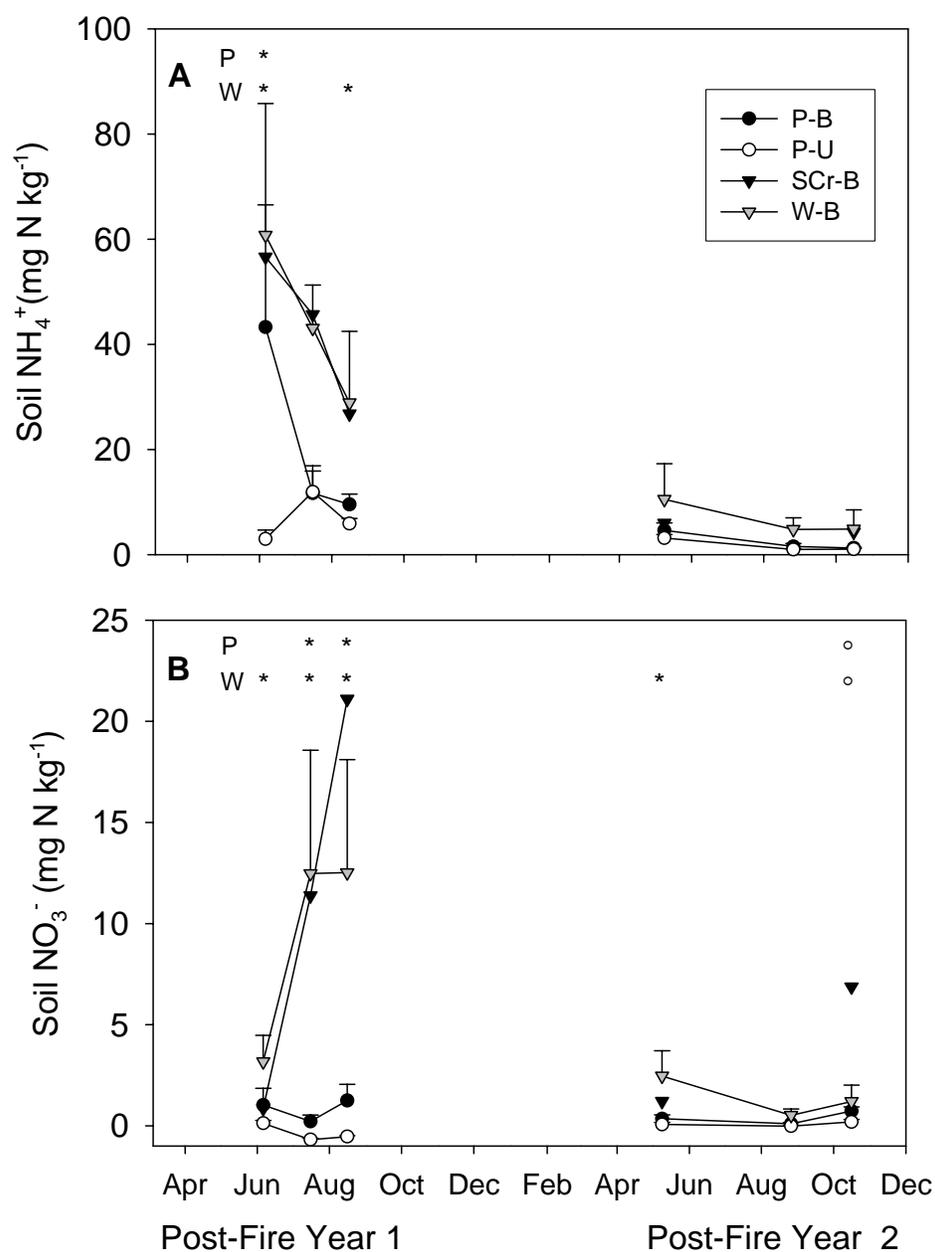
**Table 1.** Foliar N concentration (%) in burned (B) and unburned (U) watersheds of three spring prescribed burn sites and one spring test burn site in the two post-fire years (PFY). One SE is given in parentheses ( $n = 4$  sites for upland species,  $n = 3$  sites for obligate riparian species). An asterisk a statistically significant treatment effect ( $P \leq 0.05$ ) within a given species and PFY.

|                            | PFY 1     |            | PFY 2      |           |
|----------------------------|-----------|------------|------------|-----------|
|                            | U         | B          | U          | B         |
| <b>Upland Species</b>      |           |            |            |           |
| <i>Carex spp.</i>          | 1.2 (0.1) | 1.6 (0.1)* | 1.4 (0.1)  | 1.3 (0.1) |
| <i>P. malvaceus</i>        | 1.9 (0.2) | 2.7 (0.1)* | 2.6 (0.2)  | 2.4 (0.2) |
| <i>Symphoricarpos spp.</i> | 1.4 (0.1) | 2.2 (0.3)* | 1.9 (0.02) | 2.0 (0.3) |
| <i>S. betulifolia</i>      | 1.6 (0.1) | 2.6 (0.3)* | 2.1 (0.1)  | 2.2 (0.3) |
| <b>Riparian Species</b>    |           |            |            |           |
| <i>G. triflorum</i>        | 2.8 (0.1) | 2.9 (0.4)  | 2.4 (0.1)  | 2.5 (0.6) |
| <i>C. alpina</i>           | 2.4 (0.4) | 2.6 (0.6)  | 2.7 (0.2)  | 2.4 (0.5) |
| <i>C. stolonifera</i>      | 2.9 (0.5) | 2.4 (0.6)  | 2.4 (0.2)  | 2.3 (0.3) |
| <i>Rubus/Ribes</i>         | 2.6 (0.3) | 2.6 (0.7)  | 2.8 (0.3)  | 2.6 (0.6) |

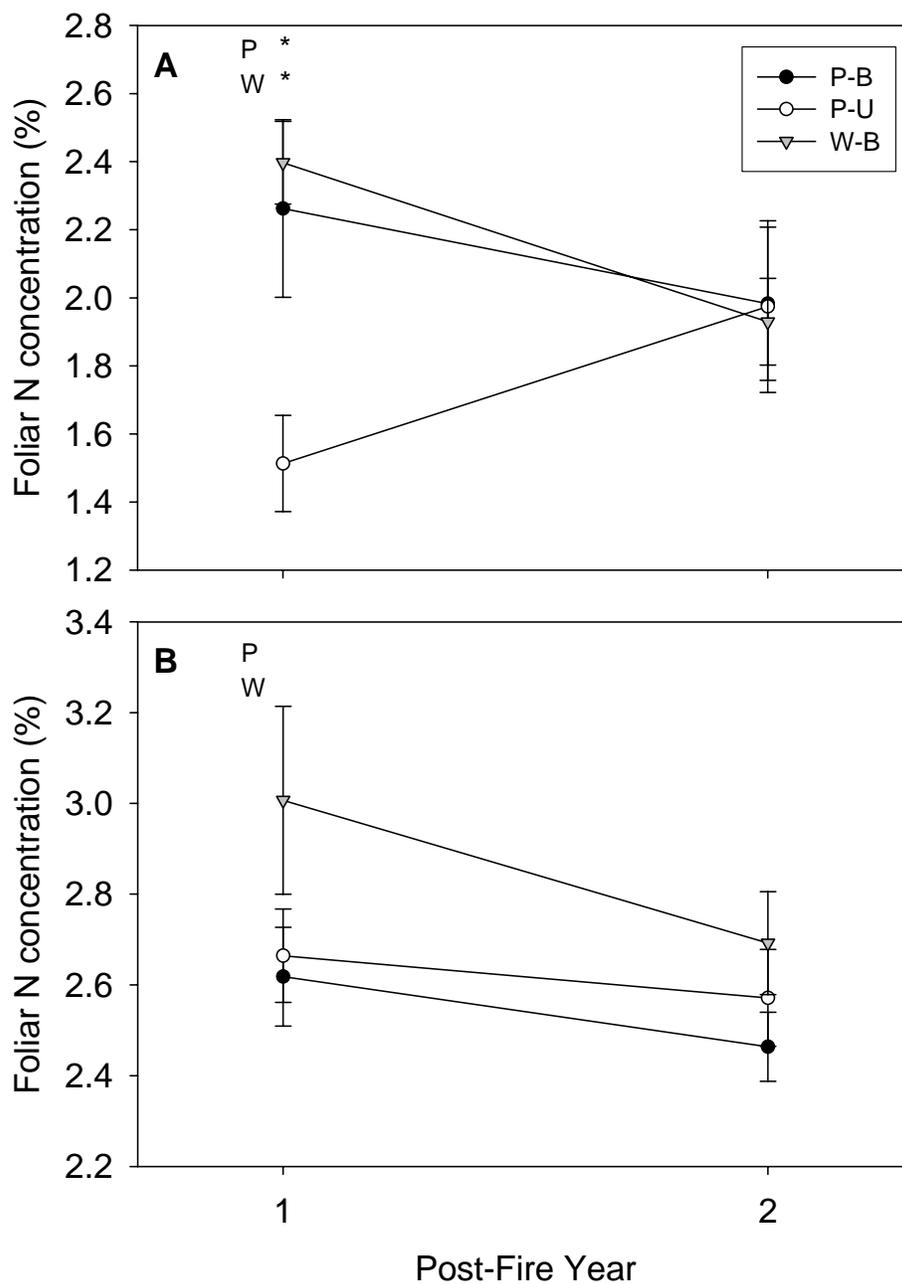
**Figure 1.** Burned watershed of the Sixbit prescribed burn representing the typical severity of the spring prescribed burns in this study.



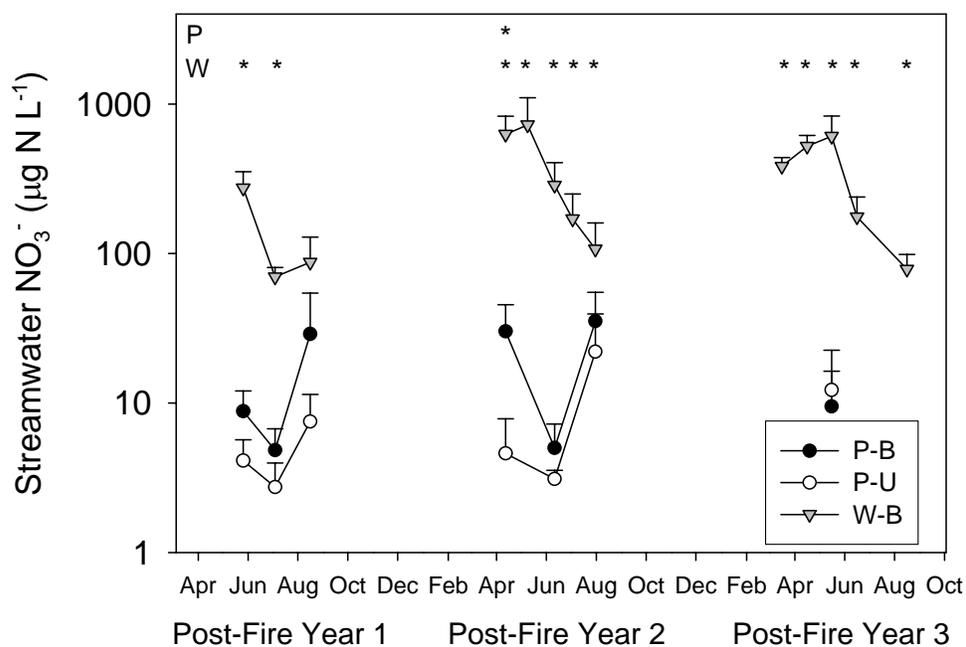
**Figure 2.** Soil a)  $\text{NH}_4^+$  and b)  $\text{NO}_3^-$  concentrations in mineral soil (0-10 cm) in burned (B) and unburned (U) watersheds across replicated spring prescribed burn sites (P), a spring test burn site (SCr), and watersheds burned by wildfire (W-B). Error bars represent 1 SE between watersheds. Wildfire data is from K. Stephan, K. L. Kavanagh, and A. Koyama (Unpublished Manuscript b). Symbols above sample dates indicate statistically significant differences, using pairwise comparisons per sample date, between treatments (B vs. U) for spring prescribed burns and wildfires, respectively (\*  $P \leq 0.05$ ; °  $P \leq 0.1$ ).



**Figure 3.** Foliar N concentrations averaged across all a) upland and b) obligate riparian species in burned and unburned watersheds of spring prescribed burn sites (including one severe spring test burn site) and wildfire-burned watersheds (K. Stephan, K. L. Kavanagh, and A. Koyama Unpublished Manuscript a). See Figure 2 for explanation of legend and symbols above sample dates and Table 1 for species-specific values in spring burn sites.



**Figure 4.** Streamwater  $\text{NO}_3^-$  concentrations in burned and unburned watersheds of spring prescribed burn sites and wildfire-burned watersheds (K. Stephan, K. L. Kavanagh, and A. Koyama Unpublished Manuscript a). Note the logarithmic scale of y-axis. See Figure 2 for explanation of legend and symbols above sample dates.



## CHAPTER 4

### INFLUENCE OF FIRE SEVERITY ON WATERSHED NITROGEN CYCLING USING $^{15}\text{N}$ NATURAL ABUNDANCE IN TERRESTRIAL AND AQUATIC ECOSYSTEM COMPONENTS

#### ABSTRACT

Fire is an integral component of ecosystem nitrogen (N) cycling in coniferous ecosystems of the Rocky Mountains. The objective of this study was to use N stable isotopes at natural abundance to study post-fire N cycling in small watersheds that experienced different fire severities. Within four wildfire, one spring test burn, and three spring prescribed burn sites we quantified and interpreted the N isotopic signatures of soil, plants, streamwater and in-stream moss, and the  $\text{NO}_3^-$  use by plants. We also discuss the role of soil N leaching. We found short-term (1-3 y) post-fire increases of  $\delta^{15}\text{N}$  in these ecosystem N pools to be correlated with fire severity. After wildfires,  $\delta^{15}\text{N}$  significantly increased in all of the studied N pools ( $P < 0.05$ ), whereas after spring burns only plant foliage  $\delta^{15}\text{N}$  significantly increased ( $P < 0.05$ ), although with a smaller magnitude than after wildfire. For example, the  $\delta^{15}\text{N}$  of foliage of upland plants was enriched by 2.9 ‰ (absolute difference between burned and unburned watersheds) in the first two years after wildfire, but only 1.3 ‰ after spring burns. The simultaneous enrichments of both shoots and roots indicated that isotopic enrichment was caused by uptake of enriched soil N. In-stream moss  $\delta^{15}\text{N}$  in wildfire-burned watersheds was increased by 1.3 ‰ relative to unburned watersheds, but there was no response in prescription-burned watersheds. The smaller or lacking isotopic response after spring prescribed burns likely reflected less volatilization of  $^{14}\text{N}$  during the lower-temperature burns and less altered N cycling processes (i.e., minor increases in net nitrification and subsequent lack of nitrate leaching) relative to wildfire. Thus, isotopic shifts in terrestrial plant foliage or in-stream moss after fire are a useful indicator of the magnitude and duration of fire effects and the fate of post-fire available N.

## INTRODUCTION

Fire is an integral component of ecosystem nitrogen (N) cycling in coniferous ecosystems of the Rocky Mountains. Through combustion, a net loss of N contained in live and dead aboveground organic matter and forest floor occurs, followed by a range of post-fire changes in N cycling associated with changes in plant cover, microbial activity, microclimate, and soil chemical environment. Despite the potentially large net loss of N through combustion (Grier 1975), short-term increases in inorganic N in the soils are commonly observed (Wan et al. 2001; K. Stephan, K. L. Kavanagh, and A. Koyama Unpublished Manuscript a,b). This N can either be retained in recovering plant and microbial biomass, or leached into deeper soil layers and eventually into streams where it, in turn, contributes to aquatic N cycling. Even though patterns of N distribution (concentrations and pool sizes) in recently burned ecosystems are commonly described (Fisher and Binkley 2000; Wan et al. 2001; Spencer et al. 2003) the changes in N cycling mechanisms leading to these patterns are not equally well understood. This is in part caused by the complexity of the N cycle.

Progress in understanding N cycling has been aided by the use of stable  $^{15}\text{N}$  isotope tracers by, e.g., quantifying gross N transformation rates in soil (Davidson et al. 1991; Stark and Hart 1997, A. Koyama, K.L. Kavanagh and K. Stephan Unpublished Manuscript), and by identifying ammonium ( $\text{NH}_4^+$ ) and nitrate ( $\text{NO}_3^-$ ) sinks and N cycling rates in terrestrial (Nadelhoffer et al. 1999; Chambers et al. 2004) and aquatic ecosystems (Mulholland et al. 2000; Peterson et al. 2001). The use of stable isotopes at natural abundance, however, offers the advantage of giving insights into the N cycle without disturbing it (Högberg 1997). Plant foliar isotopic values have been thought to indicate plant N sources but, in fact, many factors influence plant  $\delta^{15}\text{N}$  values (Högberg 1997; Robinson 2001; Evans 2001). Another current challenge to improved understanding of N cycle is the  $^{15}\text{N}$  analysis at natural abundance of small and variable pools of inorganic N in soil (Robinson 2001; K. Stephan and K. L. Kavanagh Unpublished Manuscript).

Nevertheless, recent studies have explored changes in N cycling after a disturbance using N stable isotopes at natural abundance. Foliar  $^{15}\text{N}$  enrichment after clear-cutting has been observed repeatedly (Pardo et al. 2002; Högbom et al. 2002; Högberg and Johannisson 1993) and has been linked to uptake of enriched residual soil inorganic N caused by leaching losses of depleted  $\text{NO}_3^-$ . The use of N stable isotopes in studying post-fire N cycling, however, has been sparse. In a notable exception, Grogan et al. (2000) found significant enrichment of foliage on burned sites that was attributed to reliance on  $\text{NH}_4^+$  post-fire, generated from enriched soil organic matter. Very recently, Saito et al. (2007) pointed out the possibility of using a fire-generated isotopic signal in residual soil to trace the impact of fires to aquatic systems.

The objective of our study was to use the observed responsiveness of N stable isotopes to changes in the N cycle and the potential of a fire-induced, traceable isotopic signal in the soil for improving the understanding of post-fire N cycling. We achieved this by quantifying and interpreting the N isotopic signatures of soil, plants, streamwater, and in-stream moss, the  $\text{NO}_3^-$  use by plants, and by discussing the role of soil N leaching presented in companion studies (K. Stephan, K. L. Kavanagh, and A. Koyama Unpublished Manuscript a,b).

## METHODS

### Study Sites

Our four wildfire sites (Hall, Canyon Creek, South Fork, Danskin Creek), three spring prescribed burn sites (Danskin Creek, Sixbit, Parks-Eiguren) and one spring test burn site (Squaw Creek) are located on the Boise and Payette National Forests in the Salmon River Mountains and West Mountains of central Idaho, USA (44°05'-44°57'N, 115°12'-116°21'W). The regional climate is characterized by warm dry summers and cool, moist winters. Depending on site elevation (1400-2350 m) mean annual air temperature ranges from 0.5 to 5.4 °C and mean annual precipitation (mainly as snow) ranges from 680 to 1060 mm. The geology underlying the study area is either Idaho Batholith Granitics at most sites or Columbia River Basalt at the westernmost sites Hall and Squaw Creek. Watershed areas are small (mean 64 ha, range 8-140 ha), drained by either intermittent or perennial first-order streams, and have either a westerly (all wildfires sites and Danskin Creek prescribed burn) or southerly aspect (three remaining spring burn sites). Stream channels are confined by relatively steep hill slopes (15-41°) and fringed by only a narrow strip ( $\leq 1$  m width) of obligate riparian shrubs and herbs. The overstory is comprised of mature Douglas-fir (*Pseudotsuga menziesii*) and ponderosa pine (*Pinus ponderosa*). At the highest elevation site (Canyon Creek), ponderosa pine is replaced by subalpine fir (*Abies lasiocarpa*). Three of the wildfire sites had burned in the summer of 2003, one site (Danskin Creek) had burned in 2002. The four spring burns had been carried out in April and May of 2004.

We assessed fire severity, defined as the effect of fire on the ecosystem (Ryan and Noste 1985), from satellite imagery (Landsat) before and one (wildfires) to three (spring burns) months after fires. As an index of fire severity, we used the delta Normalized Burn Ratio (dNBR) (Key and Benson 2006) mainly reflecting overstory mortality (Lentile et al. 2006). Delta NBR was derived for all watersheds where streamwater had been collected (see below). Delta NBR revealed significant and varying levels of overstory mortality in wildfire-burned watersheds, but no overstory mortality in the

prescription-burned watersheds (K. Stephan, K. L. Kavanagh, and A. Koyama Unpublished Manuscript a,b). Based on ocular estimates on the ground, understory vegetation and forest floor was completely consumed over approximately 30 to 80 % of the watersheds areas burned by wildfires. In prescription-burned watersheds, understory and forest floor was charred or consumed in relatively small patches (5 to 100 m<sup>2</sup>) covering less than one third of the total watershed area. The spring test burn had a very small extent (1 ha) so that dNBR assessment was not possible. The burn characteristics resembled those of the prescribed burns with respect to overstory mortality (very few trees killed due to fire suppression effort) but resembled those of wildfires on the ground (complete understory and forest floor consumption).

### Sample Collection and Analysis

Mineral soil (0-10 cm) and foliage samples of four upland understory species (three shrubs, one sedge) were collected from four plots located within 25 m of either side of the stream in one burned and one unburned watershed within each site. Foliage of obligate riparian plants (two perennial herbs, two shrubs) growing on the stream banks and moss growing on rocks in streams were collected from two plots per watershed (see Table 1 for species identities). Obligate riparian plants will be used interchangeably with riparian plants throughout this document, although upland plants were also collected from the riparian zone if defined more broadly by the slope distance within which plants influence stream function (via coarse and fine organic matter input, shade) (Naiman and DeCamps 1997). All vascular plants had resprouting capabilities. Per plot, each sample represents a composite of four to five soil cores (1.9 or 5 cm diameter), leaves of three to six individual plants, and three moss patches that were randomly chosen. Further details are described in K. Stephan, K. L. Kavanagh, and A. Koyama (Unpublished Manuscript a,b). Soil was collected in August 2004 and October 2005 for isotopic analysis of inorganic N. Plant foliage was collected in July/August and moss was collected in May/June for two or three post-fire years (PFYs) in spring burned and wildfire sites, respectively. Data for a fourth PFY is available for the Danskin Creek wildfire site since it burned one year prior to the other three wildfire sites. In July of 2005, fine roots (< 2 mm) of spiraea (*Spiraea betulifolia*) were collected in wildfire sites. Streamwater was collected in June 2006 for analysis of NO<sub>3</sub><sup>-</sup>-δ<sup>15</sup>N from one burned and one unburned watershed of each of the three 2003 wildfires. Due to the small area of the Squaw Creek test burn, reference plots were established ca. 100 m downstream from the test burn area and obligate riparian, streamwater, and moss from this site were not collected (not available due to ephemeral nature of streamflow).

### Plant $\delta^{15}\text{N}$

Plant material was dried at 70 °C for 24 h (all 2003 foliage, 2004 obligate riparian foliage) or freeze-dried (2004 upland foliage, all 2005 and 2006 foliage, roots, moss). Dried materials were ground to a fine powder with a ball mill, packed into a tin capsule and analyzed for their  $\delta^{15}\text{N}$  value with continuous-flow direct combustion isotope ratio mass spectrometry (IRMS) following combustion in an elemental analyzer. Analyses of roots, and upland and obligate riparian foliage collected in 2004, 2005 and 2006 were carried out in the laboratory of R. Lee at the School of Biological Sciences at Washington State University (Isoprime [Micromass Ltd. Manchester, UK] coupled to an EuroEA 3000 elemental analyzer [EuroVector S.p.A., Milan, Italy]). Foliage samples collected at Danskin Creek in 2003 and moss samples collected in 2004 and 2005 were analyzed in the University of Idaho Stable Isotope Laboratory (ISIL) (Finnigan Delta Plus [Finnigan MAT, Bremen, Germany] coupled to a Carlo Erba NC 2500 elemental analyzer [CE Instruments, Milan, Italy]). Moss collected in 2006 was analyzed in the Laboratory for Biotechnology and Bioanalysis Stable Isotope Core at Washington State University (Delta PlusXP [ThermoFinnigan, Bremen, Germany] coupled to an ECS 4010 elemental analyzer [Costech Analytical, Valencia, California, USA]). In each laboratory, analytical precision for  $\delta^{15}\text{N}$  was  $\leq 0.2$  ‰ (standard deviation) between replicates of laboratory internal reference material and between replicates of actual sample material. Duplicate moss and foliage samples run at the two laboratories analyzing these sample types had standard deviations of  $\leq 0.2$  ‰ ( $n = 2$ ) and  $\leq 0.1$  ‰ ( $n = 8$ ), respectively.

### Nitrate Reductase Activity

We were interested in the N-forms used by plants. There are no methods to quantify the uptake of organic N or  $\text{NH}_4^+$  in the field, however, nitrate reductase activity (NRA) is a good indicator for  $\text{NO}_3^-$  uptake and use by plants (Lee and Steward 1978). Foliar NRA was determined by the *in vivo* method (Stewart et al. 1973) for spiraea in each plot in June 2005 and 2006. The method was adapted to use *in situ* (i.e., no vacuum, no DMSO, no boiling). Approximately 150-300 mg fresh leaf biomass was collected by cutting two 1-cm<sup>2</sup> squares from each of a total of six leaves (young but fully developed, from six individuals) per plot, and immediately incubated in assay medium in the dark at 25-30 °C for ca. 60 minutes. The reaction was stopped by adding the color reagents (Filner 1966). Spectrophotometric determination of the nitrite produced (Filner 1966) was carried out upon returning to the laboratory within one week. Preliminary studies had shown that the color was stable for more than one week. Leaf material used in the assay was dried at 70 °C for 24 h. NRA is expressed as  $\mu\text{mol}$  nitrite produced per hour and gram dry weight of tissue.

Despite diurnal changes of NRA (Gebauer et al. 1984), the NRA assays could not be done at a

constant time of day across all sites due to logistical reasons (all our sites were remote). However, NRA in the burned and unburned watersheds for any given site was sampled within a 2 to 3 h time period to minimize the diurnal influence on NRA.

### **Soil and Streamwater $\delta^{15}\text{N}$**

Fresh soil (sieved, 4-mm sieve) was extracted with 2 M KCl while shaking for 1 h. Soil to extractant ratio was 1 : 2.7 or 1 : 2 because soil inorganic N concentrations were very low. Soil extracts were filtered through Whatman No. 42 filters and extracts were stored frozen till analysis. A modified diffusion method (Holmes et al. 1998; K. Stephan and K. L. Kavanagh Unpublished Manuscript) was used to isolate and concentrate  $\text{NH}_4^+$ -N on filter discs that were analyzed for  $\delta^{15}\text{N}$  at ISIL. Soil extracts from August 2004 contained ca. 40  $\mu\text{g}$   $\text{NH}_4^+$ -N in volumes of 20 to 50 mL, and extracts from October 2005 contained on average 48  $\mu\text{g}$  (range 10-110  $\mu\text{g}$ )  $\text{NH}_4^+$ -N in ca. 100 ml extract. Samples were diffused during a 6-d diffusion period at room temperature or at 34 °C, respectively. Recoveries of sample-N were on average 96 %  $\pm$  0.2 (SD) and 90 %  $\pm$  10 for August 2004 and October 2005 samples, respectively. Samples that had recoveries of < 99 % of expected N were corrected for fractionation during incomplete recovery (Holmes et al. 1998, K. Stephan and K. L. Kavanagh Unpublished Manuscript). Contaminant-N contributed from reagents was negligible (K. Stephan and K. L. Kavanagh Unpublished Manuscript); therefore, no correction to obtain the true target- $\text{NH}_4^+$ - $\delta^{15}\text{N}$  was necessary.

Analysis of soil extract  $\text{NO}_3^-$ - $\delta^{15}\text{N}$  was not possible in August 2004 extracts because they contained too little  $\text{NO}_3^-$ -N for accurate analysis with the IRMS. In October 2005, a larger soil volume was extracted (75 g fresh soil in 150 ml 2 M KCL). Still, only half of the samples contained sufficient  $\text{NO}_3^-$ -N to be diffused (average 47  $\mu\text{g}$ , range 13-150  $\mu\text{g}$ ). Diffusions for  $\text{NO}_3^-$ -N were carried out for 6 d at room temperature after  $\text{NH}_4^+$ -N had been trapped (see above). The method was modified from Sigman et al. (1997) and described in K. Stephan and K. L. Kavanagh (Unpublished Manuscript). During sequential diffusions,  $\text{NH}_4^+$ -N that is not trapped in the first step will be enriched and might be carried over into the subsequent nitrate diffusion. We assumed this had happened with  $\text{NH}_4^+$ -N that was not recovered in the first diffusion step. Additionally, reagents have been shown to contribute significant amounts of contaminant-N (K. Stephan and K. L. Kavanagh Unpublished Manuscript). Sample recovery (including assumed carry-over of  $\text{NH}_4^+$ -N and reagent-contaminant-N) was 75 %  $\pm$  13 (SD). We accounted for the amounts and isotopic values of carry-over  $\text{NH}_4^+$ -N and reagent-N, and fractionation due to incomplete sample-N recovery, and calculated the true target  $\text{NO}_3^-$ - $\delta^{15}\text{N}$  as detailed in K. Stephan and K. L. Kavanagh (Unpublished Manuscript).

Streamwater was analyzed for  $\text{NO}_3^-$ - $\delta^{15}\text{N}$  using the denitrifier method (Sigman et al. 2001) at the Woods Hole Oceanographic Institute, Woods Hole, Massachusetts, USA. Three subsamples of each water sample were analyzed; the standard deviation between the three replicates was  $< 0.18$  ‰.

All sample materials were stored on ice in a cooler during the collection period and transport to the laboratory. Due to the remoteness of the field sites all sample processing in the laboratory commenced one to five days after field collection.

### **Statistical Analysis**

The study design is comparable to a block design (site = block, watershed = plot). Prior to statistical analysis, values of soil and vegetation, and in-stream moss samples taken at the four upland or two riparian/aquatic sampling plots (= subplots) within watersheds were averaged.

Data were transformed if necessary and subjected to analyses of variance (ANOVA) with linear mixed-effect models in SAS (SAS 9.1, SAS Institute Inc., Cary, NC, USA) using the ‘proc mixed’ statement. The objectives of the statistical analysis were to test for the absence of a) a difference between burned and unburned watersheds for wildfires and spring burns, respectively, b) a difference in the magnitude of the post-fire response between wildfires and spring burns, and c) a change in isotopic values through time. Mixed-effects models were used because they allowed addressing the often unbalanced data and modeling the correlation structure induced by the nested design (watersheds within sites) and repeated sampling in time.

In our mixed-effect models, site was specified as random effect (‘random’ statement). That is, study sites are a random sample from a population of sites or, as with this study, selection of sites was deterministic but the effect of sites on the outcome was of stochastic nature (Schabenberger and Pierce 2002). In consequence, inference drawn from this study is not limited to the very sites studied, but applies to similar wildfires and spring prescribed burns in mid-elevation headwater watersheds within the central Idaho region. In addition to site, the watersheds nested within each site were included as random effect if permitted by the data structure. This allowed random interactions between site and treatment, i.e., the magnitude of the burn effect could vary between sites and/or the two watersheds within each site could differ from each other due to, e.g., slight variation in elevation, slope or (pre-fire) soil characteristics. As a consequence, only those treatment effects that were sufficiently strong in all sites were detected.

Serial correlation between  $\delta^{15}\text{N}$  values of samples collected through time was assumed and accounted for with repeated measures (‘repeated’ statement). Adjustments to the random effects structure and serial correlation were made (i.e., one random effect and/or the serial correlation was dropped) if the data structure did not support having all components in the model. Pairwise

comparisons for a given sample date were carried out in SAS using the Satterthwaite function to approximate degrees of freedom. In the results, model P-values and/or P-values of pairwise comparisons are presented. Means and standard errors presented in graphs and tables are based on actual data and not the model values.

Regressions were carried out using general lineal models ('proc glm' statement). Here we used plot values rather than watershed averages because the within watershed variation was larger than the variation between watersheds. This resulted in lower coefficients of determination ( $r^2$ ) and larger P-values than when watershed values were used.

Data from the Danskin Creek wildfire, that had burned one year prior to the other three wildfire sites, is analyzed in the appropriate post-fire season in analysis of NRA, foliar and root  $\delta^{15}\text{N}$  (Table 1, Figures 2 and 3). Due the high short-term temporal variability in soil, Danksin Creek wildfire data on soil  $\delta^{15}\text{N}$  was analyzed together with data from the other wildfires collected at the same sample date (Table 2, Figure 2).

## RESULTS

### Foliar $\delta^{15}\text{N}$

Foliar isotopic values were generally more enriched in burned than in unburned watersheds, but the magnitude of enrichment differed between wildfire and spring burned watersheds, and between upland and obligate riparian vegetation. All upland plant species on wildfire sites had significantly higher foliar  $\delta^{15}\text{N}$  values (enriched) in burned watersheds relative to unburned watersheds across all the three PFYs ( $P = 0.01$ ). All species responded in a similar way, i.e., the interactions of species with treatment, PFY, or both were statistically non-significant ( $P > 0.05$ ) The absolute difference in  $\delta^{15}\text{N}$  between burned and unburned watersheds in foliar enrichment across all species was on average 3.0 ‰ and 2.8 ‰ in the first and second PFY, respectively. This difference had decreased to 1.4 ‰ by PFY 3 ( $P < 0.001$ ). Data from Danskin Creek indicated that the burned-unburned differences in PFY 3 persisted in PFY 4 (Figure 1a).

N in spiraea roots were significantly enriched by 1.7 ‰ ( $P = 0.049$ ) in burned watersheds relative to unburned watersheds of the three 2003 wildfire sites. However, there was no difference in root- $\delta^{15}\text{N}$  between the burned and unburned watershed at the Danskin Creek wildfire site in PFY 3 (Table 1). Roots were isotopically depleted relative to spiraea foliage in unburned watersheds (absolute difference in  $\delta^{15}\text{N}$  1.2 ‰,  $P = 0.01$ ) and more so in burned watersheds (absolute difference in  $\delta^{15}\text{N}$  2.7 ‰,  $P < 0.0001$ ).

In the spring burn sites there was a significant burn effect on foliar  $\delta^{15}\text{N}$  values of the upland plant species ( $P = 0.04$ ) but the burn effect differed between species, i.e., there was no response by the sedge in PFY 1 and only a marginal response in PFY 2 (Table 1). The average isotopic increase (enrichment) in burned vs. unburned watersheds was 1.3 ‰ in both PFY 1 and 2 and, thus, significantly smaller ( $P = 0.01$ ) than the respective increases after wildfire (Figure 1a). In all unburned watersheds,  $\delta^{15}\text{N}$  values of species did not change through time (Figure 1a). Foliar N isotopic values of individual species did not differ between unburned watersheds of wildfire and spring burn sites with the exception of spiraea in PFY 2 ( $P = 0.05$ ) (Table 1).

The foliage of obligate riparian species responded differently than that of upland species (Figure 1a,b). With obligate riparian species in wildfire sites there was a significant treatment effect ( $P = 0.007$ ), treatment  $\times$  season interaction ( $P = 0.005$ ) and treatment  $\times$  species interaction ( $P = 0.002$ ). There was no increased foliar  $\delta^{15}\text{N}$  values in burned relative to unburned watersheds consistent across all species in PFY 1 ( $P = 0.16$ ), although the herbaceous *G. triflorum* was significantly enriched in burned sites (Table 1). In PFY 2 and 3 increases in burned relative to unburned watersheds were significant across all species, with absolute increases averaged across all species of 2.1 ‰ ( $P = 0.002$ ) and 2.4 ‰, ( $P = 0.001$ ) respectively. Data for PFY 4, available for Danskin Creek only, indicated that the magnitude of the post-fire signal might decrease to ca 1 ‰ four years after fire (Figure 1b).

With obligate riparian species of prescription-burned sites, there was a significant treatment  $\times$  species interaction ( $P = 0.003$ ). Forbs had higher post-fire  $\delta^{15}\text{N}$  values in burned relative to unburned watersheds for both post-fire seasons studied but there was no treatment effect in shrubs (Table 1). The general pattern averaged across species is shown in Figure 1b.

When comparing wildfires and spring prescribed burns, in PFY 2, obligate riparian foliage in burned watersheds of wildfire sites had significantly higher  $\delta^{15}\text{N}$  values than obligate riparian foliage of prescription-burned watersheds (absolute difference across all species 1.4 ‰,  $P = 0.0004$ ) and there was no difference among unburned watersheds. There was no difference in PFY 1 within a given treatment between wildfires and spring prescribed burns. Foliar isotopic values of obligate riparian and upland plants did not differ from each other in unburned sites despite differing species compositions.

Isotopic values of moss responded in a similar way as obligate riparian foliage. Moss in streams of wildfire-burned watersheds had significantly increased  $\delta^{15}\text{N}$  values in the second and third PFY (Table 1). Data from Danskin Creek (not shown) indicated that the increase persisted in the fourth PFY. Moss in streams of prescription-burned watersheds had  $\delta^{15}\text{N}$  values that were not different from those in unburned reference streams (Table 1).

### Nitrate Reductase Activity

NRA varied with treatment, fire type and time since fire. Spiraea leaves collected in June of PFY 2 had higher foliar NRA in burned relative to unburned watersheds across the three 2003 wildfire-burned sites ( $P = 0.04$ ) but not across all four spring burned sites ( $P = 0.78$ ). At one of the prescription-burned sites (Sixbit), however, NRA was about four times higher in the burned than in the unburned watershed and, thus, similar to that of wildfire sites. In PFY 3 there were no differences in NRA between burned and unburned watersheds of wildfire sites ( $P = 0.5$ ) (Table 1), and this lack of difference also occurred at the Danskin Creek wildfire site in PFY 4 (data not shown).

### Soil and Streamwater $\delta^{15}\text{N}$

Isotopic values of soil inorganic N were higher in burned than in unburned watersheds in wildfire sites but not in prescribed burn sites. Across all four wildfire sites,  $\text{NH}_4^+$  in soils extracts from August 2004 (PFY 1 for three sites, PFY 2 for Danskin Creek) had significantly higher  $\delta^{15}\text{N}$  values in burned relative to unburned watersheds ( $P = 0.02$ ), but there was no consistent pattern across prescribed burn sites ( $P = 0.24$ ) (Table 2). This pattern persisted in October 2005 except that in wildfire sites the absolute difference between burned and unburned sites had decreased ( $P = 0.07$ ) from on average  $5.8 \pm 1.2$  (SE) ‰ to  $3.2 \pm 0.8$  ‰. Soil  $\text{NO}_3^-$  data (Table 2) available for October 2005 had many missing data points due to low N content in extracts from unburned soil preventing the use of the diffusion technique. Comparison of treatments was therefore not possible but  $\text{NO}_3^-$  was isotopically depleted relative to  $\text{NH}_4^+$  ( $P = 0.002$ ).

Streamwater  $\text{NO}_3^-$ - $\delta^{15}\text{N}$  values of burned watersheds were higher than those of unburned watersheds at all three 2003 wildfire sites. Values for the burned vs. unburned watersheds of Hall, Canyon Creek and South Fork sites, respectively, were 4.0 ‰ vs. -10.7 ‰; 3.6 ‰ vs. 2.8 ‰, and 3.4 ‰ vs. 2.6 ‰. Burned-unburned differences were not statistically significant across sites due to the extremely large burned-unburned difference at the Hall site.

## DISCUSSION

### Upland Plant $\delta^{15}\text{N}$ Response to Fire

We found that N in soils ( $\text{NH}_4^+$ ), streamwater ( $\text{NO}_3^-$ ), plant foliage, and moss in streams are enriched following a fire, especially after wildfire. This enrichment across interdependent N pools highlights the potential for tracking the fate of N released post-fire. In our study, the enrichment of upland plant foliage in wildfire-burned watersheds in PFY 1 (3 ‰) is lower compared to a foliar

isotopic enrichment of about 6 ‰ after a wildfire in a Californian bishop pine forest (Grogan et al. 2000). In spring burn sites, post-burn isotopic increases were either of smaller magnitude relative to wildfire sites or did not occur.

#### Mechanisms of Post-Fire Foliar Enrichment

The most likely mechanism for foliar  $^{15}\text{N}$  enrichment post-fire is the uptake of soil N that was enriched due to fractionation occurring during volatilization and/or combustion of N in organic matter. During pyrolysis (chemical heat degradation) of solid particles, the first stage of combustion, low molecular weight species (e.g., amides, amines) are given off and convected into the oxidizing portion of the flame (DeBano et al. 1998; Raison 1979). Additionally, dissolved inorganic N and low weight organic molecules vaporize (volatilize) at relatively low temperatures (Fisher and Binkley 2000). These processes fractionate against  $^{15}\text{N}$  so that  $^{14}\text{N}$  is preferentially lost. This has been demonstrated by Saito et al. (2007), by heating organic and mineral soil in a muffle furnace at different temperatures and durations. The highest isotopic enrichment of residual N (2.5 ‰) occurred at the highest combustion temperatures and durations. Furthermore, enrichment correlated with proportion of N lost. In one of our wildfire study sites (Danskin Creek), ash was enriched by about 4 ‰ relative to the unburned forest floor (A. Koyama, K. L. Kavanagh and K. Stephan Unpublished Manuscript). Specific N-forms in residual organic N can be more highly enriched than the enrichment observed in bulk organic residual or ash; e.g., the high fractionation factor of  $\text{NH}_3$  volatilization (40-60 ‰) (Robinson 2001) can leave residual  $\text{NH}_4^+$  significantly enriched depending on the proportion of substrate volatilized (e.g., enrichment of 28‰ when 50% volatilized).

Increased rates of net nitrification have also been associated with enriched foliar  $\delta^{15}\text{N}$  (Garten and Van Miegrot 1994) with the theoretical foundation provided by Shearer et al. (1974). Due to the larger fractionation associated with nitrification than with microbial  $\text{NH}_4^+$  immobilization, both  $\text{NH}_4^+$  and  $\text{NO}_3^-$  pools become enriched with increasing proportion of  $\text{NH}_4^+$  nitrified. In laboratory incubations,  $\text{NH}_4^+$ - and  $\text{NO}_3^-$ - $\delta^{15}\text{N}$  became enriched early and later during the incubation period, respectively, in burned relative to unburned chaparral soils (Herman and Rundel 1989). Furthermore, if  $\text{NO}_3^-$  is lost via leaching, the residual N will be enriched relative to the  $\text{NO}_3^-$  lost, causing additional increases in  $\delta^{15}\text{N}$  of shallow-rooted plants. Both increased net nitrification and leaching losses of  $\text{NO}_3^-$  are generally observed in burned areas or after other vegetation disturbances, and increased foliar  $\delta^{15}\text{N}$  has been attributed to these mechanisms (Grogan et al. 2000; Pardo et al. 2002; Högbom et al. 2002).

All of the above mechanism could contribute to foliar  $^{15}\text{N}$  enrichment and explain differences in foliar isotopic response between wildfires and spring burns. The hypothesis of soil inorganic N

enrichment due to leaching of isotopically lighter  $\text{NO}_3^-$  is supported by very high streamwater  $\text{NO}_3^-$  concentrations found in wildfire-burned watersheds relative to unburned watersheds. Leaching of  $\text{NO}_3^-$  into streams occurred during the three post-fire seasons at about equal magnitude, whereas little or no leaching occurred after spring prescribed burns (K. Stephan, K. L. Kavanagh, and A. Koyama Unpublished Manuscript a,b). Since the patterns of streamwater  $\text{NO}_3^-$  concentrations post-fire (i.e., the duration and magnitude of concentration increases) corresponded with the pattern in foliar  $\delta^{15}\text{N}$ , leaching could in part explain the differences in foliar isotopic response between wildfires and spring prescribed burns. Additionally, lower combustion temperatures (DeBano et al. 1998) and incomplete removal of the forest floor (Schoch and Binkley 1986) are commonly observed with spring prescribed burns. Whereas we did not measure the former but did observe the latter, these factors could also explain the lower isotopic shifts of plant foliage post-prescribed burn relative to wildfires in our study. With spring prescribed burns, less volatilization of  $^{14}\text{N}$  had occurred and resprouting plants could still acquire N mineralized from the partially charred, residual litter layer.

#### Enriched Soil Inorganic N

The most decisive evidence of post-fire enriched plant foliage being caused by the above mentioned mechanisms is the presence of post-fire enriched plant available N. However,  $\delta^{15}\text{N}$  of soil inorganic N is rarely quantified, largely due to analytical difficulties (K. Stephan and K. L. Kavanagh Unpublished Manuscript). We show that post-fire  $\text{NH}_4^+$  in soil extracts was enriched across sites burned by wildfire for at least two years (Table 2). In fact, soil  $\text{NH}_4^+$  isotopic values could explain about one third of the variability of spiraea foliar  $\delta^{15}\text{N}$  (Figure 2a,b) (since the foliage of the other upland species had similar pattern in their foliar  $\delta^{15}\text{N}$ , only spiraea is presented). The correlation might have been even higher if data on soil  $\text{NH}_4^+$ - $\delta^{15}\text{N}$  had been available throughout the growing season, instead of one point measurement, and if soil sampling locations would have exactly represented the rhizosphere of the plants collected for foliar  $\delta^{15}\text{N}$ .

Unfortunately, no conclusive isotopic data for soil  $\text{NO}_3^-$  was available. If soil  $\text{NH}_4^+$  is enriched post-fire, however,  $\text{NO}_3^-$  derived from it can also be expected to be enriched relative to unburned conditions under the assumption that denitrification (with high fractionation factors; Robinson 2001) is negligible. Nitrate reductase activity, and thus  $\text{NO}_3^-$  use by plants, was significantly higher in the second growing season after wildfire (Table 1) and explained about one third to half of the variation in foliar N concentration in prescribed burn sites and wildfire sites, respectively (Figure 3a,b). Higher NRA after fire has also been documented by Stewart et al. (1993). In fact, NRA in our wildfire-burned sites also correlated positively with foliar  $\delta^{15}\text{N}$  ( $P = 0.0004$ ) (Figure 4), indicating that soil  $\text{NO}_3^-$  is enriched. If soil  $\text{NO}_3^-$  was not enriched post-fire, higher  $\text{NO}_3^-$  uptake by plants could have

been balanced by simultaneously higher uptake of enriched soil  $\text{NH}_4^+$ .

Högbom et al. (2002) also observed a positive correlation of NRA and foliar  $\delta^{15}\text{N}$  after clear-cutting. These authors attributed foliar enrichment to uptake of N from an enriched residual pool as a consequence of  $\text{NO}_3^-$  leaching. In their study, increased NRA after clear-cutting persisted for at least five years. In contrast, we could not detect treatment differences in NRA in wildfire sites in PFY 3 even though foliar  $\delta^{15}\text{N}$  was still enriched and  $\text{NO}_3^-$  leaching into streams occurred as evidenced by high streamwater  $\text{NO}_3^-$  concentrations (K. Stephan, K. L. Kavanagh, and A. Koyama Unpublished Manuscript a) and enriched in-stream mosses. Temperature and light intensity influence NRA (Gebauer 1984; Högbom 1991), so that a treatment effect in PFY 3 might have been obscured by abiotic conditions at the time of sampling.

The two burned plots with the highest NRA, also having some of the highest foliar N concentrations (Figure 3a), do not fall on the regression line produced by the other plots in Figure 4. This might be explained by a higher proportional uptake of  $\text{NO}_3^-$ , that is less enriched than the post-fire  $\text{NH}_4^+$ , relative to other plots. This switch in proportional use of N-forms could explain contrasting relationships of NRA and foliar  $\delta^{15}\text{N}$  observed in the literature (Nadelhoffer et al. 1996).

#### Location of $\text{NO}_3^-$ assimilation

An alternative explanation for the correlation of foliar  $\delta^{15}\text{N}$  and NRA is plant internal fractionation. Since  $\text{NO}_3^-$  can be reduced and assimilated in roots or foliage,  $\text{NO}_3^-$  translocated to foliage will be enriched relative to the  $\text{NO}_3^-$  assimilated in the roots. This is because nitrate reductase in roots fractionates against  $^{15}\text{N}$  and the  $\text{NO}_3^-$  translocated to shoots originates from an enriched residual root  $\text{NO}_3^-$  pool (Evans et al. 1996). Since foliar NRA was higher in wildfire-burned watersheds compared to unburned watersheds (Table 1, x-axis of Figure 3a), location of assimilation could explain enriched foliar  $\delta^{15}\text{N}$  post-fire. If this mechanism was the major cause of foliar enrichment (i.e., no change in soil inorganic  $\delta^{15}\text{N}$ ), root  $\delta^{15}\text{N}$  would have been expected to decrease (preserving mass balance). However, both leaves and roots were enriched after wildfire (Table 1). Therefore we suggest that the post-fire enrichment in both roots and leaves is mainly a consequence of uptake of enriched soil N. The larger root-leaf isotopic difference observed in burned sites (2.5 ‰,  $P < 0.0001$ ) compared to that in unburned sites (1.2 ‰,  $P = 0.004$ ) would also be expected with an increased proportion of  $\text{NO}_3^-$  assimilated in leaves. However, in our study it could be the consequence of foliar N being largely from recent uptake of enriched post-fire soil N whereas root N represents a mix of lighter pre-burn N with enriched post-fire N.

### Alternative Explanations for Post-Fire Foliar Enrichment

Overall, the relatively low coefficients of determination in regressions of foliar  $\delta^{15}\text{N}$  against soil  $\text{NH}_4^+ - \delta^{15}\text{N}$  (Figure 2) and NRA (Figure 4) separately or together ( $R^2 = 0.48$ ,  $P = 0.0003$ ) for wildfire sites underline that the isotopic values of source N are not the only determinants of foliar  $\delta^{15}\text{N}$ .

Several other factors can influence plant foliar  $\delta^{15}\text{N}$  (Högberg 1997): origin(s) of source N (soil N, precipitation, foliar N uptake,  $\text{N}_2$ -fixation), rooting depth, influences by mycorrhizal symbioses, and fractionations during N uptake by plants.

So far we assumed that plant N reflects soil N. In our study region, N in precipitation is very low ( $1.4 \text{ kg N ha}^{-1} \text{ y}^{-1}$ ) (NADP 2006) and about one to two orders of magnitude lower than the annual requirement by understory vegetation (Vanderschaaf 1999). Foliar uptake of  $\text{NO}_x$  and  $\text{NH}_3$ , that can be substantial in areas with air pollution (Vallano and Sparks 2006), is likely negligible due to the high air quality in our remote study area. N-fixing plants were rare, so that N derived from this source is also a minor proportion of soil N. None of these potential N sources changed after fire so that our assumption of soil N (derived from mineralization) as the dominant N source for plants is reasonable. Although free-living N-fixers can increase post-fire (Wei and Kimmins 1998) their contributions are likely too small to change  $\delta^{15}\text{N}$  values of the soil inorganic N pool; if contributions were significant (with inputs  $\approx 0 \%$ ), they would decrease soil  $\delta^{15}\text{N}$  which is the opposite of what we observed post-fire.

Given that soil  $\delta^{15}\text{N}$  generally increases with soil depth (Nadelhoffer and Fry 1988, 1994; Nadelhoffer et al. 1996; Evans and Ehleringer 1993), it has been suggested that increased plant  $\delta^{15}\text{N}$  after fire reflects changes in root location; i.e., after the removal of the litter layer, plants would root and take up N in the mineral soil where N is derived from the mineralization of heavier soil organic matter as opposed to N mineralized from the isotopically lighter litter layer (Högberg 1997). The fact that the  $\delta^{15}\text{N}$  of foliage in burned watersheds became more similar to that of bulk mineral soil (0-10 cm) (ranging between 2.6 and 4.9 ‰; A. Koyama Unpublished Data) would support this hypothesis. However, the isotopic value of bulk soil does not necessarily represent the isotopic value of plant available N (Evans 2007). Due to the relatively low productivity of the dry forests of central Idaho, not all plots within unburned watersheds had an organic horizon, especially those of southerly aspect in wildfire sites. Therefore, changes in rooting depth of post-fire vegetation as major cause for foliar enrichment seem unlikely at least for the southerly aspect.

Mycorrhizal associations can result in  $^{15}\text{N}$ -depleted plant foliage (Hobbie et al. 2000), particularly with ecto- and ericoid mycorrhizae (Högberg 1997) but maybe less so for arbuscular mycorrhizae (Handley et al. 1993). Whether this is due to fractionation during N transfer (Hobbie et al. 2000) or

due to the use of different N forms by different groups of mycorrhizae (Michelsen et al. 1998) is currently not clear. Since most of the species we studied likely had mycorrhizae, mycorrhizal association potentially contributed to relatively low foliar  $^{15}\text{N}$  values in unburned watersheds. The high post-fire soil inorganic N availability could have led to reduced degrees of mycorrhizal infection (Wallenda and Kottke 1998). Therefore, a reduced role of mycorrhizae in plant N supply could have contributed to increased foliar isotopic values post-fire.

Fractionation during N uptake by roots not colonized by mycorrhizae can also influence plant  $\delta^{15}\text{N}$ . Since  $^{14}\text{N}$  is assimilated preferentially, at high external nitrogen supply relative to plant uptake, enriched N from the unassimilated root inorganic N pool (Mariotti et al. 1982) or root organic N pool (Robinson et al. 1996) might efflux from the root. Under N-limiting conditions virtually all available N is taken up and assimilated, resulting in no or negligible discrimination (Evans et al. 1996). N limitation is a reasonable assumption for our unburned watersheds, and in PFY 2 and 3 in burned watersheds, as soil N concentrations were low, averaging  $4 \text{ mg NH}_4^+\text{-N kg}^{-1}$  and  $< 1 \text{ mg NO}_3^-\text{-N}$  (K. Stephan, K. L. Kavanagh, and A. Koyama Unpublished Manuscript a,b). In the first growing season post-fire, however, soil inorganic N concentrations had increased markedly (K. Stephan, K. L. Kavanagh, and A. Koyama Unpublished Manuscript a,b). Assuming that all  $\text{NO}_3^-$  extracted with salt solution originated from soil solution, some of the burned plots (especially at Hall and Canyon Creek wildfire sites) had soil solution  $\text{NO}_3^-$  concentrations in the range of 2 to 24 mM, at which discrimination during uptake had been reported (Mariotti et al 1982; Evans 2001; Högberg 1997). At the same time soil  $\text{NH}_4^+$  concentrations were even higher than those of  $\text{NO}_3^-$ , though most of it likely was extracted from cation exchange sites rather than in solution. Even though inorganic N concentrations extracted from soil cores were high, N concentrations of bulk soil extracts do not necessarily represent the immediate root environment (depletion zone) (Nye and Tinker 1977) so that plants might still have been N limited. Nevertheless, fractionation during uptake might have occurred in some wildfire-burned locations. If fractionation during uptake post-fire had been a major determinant of foliar  $\delta^{15}\text{N}$ , values of foliar  $\delta^{15}\text{N}$  would have been expected to decrease. Since we found the opposite, fractionation during uptake would only have decreased the magnitude of post-fire foliar enrichment that would have been otherwise observed.

In sum, observed pattern in foliar isotopic values are result of several superimposed processes (Högberg 1993; Nadelhoffer et al. 1996). Fire-induced enrichment of source inorganic N via volatilization and  $\text{NO}_3^-$  leaching and subsequent cycling of enriched residual N are likely the major factors.

### **Obligate Riparian versus Upland Plant $\delta^{15}\text{N}$ Response to Fire**

N cycling processes in drier upland areas and moister riparian areas are likely to differ. Garten (1993) found higher soil inorganic N concentrations, higher net mineralization and nitrification rates in valley bottoms relative to slopes. In our study sites, these differences were reflected in the different species compositions and higher foliar N concentrations (K. Stephan, K. L. Kavanagh, and A. Koyama Unpublished Manuscript a,b) in the narrow riparian fringe relative to the upland areas (although only maximally 30 m upslope). Despite these presumable differences between riparian and upland plots, foliar isotopic values in unburned watersheds did not appear to differ even though a direct comparison is confounded by differing species identities.

Similar to upland species, obligate riparian species also showed foliar enrichment post-fire (wildfire) relative to unburned watersheds, even though with a one year delay. In PFY 3, when post-fire isotopic enrichment in upland species started to decrease, the post-fire isotopic enrichment in obligate riparian species was unchanged relative to that of the previous year.

The interpretation of the response of the obligate riparian plants is complicated because riparian plots had been impacted by fire to a differing degree; about half the plots in wildfire-burned watersheds had been burned directly, whereas the other half was unburned but adjacent to burned upland area. Since our main study goal was not to specifically address differences in N cycling in different habitats, we did not collect any data on soil N in riparian plots, making the interpretation of post-fire observed changes of obligate riparian foliar  $\delta^{15}\text{N}$  more difficult. One possible interpretation of the riparian response is that source-N isotopic signatures are altered similarly to that of upland areas. However, this would not explain the temporal pattern observed. Alternatively, riparian areas likely also receive N via leaching from upland areas. Due to the ample  $\text{NO}_3^-$  supply (indicated by increased streamwater concentrations in wildfire-burned watersheds; K. Stephan, K. L. Kavanagh, and A. Koyama Unpublished Manuscript a) and high soil moisture content, increased denitrification (Ullah and Zinati 2006) and associated fractionation (Robinson 2001) could contribute to obligate riparian foliar enrichment after wildfire. Streamwater  $\text{NO}_3^-$  concentrations were as high in PFY 3 as in PFY 2 (K. Stephan, K. L. Kavanagh, and A. Koyama Unpublished Manuscript a), which coincides with the pattern in riparian plant foliar enrichment. In PFY 1, streamwater  $\text{NO}_3^-$  concentrations were not collected during peak flow, so that  $\text{NO}_3^-$  leaching might have been less than in the following years (Bernhardt et al. 2003) explaining the delayed response in obligate riparian plant foliar  $\delta^{15}\text{N}$ .

In contrast to wildfire sites, only herbaceous riparian species showed an increase in foliar  $\delta^{15}\text{N}$  in prescribed burn sites. This was observed despite a general lack in streamwater  $\text{NO}_3^-$  response (K. Stephan, K. L. Kavanagh, and A. Koyama Unpublished Manuscript b) and might reflect localized burn effects on soil rather than denitrification of leached  $\text{NO}_3^-$ . In both wildfire and prescription-

burned sites, herbaceous riparian species responded more quickly and with higher magnitude than shrubs, possibly indicating a heavier reliance on  $\text{NO}_3^-$ .

### **Fire and Aquatic N Cycling**

Saito et al. (2007) suggested that the fire-induced changes in bulk soil  $\delta^{15}\text{N}$  may be a useful means for tracing impacts of fire on the aquatic food web. To our knowledge, this is the first study showing that the fire-induced change in soil isotopic signature can be traced into stream water and in-stream moss. Spencer et al. (2003) found higher  $\delta^{15}\text{N}$  values in fish and aquatic macroinvertebrates after a large wildfire in Montana, but the authors being unaware of the terrestrial fire signal attributed this to a change from terrestrial to aquatic food sources rather than a change in terrestrial food's  $\delta^{15}\text{N}$ . In our study, in-stream moss of wildfire sites had higher foliar N concentrations starting with PFY 1 (K. Stephan, K. L. Kavanagh, and A. Koyama Unpublished Manuscript a) and increased  $\delta^{15}\text{N}$  starting with PFY 2. In  $^{15}\text{N}$  tracer additions to streams, moss was identified as an important sink for both  $\text{NH}_4^+$  and  $\text{NO}_3^-$  (Ashkenas 2004; D. J. Sobota Personal Communication 2007). In our wildfire-burned watersheds, streamwater  $\text{NO}_3^-$  but not  $\text{NH}_4^+$  concentrations increased (K. Stephan, K. L. Kavanagh, and A. Koyama Unpublished Manuscript a), indicating that moss foliar enrichment resulted from the uptake of post-fire abundant and isotopically heavier  $\text{NO}_3^-$  leached into streams. A single direct measurement of streamwater  $\text{NO}_3^-$ - $\delta^{15}\text{N}$  supported this hypothesis. More extensive analysis of streamwater  $\text{NO}_3^-$ - $\delta^{15}\text{N}$  was not possible due to the high cost associated with analysis by service laboratories using the denitrifier method. Post-fire increases in streamwater  $\text{NH}_4^+$  concentrations, however, have been reported for the first PFY after a wildfire (Hauer and Spencer 1998). Preferential  $\text{NH}_4^+$  uptake by moss might therefore have contributed to increased moss  $\delta^{15}\text{N}$  and would explain the lack of increased streamwater  $\text{NH}_4^+$  concentrations. Alternatively, rather than reflecting a terrestrial isotope signal, altered in-stream N cycling (due to likely higher light availability and water temperatures) could have contributed to increased moss  $\delta^{15}\text{N}$  after wildfire. If the post-fire increased moss  $\delta^{15}\text{N}$  largely reflected a terrestrial isotope signal it would highlight the importance of terrestrial N inputs for aquatic productivity in N limited streams. It would further demonstrate retention of part of the leached terrestrial N in in-stream biota, for subsequent fueling of stream-internal N cycling and the potential of reciprocal exchanges with the land via stream water N uptake by riparian plants (Ashkenas et al. 2004) or via interdependent food webs (Sanzone et al. 2003; Nakano and Murakami 2001). Terrestrial N was not exported into streams after spring prescribed burns as was reflected in the lack of a fire signal in moss and corroborated by non-increased streamwater  $\text{NO}_3^-$  concentrations (K. Stephan, K. L. Kavanagh, and A. Koyama b). Thus, low severity spring prescribed burns do not

provide the stream ecosystem with potentially important nutrient pulses.

## CONCLUSIONS

Fire resulted in an increase of foliar  $\delta^{15}\text{N}$  in upland understory plants. Fire severity was reflected in a large isotopic enrichment after wildfires but only a minor shift after spring burns. A likely major cause of post-fire increased plant foliar  $\delta^{15}\text{N}$  is the increased  $\delta^{15}\text{N}$  of soil inorganic N caused by volatilization of  $^{14}\text{N}$  during the fire and altered N cycling processes (i.e., higher net nitrification and subsequent leaching of  $\text{NO}_3^-$ ). Other potential contributing factors to post-fire foliar enrichment are increased plant internal fractionations and a decreased role of mycorrhizae. In riparian areas, the increased nitrification following  $\text{NO}_3^-$  import from upland areas could be an important factor in post-fire foliar enrichment. The longevity of the isotopic signal in deciduous foliage appears to be a better indicator of the duration of altered terrestrial N cycling than soil inorganic or foliar N concentrations. This is the first study to demonstrate that aquatic biota can possibly reflect changes in terrestrial N cycling due to fire, although the role of post-fire altered in-stream N cycling needs to be clarified. Isotopic shifts in terrestrial plant foliage or aquatic biota post fire are a useful indicator of the magnitude of fire effects and the fate of post-fire available N.

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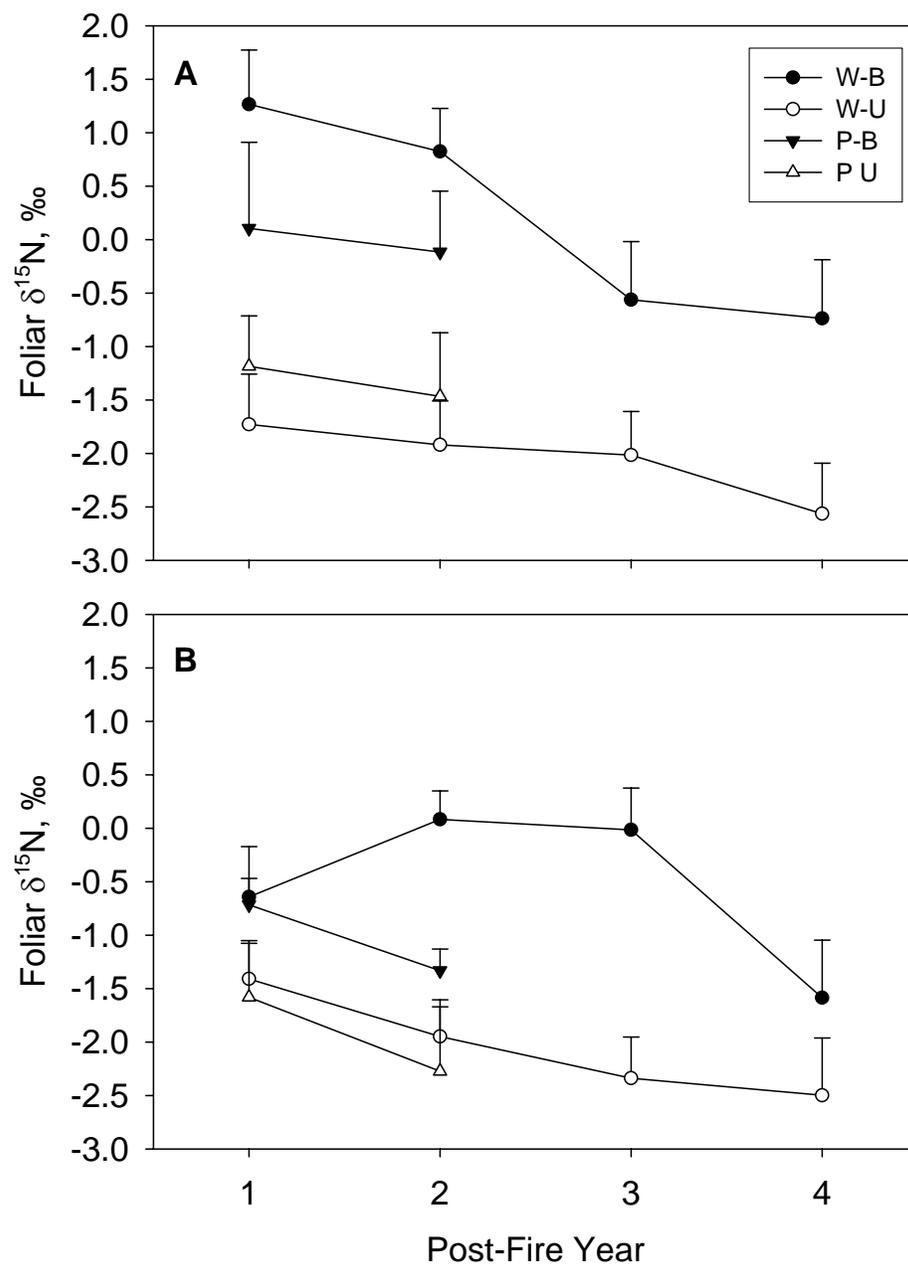
**Table 1.** Foliar  $\delta^{15}\text{N}$  (‰), spiraea root  $\delta^{15}\text{N}$  (‰), and nitrate reductase activity (NRA,  $\mu\text{mol g}^{-1}$  dry wt  $\text{h}^{-1}$ ) in burned (B) and unburned (U) watersheds in each post-fire year (PFY). One SE is given in parentheses (n = 3 or 4 sites), each n represents average of four (upland) or two (riparian/moss) subsamples. Superscripts<sup>\*.o, a,b</sup> denote individual pairwise comparisons: <sup>\*/o</sup> denote  $P \leq 0.05/0.10$  for treatment comparisons within a given species and PSF; <sup>a,b</sup> denote  $P < 0.05$  for comparison between PFYs of burned watersheds and each species for wildfire and spring burns, respectively. Values from unburned watersheds did not differ between years in most species, except for *G. triflorum* in prescribed burn watersheds. <sup>x</sup> Data in wildfire sites of PFY 2 comprises the three 2003 wildfire sites, in PFY 3 just the Danskin Creek site. Nc, data not collected.

|   | Wildfires                |            |                           |                        |                          |            | Spring Burns             |             |                          |            |
|---|--------------------------|------------|---------------------------|------------------------|--------------------------|------------|--------------------------|-------------|--------------------------|------------|
|   | PFY 1                    |            | PFY 2                     |                        | PFY 3                    |            | PFY 1                    |             | PFY 2                    |            |
|   | B                        | U          | B                         | U                      | B                        | U          | B                        | U           | B                        | U          |
| <b>Upland</b>                           |                          |            |                           |                        |                          |            |                          |             |                          |            |
| <i>Carex spp.</i>                       | 1.3 (1.5) <sup>*a</sup>  | -3.9 (0.3) | -0.2 (0.1) <sup>*ab</sup> | -3.1 (0.6)             | -1.7 (0.5) <sup>b</sup>  | -3.1 (0.5) | -2.3 (0.4) <sup>a</sup>  | -2.5 (0.4)  | -1.8 (0.3) <sup>oa</sup> | -2.9 (0.3) |
| <i>Physocarpus malvaceus</i>            | -0.2 (0.6) <sup>ab</sup> | -1.6 (0.9) | 1.0 (0.5) <sup>*a</sup>   | -1.2 (0.4)             | -1.3 (0.6) <sup>b</sup>  | -2.0 (0.6) | 1.1 (0.3) <sup>*a</sup>  | -0.5 (0.01) | 0.6 (0.4) <sup>oa</sup>  | -0.8 (0.6) |
| <i>Symphoricarpos spp.</i>              | 2.2 (0.8) <sup>*a</sup>  | -1.7 (0.8) | 0.9 (0.1) <sup>*ab</sup>  | -1.9 (0.8)             | 0.3 (0.6) <sup>ob</sup>  | -1.7 (1.3) | 0.5 (0.4) <sup>*a</sup>  | -1.2 (0.5)  | 0.0 (0.2) <sup>*a</sup>  | -2.0 (0.9) |
| <i>Spiraea betulifolia</i>              | 1.7( 0.6) <sup>*a</sup>  | -0.7 (0.5) | 1.7 (0.2) <sup>*a</sup>   | -1.5 (0.3)             | 0.4 (0.4) <sup>*b</sup>  | -1.2 (0.4) | 1.1 (0.5) <sup>oa</sup>  | -0.5 (0.6)  | 0.7 (0.6) <sup>a</sup>   | -0.2 (0.7) |
| <i>S. betulifolia</i> root <sup>x</sup> | nc                       | nc         | -1.0 (0.1) <sup>a</sup>   | -2.7 (0.5)             | -2.3                     | -2.4       | nc                       | nc          | nc                       | nc         |
| <i>S. betulifolia</i> NRA <sup>x</sup>  | nc                       | nc         | 1.6 (0.3) <sup>a</sup>    | 0.5 (0.1) <sup>b</sup> | 0.6 (0.1)                | 0.5 (0.1)  | nc                       | nc          | 0.8 (0.3)                | 0.7 (0.2)  |
| <b>Riparian</b>                         |                          |            |                           |                        |                          |            |                          |             |                          |            |
| <i>Galium triflorum</i>                 | 0.5 (0.6) <sup>*a</sup>  | -1.4 (0.4) | 0.3 (0.5) <sup>*a</sup>   | -2.0 (0.3)             | -0.8 (0.6) <sup>*b</sup> | -2.6 (0.4) | 0.0 (0.3) <sup>*a</sup>  | -1.6 (0.5)  | -1.4 (0.2) <sup>ob</sup> | -2.4 (0.4) |
| <i>Circaea alpina</i>                   | -0.6 (0.9) <sup>a</sup>  | -2.0 (0.7) | 0.4 (0.8) <sup>*a</sup>   | -2.7 (0.5)             | 1.0 (1.5) <sup>*a</sup>  | -3.3 (0.5) | -0.7 (0.4) <sup>*a</sup> | -2.7 (0.2)  | -1.8 (0.4) <sup>*a</sup> | -3.8 (0.6) |
| <i>Cornus stolonifera</i>               | -1.8 (0.4) <sup>a</sup>  | -1.8 (0.5) | -0.7 (0.5) <sup>b</sup>   | -1.9 (0.5)             | -0.4 (0.5) <sup>ob</sup> | -1.8 (0.6) | -1.2 (0.6) <sup>a</sup>  | -1.8 (0.8)  | -1.4 (0.3) <sup>a</sup>  | -2.4 (0.6) |
| <i>Rubus/Ribes spp.</i>                 | -0.8 (0.4) <sup>a</sup>  | -0.5 (0.4) | 0.3 (0.5) <sup>oa</sup>   | -1.3 (0.7)             | 0.1 (0.7) <sup>*a</sup>  | -1.7 (0.5) | -0.9 (0.5) <sup>a</sup>  | -0.2 (0.9)  | -0.8 (0.5) <sup>a</sup>  | -0.5 (0.5) |
| <b>In-stream moss</b>                   | 0.2 (0.2) <sup>a</sup>   | -0.3 (0.5) | 1.8 (0.4) <sup>*b</sup>   | 0.5 (0.4)              | 0.9 (0.2) <sup>*ab</sup> | -1.2 (0.2) | 0.0 (0.7) <sup>a</sup>   | 1.3 (0.7)   | 0.9 (0.5) <sup>a</sup>   | 0.0 (0.5)  |

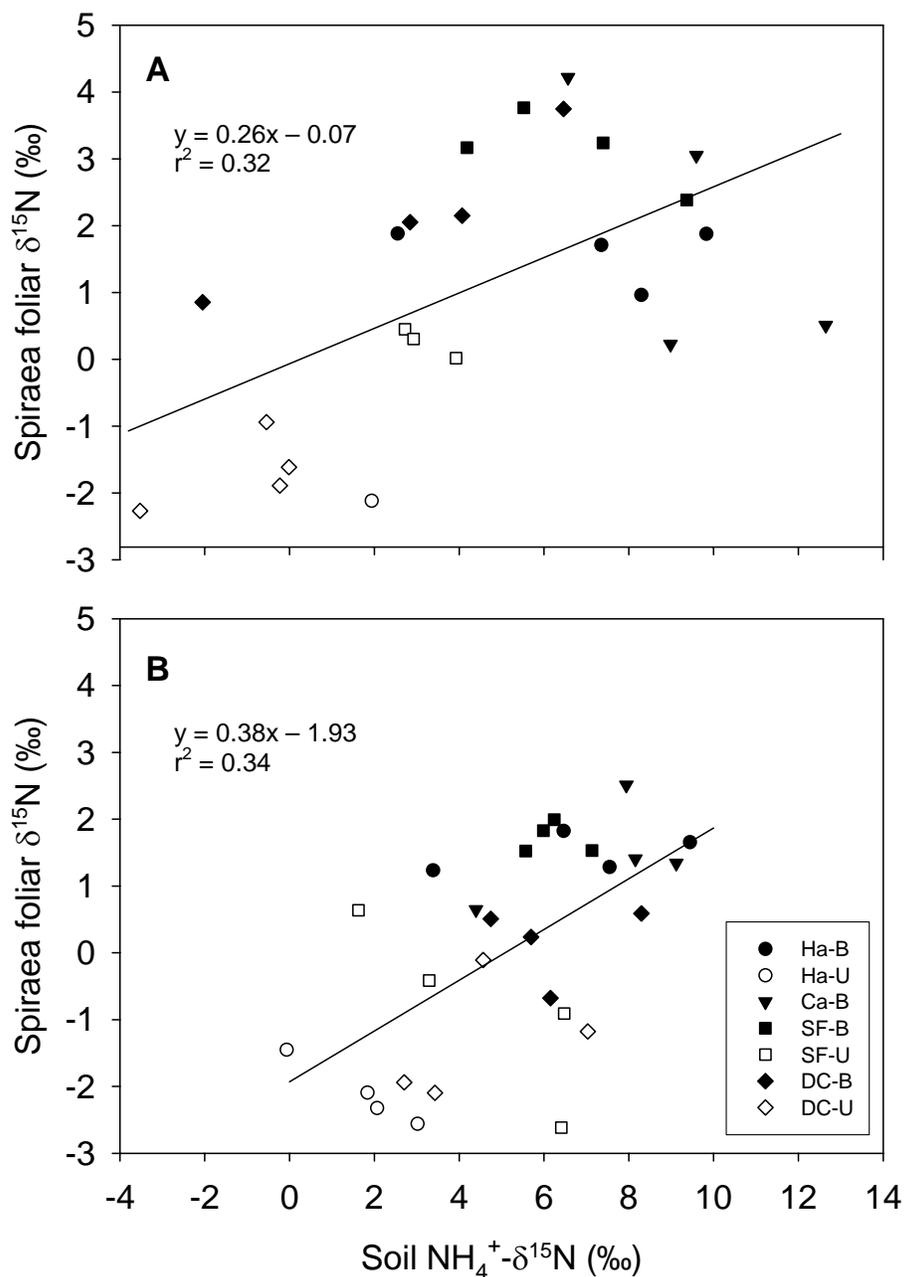
**Table 2.** Mean  $\delta^{15}\text{N}$  (‰) values of soil inorganic N in burned and unburned watersheds at two sample dates. One SE is given in parentheses ( $n = 4$  sites), each  $n$  represents the average of generally four subsamples<sup>x,y</sup>. <sup>x</sup> Value represents only one plot from one watersheds, <sup>y</sup> value represents only six plots from two watersheds, hence statistical analysis of treatment differences not performed. <sup>a,b</sup> Superscripts denote significant treatment difference at  $P \leq 0.05$ .

|  | Wildfires              |                        | Spring burns |                        |
|--|------------------------|------------------------|--------------|------------------------|
|  | Burned                 | Unburned               | Burned       | Unburned               |
| $\text{NH}_4^+ \delta^{15}\text{N}$ , Aug 2004 | 6.5 (1.4) <sup>a</sup> | 0.7 (0.7) <sup>b</sup> | 2.5 (0.4)    | 1.2 (0.9)              |
| $\text{NH}_4^+ \delta^{15}\text{N}$ , Oct 2005 | 7.0 (0.2) <sup>a</sup> | 3.8 (0.6) <sup>b</sup> | 6.4 (1.4)    | 5.2 (0.6)              |
| $\text{NO}_3^- \delta^{15}\text{N}$ , Oct 2005 | 2.4 (1.4)              | -0.2 <sup>x</sup>      | 1.9 (1.1)    | 0.4 (3.1) <sup>y</sup> |

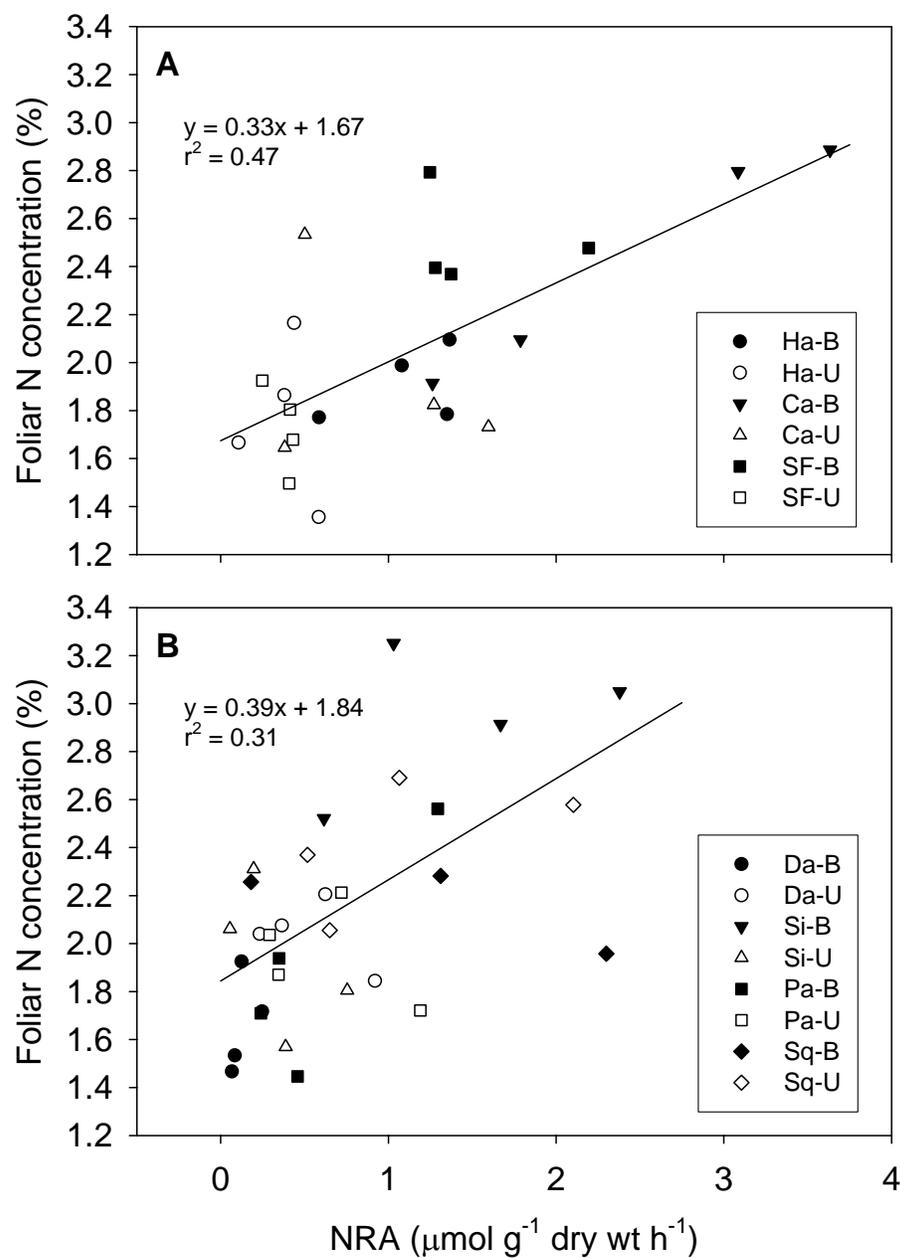
**Figure 1.** Foliar  $\delta^{15}\text{N}$  values across all a) upland and b) obligate riparian species in burned (B) and unburned (U) watersheds of spring burns (P) and wildfires (W). Error bars represent 1 SE across four species. Each species' value is the average across generally four sites; the variability across sites per species is presented in Table 1. Data for the fourth post-fire year represents only the Danskin Creek wildfire site. Note that averaging across species obscures species  $\times$  treatment interactions to some extent (see text).



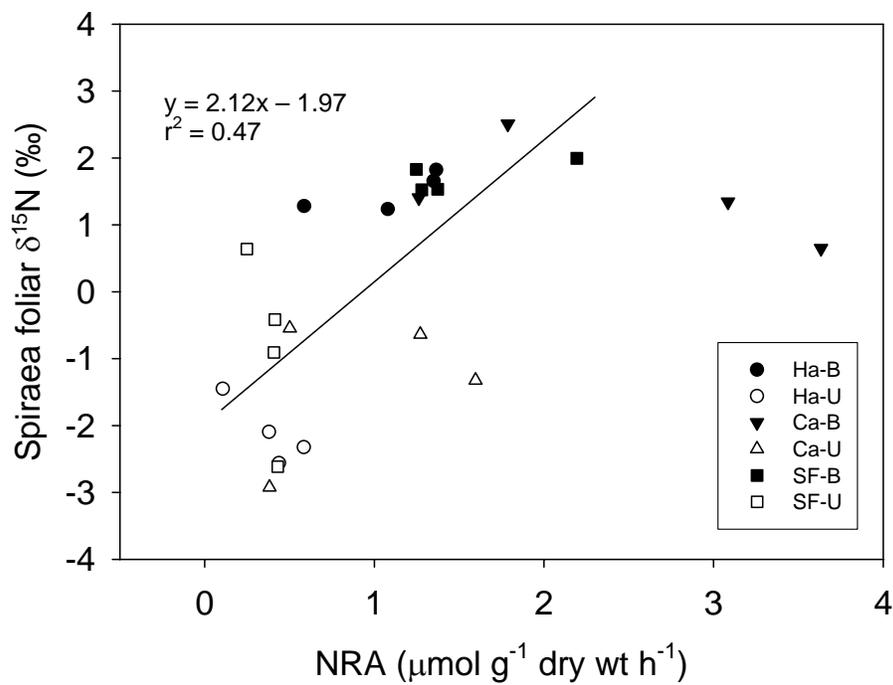
**Figure 2.** Relationship between soil  $\text{NH}_4^+ - \delta^{15}\text{N}$  collected in a) August 2004 and b) October 2005 and spiraea foliar  $\delta^{15}\text{N}$  in the three 2003 wildfire sites. Each data point represents one plot since variability within watersheds was higher than variation between watersheds. Open and filled symbols represent unburned (U) and burned (B) plots, respectively. Ha, Hall site, Ca, Canyon Creek site, SF, South Fork site, DC, Danskin Creek site. No data was available for Ca-U.



**Figure 3.** Relationship between spiraea foliar nitrate reductase activity (NRA) and spiraea foliar N concentration in a) the three 2003 wildfire sites and b) spring burn sites in the second post-fire year. See Figure 2 for explanations of legend.



**Figure 4.** Relationship between spiraea foliar nitrate reductase activity (NRA) and spiraea foliar  $\delta^{15}\text{N}$  in the three 2003 wildfire sites in the second post-fire year. Regression line excludes the two points with the highest NRA values. See Figure 2 for explanations of legend.



## CHAPTER 5

### CONCLUSIONS

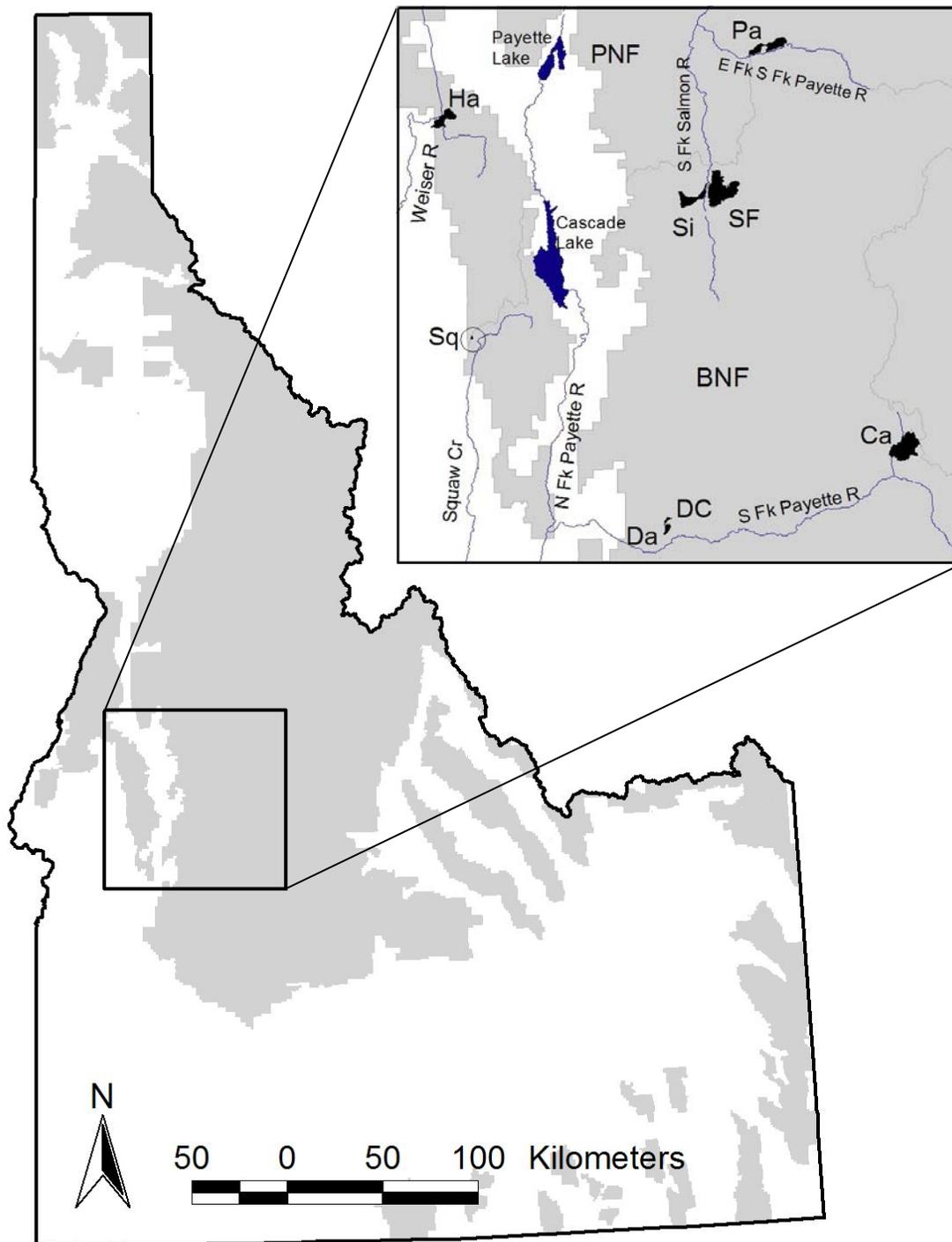
The watershed-ecosystem approach furthered the understanding of post-fire N dynamics between the terrestrial and aquatic ecosystem components. In addition to complex interactions of inorganic N production, consumption, and transport, watershed N cycling has a strong seasonality as a result of the seasonal moisture and temperature regimes. Changes in temperature and moisture influence the activity of photosynthetic and heterotrophic organisms and the hydrologic connectivity of upland soils and streams. After a severe fire, terrestrial N cycling mechanisms are profoundly altered in the short term, most noticeably reflected in hydrologic  $\text{NO}_3^-$  export with snowmelt. More studies are necessary on gross rates of microbial N transformations after fire. When collected during the growing season, these data could provide the mechanism for often observed post-fire increases in soil inorganic N and higher foliar N concentrations. Studying gross N transformations during winter would contribute to a better understanding of the causes of the spring  $\text{NO}_3^-$  flush into streams. In this respect, more research is needed on the role of hydrological connectivity with uplands in determining streamwater chemistry. This would be the initial step toward quantifying the contribution of in-stream N retention and mineralization to the hitherto assumed terrestrial imprint on streamwater chemistry.

The use of stable N isotopes at natural abundance has proven to be a useful tool in tracking a fire-induced signal in the soil to subsequent N pools. Overcoming analytical challenges in analyzing  $\delta^{15}\text{N}$  of small and variable pools of soil inorganic N is key for improving understanding of N cycling after fire and in general. Knowing the  $\delta^{15}\text{N}$  values of plant available  $\text{NH}_4^+$  and  $\text{NO}_3^-$  and a better understanding of plant internal fractionations as well as the role of mycorrhizae in determining plant foliar  $\delta^{15}\text{N}$  will improve the utility of foliar  $\delta^{15}\text{N}$  values as indicators of the degree to which N dynamics are altered after fire. Finally, long-term data on post-fire N dynamics are lacking, especially at the watershed scale. Additionally, the extent of legacy effects of fire suppression on currently observed post-fire N cycling is unknown. These future research questions will be a challenge in the light of impending climate change. At the same time this research will be critical for predicting the future of our natural resources.

# **APPENDICES**

**APPENDIX A.** Map of study sites.

Shaded areas denote National Forest land (BNF, Boise National Forest; PNF, Payette National Forest). Areas in black denote fire sites. Wildfire sites are Hall (Ha), Canyon Creek (Ca), South Fork (SF), and Danskin Creek (DC); spring prescribed burn sites are Danskin Creek (Da), Sixbit (Si), and Parks-Eiguren (Pa); and the spring test burn site is Squaw Creek (Sq).



**APPENDIX B.** Description of study sites.

Soil, plants, streamwater and aquatic biota were sampled in watersheds labeled B1 (burned) and U1 (unburned). Additional streamwater samples were collected from watersheds B2/U2.

<sup>a</sup>Two additional watersheds were sampled; <sup>b</sup>Danskin Creek wildfire and prescribed burn sites shared the same unburned watersheds; <sup>c</sup>Squaw Creek burned and unburned areas were adjacent to each other in the same watershed.

|                                  | Wildfires                 |                       |                           |                           | Spring Prescribed Burns   |                           |                           | Test Burn             |
|----------------------------------|---------------------------|-----------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|-----------------------|
|                                  | Canyon Creek              | Hall Fire             | South Fork                | Danskin Creek             | Danskin Creek             | Parks-Eiguren             | Sixbit                    | Squaw Creek           |
| Fire Name                        | Canyon Creek              | Hall Fire             | South Fork                | Danskin Creek             | Danskin Creek             | Parks-Eiguren             | Sixbit                    | Squaw Creek           |
| Abbreviation                     | Ca                        | Ha                    | SF                        | DC                        | Da                        | Pa                        | Si                        | Sq                    |
| National Forest                  | Boise NF                  | Payette NF            | Boise NF                  | Boise NF                  | Boise NF                  | Payette NF                | Boise NF                  | Boise NF              |
| Ranger District                  | Lowman                    | Council               | Cascade                   | Emmett                    | Emmett                    | Krassel                   | Cascade                   | Emmett                |
| Time of Fire                     | Aug 2003                  | Aug 2003              | Aug 2003                  | Jul 2002                  | Apr 2004                  | May 2004                  | May 2004                  | May 2004              |
| Coordinates of B1 (NAD27, UTM11) | E 641605<br>N 4895979     | E 551277<br>N 4965338 | E 600478<br>N 4951091     | E 595393<br>N 4883615     | E 595320<br>N 4882216     | E 613360<br>N 4979754     | E 599883<br>N 4948290     | E 555770<br>N 4920642 |
| Mean Elevation (B1/U1), m        | 2100/2190                 | 1420/1400             | 1940/1770                 | 1440/1540                 | 1410/1540                 | 1880/1840                 | 1960/1790                 | 1470 <sup>c</sup>     |
| Watershed aspect B1/U1           | NW/W                      | W/W                   | W/SW                      | N-NW/NW                   | W-SW/NW                   | S-SE/S-SW                 | SE/S                      | S <sup>c</sup>        |
| Watershed area B1/U1, ha         | 115/38                    | 143/81                | 82/48                     | 8/13                      | 53/13 <sup>b</sup>        | 89/35                     | 33/12                     | 37 <sup>c</sup>       |
| Watershed area B2/U2, ha         | 153/49                    | 105/480               | 125/71                    | 8,7/11,17 <sup>a</sup>    | 44/11,17 <sup>b</sup>     | 46/189                    | 50/97                     | NA                    |
| Distance B1-U1, m                | 6800                      | 600                   | 9500                      | 650                       | 1600                      | 8500                      | 5500                      | 100 <sup>c</sup>      |
| Bedrock Type                     | Idaho Batholith Granitics | Columbia River Basalt | Idaho Batholith Granitics | Columbia River Basalt |

**APPENDIX C.** Photographs of a spring prescribed burn site (Sixbit) (a) and a wildfire site (Canyon Creek) (b) representing the fire severities typical for the spring prescribed burns and wildfires of this study; the Squaw Creek spring test burn site is shown in (c). (Photographs by K. Stephan.)



APPENDIX C. Continued.



**APPENDIX D.** Suitability of the diffusion method for low concentrations of ammonium and nitrate in KCl extracts for  $^{15}\text{N}$  analysis at natural abundance.

**SUITABILITY OF THE DIFFUSION METHOD FOR LOW CONCENTRATIONS OF AMMONIUM  
AND NITRATE IN KCL EXTRACTS FOR  $^{15}\text{N}$  ANALYSIS AT NATURAL ABUNDANCE**

**ABSTRACT**

We tested the suitability of the diffusion method, commonly applied to samples containing  $^{15}\text{N}$  at tracer level, for KCl extracts containing low amounts of ammonium and nitrate at natural  $^{15}\text{N}$  abundance. We experimentally assessed the effects of N contamination from reagents and incomplete N recovery from the KCl solution on target- $\delta^{15}\text{N}$  values, and we discuss based on published literature the potential for decomposing labile soil organic N to alter target- $\delta^{15}\text{N}$  values. We found contamination in reagents to be negligible (0.8-2 % of total sample-N) for ammonium diffusions but to be considerable (7-13.4 % of total sample-N) in nitrate diffusions containing 50  $\mu\text{g}$  target-N. Ignoring the isotopic values of contaminants, which we found to be depleted by 10 ‰ relative to target- $\delta^{15}\text{N}$ , in nitrate diffusions would have resulted in underestimating target- $\delta^{15}\text{N}$  by 0.8 to 1.6 ‰ depending on the amounts of reagents used. With incomplete recovery of sample N, the resulting underestimation of sample- $\delta^{15}\text{N}$  can be easily calculated. Breakdown of organic N can be a problem with prolonged diffusion durations, increased temperatures, and in the presence of Devarda's alloy (in nitrate diffusions). We conclude that the diffusion method is an appropriate tool for analyzing the  $^{15}\text{N}$  concentration at natural abundance of soil ammonium while other methods might represent better alternatives for soil nitrate- $\delta^{15}\text{N}$ .

## INTRODUCTION

Knowing the isotopic signature of inorganic nitrogen (N) in soil is an important component in understanding nitrogen cycling processes. In particular, the accurate determination of soil ammonium ( $\text{NH}_4^+$ -N) and nitrate ( $\text{NO}_3^-$ -N)  $\delta^{15}\text{N}$  values would greatly improve the ability to identify or model rates of soil N transformations *in situ* (Garten and Van Miegroet 1994; Houlton et al. 2006), plant N sources (Koba et al. 2003), or plant-mycorrhizal interactions (Hobbie and Hobbie 2006). Because of methodological difficulties for measuring soil ammonium and nitrate  $\delta^{15}\text{N}$  values directly, researchers often draw inferences from plant foliar  $\delta^{15}\text{N}$  (Grogan et al. 2000) or bulk soil  $\delta^{15}\text{N}$  (Garten, 1993). Several methods are used to isolate and concentrate inorganic N from solutions for isotopic analysis. Steam distillation (Mulvaney 1993, and references therein) is the oldest. However, this method has several limitations; it is labor intensive, there is potential for fractionation due to incomplete distillation or loss of N during collection (Mulvaney 1993) and organic N might decompose at the high distillation temperatures (Mulvaney and Khan 1999; Saghir et al. 1993a). Thus, steam distillation has been largely replaced by the diffusion method (also known as micro-diffusion). Brooks et al. (1989) described and tested the diffusion method for determining N isotopes in KCl soil extracts with low N and Stark and Hart (1996) expanded the method assessment to inorganic N in salt solutions, Kjeldahl digests and persulfate digests.

Most commonly, as in Brooks et al. (1989), Stark and Hart (1996), Saghir et al. (1993a, 1993b), and Mulvaney et al. (1997), the diffusion method has been applied to soil extracts at  $^{15}\text{N}$  tracer level. While not in soil, marine scientists have successfully developed diffusion protocols for determination of isotopic values of oceanic or freshwater ammonium (Holmes et al., 1998) and nitrate (Sigman et al. 1997) at natural  $^{15}\text{N}$  abundance. In soil N studies, however, the diffusion method at natural  $^{15}\text{N}$  abundance has only been occasionally applied (e.g., Robinson and Conroy 1999; Koba et al. 2003; Pritchard and Guy 2005; Hobbie and Hobbie 2006). The hesitation in adopting the diffusion method at natural  $^{15}\text{N}$  abundance is founded in uncertainties related to error associated with incomplete recovery, alteration of target- $\delta^{15}\text{N}$  by contamination from reagents or breakdown of labile soil organic N (Robinson 2001). These uncertainties have either been only partially addressed or ignored in the studies to date, rendering the results susceptible to criticism.

There are a few non-diffusion methods to determine the natural abundance isotopic values of inorganic N. Those involving ion exchange resins (Lehman et al. 2001; Silva et al. 2000; Stickrod and Marshall 2000) are suitable for freshwater but not for KCl soil extracts because their high ion concentrations interfere with the ion exchange capacity of the resin. Another approach suitable for soil extracts and fresh- and ocean water involves incorporating  $\text{NO}_3^-$  or  $\text{NH}_4^+$  into an organic

derivative that can be selectively isolated from the bulk sample. The derivatization methods developed by Johnston et al. (1999, 2003), though desirably target specific, require corrections for fractionation during derivatization (for  $\text{NO}_3^-$  method) or corrections for reagent blanks ( $\text{NH}_4^+$  method). Presumably due to the complex chemistry of derivatization, soil ecologists have not yet adopted it. Another relatively new and reliable method to analyze nitrate- $\delta^{15}\text{N}$  is the denitrifier method (Sigman et al. 2001) developed for fresh- and ocean water samples. This method involves cultivating denitrifying bacteria that convert nitrate to nitrous oxide which is trapped and subsequently analyzed with an isotope ratio mass spectrometer (IRMS). The major advantage of this method is that only very small amounts of N (10-20 nmoles) are needed. To date, the denitrifier method is used or being established in a number of laboratories only few of which also provide this service to users. The cost of this service, however, is substantial (ca \$200 per sample). A novel counterpart to the denitrifier method is the chemical conversion of nitrate and nitrite to nitrous oxide using sodium azide (McIlvin and Altabet 2005). The advantages of this method include no need to maintain bacterial cultures and the applicability to a wider range of sample types. The main disadvantage is the volatility and toxicity of azide. Thus, even though the denitrifier and azide methods might be applicable to the analysis soil nitrate- $\delta^{15}\text{N}$ , there is no reliable, readily applied alternative to the diffusion method for analyzing soil ammonium- $\delta^{15}\text{N}$  at natural abundance.

The diffusion method has several advantages compared to derivatization, denitrifier and azide methods. It is relatively simple, inexpensive and non-toxic. Because of these advantages we rigorously assessed the suitability of the diffusion method for determining nitrate and ammonium isotopic values at natural abundance  $^{15}\text{N}$  in low-N soil or resin KCl extracts. We experimentally addressed two of the aforementioned uncertainties of the diffusion method by quantifying (I) N contamination of reagents, (II)  $\delta^{15}\text{N}$  of contaminants, and (III) error in sample  $\delta^{15}\text{N}$  due to incomplete recovery. Our goal was to provide means for correcting for those three sources of error in order to accurately estimate target ammonium- and nitrate- $\delta^{15}\text{N}$ . The last uncertainty, i.e., breakdown of organic N in natural samples, will be discussed based on published literature although it was not relevant to our inorganic experimental KCl solutions.

## **MATERIALS AND METHODS**

### **General Outline of the Procedure**

We diffused ca 50  $\mu\text{g}$  of standard ammonium-N (as  $(\text{NH}_4)_2\text{SO}_4$ ) or nitrate-N (as  $\text{KNO}_3$ ),

respectively, in various volumes of 2 M KCl (20 mL to 120 mL) in either 150-mL or 240-mL polypropylene vessels. Magnesiumoxide (MgO), a mild alkali, is added to ammonium diffusions to raise the pH above 9.2 to 9.5 at which ammonium dissociates. The resulting ammonia gas diffuses into the headspace of the tightly sealed diffusion vessel and subsequently onto two acidified quartz fiber filter discs (referred to as an acid trap) sandwiched between the gas permeable PTFE (Teflon) tape floating on top of the sample solution. Devarda's alloy (DA) and MgO are added to nitrate diffusions. The alloy catalyzes the reduction of  $\text{NO}_3^-$  to  $\text{NH}_3$  and MgO raises the pH which aids in the  $\text{NO}_3^-$  reduction and  $\text{NH}_3$  diffusion into the headspace. Prior to nitrate diffusion, sample- or reagent- $\text{NH}_4^+$ -N (if present) is removed by diffusing it into the air or trapping it onto filter discs. The amounts of MgO and Devarda's alloy added were adopted from Holmes et al. (1998) (3 g MgO per 1 L of sample) and Sigman et al. (1997) (0.2 g Devarda's alloy per 50 mL of sample). Prior to use, MgO was ashed to remove potential N contamination. Diffusion durations were 1 to 10 d or 6 d depending on the experimental goal. Most experiments were carried out at room temperature but a few experiments were carried out at higher temperatures in order to increase recovery. Vessels were gently swirled and inverted every other day starting on day one. After termination of diffusions filter discs were dried and packed into tin capsules and analyzed for total N and isotopic value with a continuous-flow direct combustion IRMS at the University of Idaho Stable Isotope Laboratory (ISIL) (natural abundance samples; Finnigan Delta Plus, Finnigan MAT, Bremen, Germany) and at UC Davis Stable Isotope Facility (tracer samples; Europa Scientific Integra, PDZ Europa Ltd., Crewe, UK). At ISIL, repeated analysis of the internal acetanilide standard ( $\delta^{15}\text{N} = 0.04 \text{ ‰}$ , N concentration = 10.36 %) for both isotopic value and N concentration had standard deviations of  $< 0.2$ . A baseline for standard- $\delta^{15}\text{N}$  values and N amount added to diffusions was provided by directly pipetting the same volume of standard-N solution used in diffusions onto acidified (for  $\text{NH}_4^+$ -N) or plain (for  $\text{NO}_3^-$ -N) filter discs. These non-diffused standards were analyzed as described above. More details on methods are provided with the description of the specific experiments (see below) and in an annotated protocol available at request. Throughout the paper we will use the terms sample-N, target-N and reagent-N in the following definitions. Sample-N refers to all dissolved inorganic N in the diffusion vessel; it is the sum of target-N and reagent-N. Target-N refers to the inorganic N form of interest in soil extracts; it is synonymous with standard-N in our experiments. Reagent-N refers to N contamination in reagents and is synonymous with blank-N (or short: blank). In actual soil or resin extracts there are sources other than reagents for contamination, i.e., breakdown of organic N and resin impurities, respectively. The former will be discussed at the end of the paper, the latter needs to be quantified in the same way as reagent-N described below.

## I. Quantifying Contamination

KCl solution (J.T. Baker, 2 M KCL) was analyzed colorimetrically for its N concentration. KCl samples of several lots used during this study were analyzed with a Lachat QuikChem 8000 (Zellweger Analytics Inc., Milwaukee, Wisconsin, USA, method 31-107-06-5-A for ammonia, method 31-107-04-1-A for nitrate + nitrite) at the Marine Science Institute (MSI), UC Santa Barbara. The deionized (DI) water used to prepare KCl solutions was produced by a customized in-house filtering system (ion exchange resins/ charcoal/ UV light/ 0.2  $\mu\text{m}$  filter, 9 M $\Omega$ ) and was also analyzed colorimetrically at MSI for possible N contamination.

### I.a Contamination in Ammonium Diffusions

In ammonium diffusions, the only other source of contaminant- $\text{NH}_4^+\text{-N}$  (besides KCl and DI water) is MgO.

**Experiment 1: Determining  $\text{NH}_4^+\text{-N}$  contamination in MgO (MgO- $\text{NH}_4^+\text{-N}$ ) with tracer-dilution method.** If contaminant-N, assumed to be at natural abundance of ca 0.366 atom %, is present in MgO it would dilute the  $^{15}\text{N}$  concentration of a highly enriched standard-N. Therefore, 5  $\mu\text{L}$  of 8,000 ppm (i.e., 40  $\mu\text{g}$ ) tracer standard- $\text{NH}_4^+\text{-N}$  at 4.8 atom % were pipetted into 20, 40, 60, 80, and 160 mL 2 M KCL ( $n = 3$  per volume). Approximately 0.25 g MgO were added per replicate and samples were diffused for 6 d at room temperature. Additionally, non-diffused standards were prepared by pipetting 5  $\mu\text{L}$  of the tracer standard-N directly onto filter discs.

The amount of total N contamination can be quantified by rearranging a mixing model (Kelley et al., 1991; Stark and Hart, 1996) (Eq. [1]).

$$M_b = M_{\text{std}}(E_m - E_{\text{std}}) / (E_b - E_m) \quad [1]$$

$M_b$  is the mass of total blank-N (i.e., reagent-N),  $M_{\text{std}}$  is the mass of N added with the standard,  $E_{\text{std}}$  is the  $^{15}\text{N}$  enrichment in non-diffused standards,  $E_m$  is the enrichment measured by diffusing standards, and  $E_b$  is the enrichment of the blank, assumed to be 0.366 atom %. Calculated blanks ( $M_b$ , Eq. [1]), representing the total  $\text{NH}_4^+\text{-N}$  contamination of KCl solution and MgO, were regressed against the amount of KCl. The intercept of the regression yields the amount of  $\text{NH}_4^+\text{-N}$  contamination from MgO.

**Experiment 2: Determining  $\text{NH}_4^+$ -N contamination in MgO (MgO- $\text{NH}_4^+$ -N) with measured blank.** We also measured MgO- $\text{NH}_4^+$ -N directly. Two g MgO (about 8-10 fold of regular amount) were added to 50 mL KCl without adding any standard- $\text{NH}_4^+$ -N and samples were diffused for 6 days at 34 °C (n = 3). Acid traps were analyzed for total N, based on the magnitude of the ion beam from the mass spectrometer. The known contribution from KCl- $\text{NH}_4^+$ -N (determined colorimetrically) was subtracted from the N on the acid trap; the difference represents  $\text{NH}_4^+$ -N contamination in MgO.

### **I.b Contamination in Nitrate Diffusions**

In nitrate diffusions, sources of contaminant- $\text{NO}_3^-$ -N (besides KCl and DI water) are MgO and Devarda's alloy. The actual N form in Devarda's alloy is not known but we will refer to it as reagent- $\text{NO}_3^-$ -N because it would only affect nitrate diffusions. In the following experiments, reagent- $\text{NH}_4^+$ -N had either been removed prior to nitrate diffusions (Exp. 3) or, if not, the known amounts of reagent- $\text{NH}_4^+$ -N have been subtracted from total reagent-N in order to assess reagent- $\text{NO}_3^-$ -N (Exp. 4).

**Experiment 3: Determining  $\text{NO}_3^-$ -N contamination in MgO and N contamination in Devarda's alloy combined (MgO- $\text{NO}_3^-$ -N + DA-N) with tracer dilution and using variable amounts of KCl.** This experiment is analogous to Experiment 1. Three replicates each of 20, 40, 60, 80, and 160 mL of 2 M KCl containing 40  $\mu\text{g}$  of tracer standard- $\text{NO}_3^-$ -N (4.93 atom %) were diffused at room temperature for 6 d under addition of 0.25 g MgO and 0.2 g Devarda's alloy (following a prior 6 d open period with 0.25 g MgO). Calculated blanks ( $M_b$ , Eq. [1]) were regressed against the amount of KCl. The intercept of the regression yields the amount of  $\text{NO}_3^-$ -N contamination from MgO and Devarda's alloy combined.

**Experiment 4: Determining  $\text{NO}_3^-$ -N contamination in MgO (MgO- $\text{NO}_3^-$ -N) and N contamination in Devarda's alloy (DA-N) with tracer dilution and using variable amounts of MgO and Devarda's alloy.** In order to tell apart how much contaminant- $\text{NO}_3^-$ -N originates from either MgO or Devarda's alloy, 37  $\mu\text{g}$  tracer  $\text{NO}_3^-$ -N at 4.98 atom % were diffused with different amounts of the two reagents in 50 mL KCl without prior open period for 6 days at room temperature. In the MgO experiment, three replicates each with 0.25 g, 0.5 g, 1.5 g and 3 g MgO were diffused with a fixed amount (0.2 g) of Devarda's alloy. In the Devarda's alloy experiment, three replicates each with 0.2 g, 1.5 g, and 3 g Devarda's alloy were diffused with a fixed amount (0.25 g) of MgO. Using Eq. [1], the total N contamination of reagents ( $M_b$ ) was calculated and the known contributions of KCl- $\text{NO}_3^-$ -N (from colorimetric analysis) and reagent- $\text{NH}_4^+$ -N (KCl- $\text{NH}_4^+$ -N from colorimetric

analysis and  $\text{MgO-NH}_4^+\text{-N}$  from Exp. 2) were subtracted. Resulting values represent the sum of  $\text{MgO-NO}_3^-\text{-N}$  and  $\text{DA-N}$ ; they were plotted and regressed against the respective amount of the variable reagent. The y-intercept of each regression line yields the  $\text{NO}_3^-\text{-N}$  contribution of the fixed reagent; slopes estimate the  $\text{NO}_3^-\text{-N}$  contribution per 1 g of the variable reagent. Thus, each of the two regression lines (i.e., one with variable  $\text{MgO}$  amount and the other with variable Devarda's alloy amount on the x-axes) yields independent solutions for both the amounts of  $\text{MgO-NO}_3^-\text{-N}$  and  $\text{DA-N}$ .

## II. $\delta^{15}\text{N}$ of Contaminants

**Experiment 5: Determining the proportional contribution and  $\delta^{15}\text{N}$  values of contaminants ( $\text{KCl-NO}_3^-\text{-N}$ ,  $\text{MgO-NO}_3^-\text{-N}$ ,  $\text{DA-N}$ ) in nitrate diffusions.** Samples with large amounts of reagents but no addition of standard- $\text{NO}_3^-\text{-N}$  were diffused in order to recover enough reagent- $\text{NO}_3^-\text{-N}$  for isotopic analysis. Table 1 summarizes the diffusion conditions and the reagent amounts per diffusion vessel. In Exp. 5a the amount of reagent-N captured in acid traps was either not or just barely sufficient to be within linear (i.e., precise) range of the IRMS. Therefore, Exp. 5b was conducted, where acid traps of two diffusion vessels were combined in order to produce a larger ion beam.

Using simple algebra, the measured amounts of reagent-N in Exp. 5a-I and II, or 5b-I and II, (see Table 1) are used to determine the proportion (P) of N in  $\text{MgO}$  and in Devarda's alloy (Eq. [2]), and to calculate the proportional contributions (PC) of  $\text{MgO-NO}_3^-\text{-N}$  and  $\text{DA-N}$  to the total measured reagent-N (Eq. [3]). Finally, using the measured isotopic values of the total reagent-N, three mass balance equations with three unknowns were solved for the isotopic values of each reagent's N (Eq. [4]). Each of the three mass balance equations represents a certain combination of reagent amounts, i.e. equation I is from Exp. 5a-I (or 5b-I), equation II is from Exp. 5a-II (or 5b-II), and equation III is from Exp. 5c). Ideally, an open period prior to nitrate diffusion would have removed reagent- $\text{NH}_4^+\text{-N}$ . Due to the lack of such an open period in Exp. 5a and 5b,  $\text{MgO-}$  and  $\text{KCl-NH}_4^+\text{-N}$  will have been measured along with the reagent- $\text{NO}_3^-\text{-N}$ . We did not attempt to account for reagent- $\text{NH}_4^+\text{-N}$  because of its small contribution (see results).

$$\text{I } M_b = m_{\text{MgO}} * P_{\text{MgO-NO}_3\text{-N}} + m_{\text{DA}} * P_{\text{DA-N}} + m_{\text{KCl}} * P_{\text{KCl-NO}_3\text{-N}} \quad (5a \text{ or } 5b\text{-I}) \quad [2]$$

$$\text{II } M_b = m_{\text{MgO}} * P_{\text{MgO-NO}_3\text{-N}} + m_{\text{DA}} * P_{\text{DA-N}} + m_{\text{KCl}} * P_{\text{KCl-NO}_3\text{-N}} \quad (5a \text{ or } 5b\text{-II})$$

$M_b$  is the mass of measured N blank,  $m$  is the mass of the reagent, and  $P$  is the proportion of N in the reagent (mass N in reagent / mass reagent). The two equations are solved for  $P_{\text{MgO-NO}_3\text{-N}}$  and  $P_{\text{DA-N}}$ ;  $P_{\text{KCl-NO}_3\text{-N}}$  is known from colorimetric analysis. Exp. 5c served as validation for the algebraic solutions

of Eqs.[2] that are based on Exp. 5a and 5b.

$$\begin{aligned} PC_{MgO-NO_3-N} &= m_{MgO} * P_{MgO-NO_3-N} / M_b \\ PC_{DA-N} &= m_{DA} * P_{DA-N} / M_b \\ PC_{KCl-NO_3-N} &= m_{KCl} * P_{KCl-NO_3-N} / M_b \end{aligned} \quad [3]$$

PC is the proportional contribution of each reagent's N to the total measured blank.

$$\begin{aligned} \text{I } E_b &= PC_{MgO-NO_3-N} * \delta_{MgO-NO_3-N} + PC_{DA-N} * \delta_{DA-N} + PC_{KCl-NO_3-N} * \delta_{KCl-NO_3-N} \quad (5a \text{ or } 5b-I) \\ \text{II } E_b &= PC_{MgO-NO_3-N} * \delta_{MgO-NO_3-N} + PC_{DA-N} * \delta_{DA-N} + PC_{KCl-NO_3-N} * \delta_{KCl-NO_3-N} \quad (5a \text{ or } 5b-II) \\ \text{III } E_b &= PC_{MgO-NO_3-N} * \delta_{MgO-NO_3-N} + PC_{DA-N} * \delta_{DA-N} + PC_{KCl-NO_3-N} * \delta_{KCl-NO_3-N} \quad (5c) \end{aligned} \quad [4]$$

$E_b$  is the measured isotopic value of the total blank. The three equations are solved for the unknown isotopic value ( $\delta$ ) of each reagent's N.

### III. Error in $\delta^{15}N$ with Incomplete Recovery

#### Experiment 6: Diffusion of natural abundance standard-N using variable diffusion

**durations.** We tested the effect of incomplete recovery on  $\delta^{15}N$  values of diffused standards with sample volumes of 50 mL and 120 mL. These volumes were chosen because they contained sufficient N (especially  $NH_4^+$ ) for analysis in forest soil extracts and they represent the upper limit for diffusions with relatively short diffusion durations at room temperature and without constant shaking. Five  $\mu L$  of 10,000 ppm (i.e., 50  $\mu g$ , roughly twice the amount necessary for precise measurements with IRMS) standard- $NH_4^+$ -N or standard- $NO_3^-$ -N solution were pipetted into a diffusion vessel containing KCl solution. Approximately 0.25 g or 0.5 g MgO for 50-mL and 120-mL diffusion volumes, respectively, were added to each diffusion vessel. Standard- $NH_4^+$ -N was diffused at room temperature. Samples containing standard- $NO_3^-$ -N diffused openly for 6 d at 34 °C, followed by the addition of another 0.25 g of MgO and 0.2 g or 0.4 g Devarda's alloy (for 50-mL and 120-mL diffusion volumes, respectively) for the actual nitrate diffusion at room temperature. Three sets of samples were diffused at different times (Nov'05 = Set 1, Jan'06 = Set 2, Mar'06 = Set 3) with generally one replicate per N-form  $\times$  volume  $\times$  duration in each Set. Sets 1 and 3 were diffused in brand new vessels, Set 2 in re-used acid-washed containers. The 120-mL samples of Set 1 were diffused in 150-mL vessels, those of Set 2 and 3 in taller 240-mL vessels. Diffusions were terminated after 1, 2, 4, 6, 8, and 10 days, and acid traps were dried and analyzed. Non-diffused standards were prepared by pipetting 5  $\mu L$  standard-N onto

filter discs. Table 2 shows the measured N amounts and isotopic values of non-diffused standards and the isotopic values of the salts from which standard solutions were made.

Recoveries were calculated as

$$\text{Recovery} = 100 * (\text{observed sample-N mass} / \text{actual sample-N mass}). \quad [5]$$

Error in  $\delta^{15}\text{N}$  (i.e., underestimation) due to incomplete recovery is calculated as

$$\text{Error}_{\text{recovery}} = \text{actual sample-}\delta^{15}\text{N} - \text{observed sample-}\delta^{15}\text{N}. \quad [6]$$

The observed-sample value is the N measured in the acid traps comprising the diffused standard-N and reagent-N; the actual-sample value is the sum (for N mass) or weighted average (for  $\delta^{15}\text{N}$ ) of non-diffused standard-N and expected reagent-N. In nitrate diffusions, reagent- $\text{NO}_3^-$ -N is known from colorimetric analysis for KCl and from Exp. 4 for MgO and Devarda's alloy; in ammonium diffusions, reagent- $\text{NH}_4^+$ -N is also known from colorimetric analysis for KCl and from Exp. 2 for MgO. Because reagent- $\text{NH}_4^+$ -N was such a small proportion of sample- $\text{NH}_4^+$ -N (see results), standard- $\text{NH}_4^+$ -N values rather than sample- $\text{NH}_4^+$ -N values were used in Eqs. [5] and [6].

## RESULTS

### I. Quantifying Contamination

The N contamination in KCl lot B01315 used in Experiments 2, 4, and 5 amounted to on average (SD, n)  $8.2 (1.81, 2) \mu\text{g NH}_4^+\text{-N L}^{-1}$  and  $50.2 (0.98, 2) \mu\text{g NO}_3^-\text{-N L}^{-1}$  in 2 M solution. KCl solution from lot A04338 used in Experiments 1 and 3 had similar N concentrations, i.e.,  $6.8 (1.0, 3) \mu\text{g NH}_4^+\text{-N L}^{-1}$  and  $61.1 (1.1, 3) \mu\text{g NO}_3^-\text{-N L}^{-1}$ . Contributions to N in KCl solution from DI water were  $4.5 (0.9, 4) \mu\text{g NH}_4^+\text{-N L}^{-1}$  and  $0.9 (0.5, 4) \mu\text{g NO}_3^-\text{-N L}^{-1}$ .

#### I.a Contamination in Ammonium Diffusions

**Experiment 1: Determining  $\text{NH}_4^+$ -N contamination in MgO (MgO- $\text{NH}_4^+$ -N) with tracer-dilution method.** When assessing the  $\text{NH}_4^+$ -N contamination in MgO, the calculated blanks ( $M_b$ , Eq.

[1]) based on the dilution of tracer  $\text{NH}_4^+\text{-N}$  were regressed against a variable amount of KCl. This resulted in the following relationship:  $\mu\text{g reagent-NH}_4^+\text{-N} = 0.003 [\text{mL KCl diffused}] - 0.085$  ( $r^2 = 0.80$ ,  $P = 0.04$ ). The negative intercept and its 95 % C.I. ( $\pm 0.24$ ) indicate that  $\text{NH}_4^+\text{-N}$  contamination in 0.25 mg MgO is very small if at all present.

**Experiment 2: Determining  $\text{NH}_4^+\text{-N}$  contamination in MgO (MgO- $\text{NH}_4^+\text{-N}$ ) with measured blank.** When directly measuring  $\text{NH}_4^+\text{-N}$  in MgO by diffusing large amounts of MgO without any standard- $\text{NH}_4^+\text{-N}$  the total  $\text{NH}_4^+\text{-N}$  (i.e., MgO- $\text{NH}_4^+\text{-N}$  and KCl- $\text{NH}_4^+\text{-N}$ ) in the acid trap of one diffusion vessel was too small to be detected by the IRMS. Therefore, the acid traps of the remaining two vessels were combined in one tin capsule and analyzed. The MgO- $\text{NH}_4^+\text{-N}$  obtained was 0.96  $\mu\text{g NH}_4^+\text{-N}$  from 4 g MgO, i.e., 0.06  $\mu\text{g NH}_4^+\text{-N}$  per 0.25 g MgO that we used for 50-mL sample volumes).

### I.b Contamination in Nitrate Diffusions

**Experiment 3: Determining  $\text{NO}_3^-\text{-N}$  contamination in MgO and N contamination in Devarda's alloy combined (MgO- $\text{NO}_3^-\text{-N}$  + DA-N) with tracer dilution and using variable amounts of KCl.** When quantifying the dual contamination of  $\text{NO}_3^-\text{-N}$  in MgO and N in Devarda's alloy by means of tracer dilution, the calculated blanks ( $M_b$ , Eq. [1]) were regressed against a variable amount of KCl. This resulted in the following relationship:  $\mu\text{g reagent-NO}_3^-\text{-N} = 0.038 [\text{mL KCl diffused}] + 1.85$  ( $r^2 = 0.90$ ,  $P = 0.01$ ). The intercept indicates that  $\text{NO}_3^-\text{-N}$  contamination in reagents ( $2 \times 0.25$  g MgO and 0.2 g Devarda's alloy) averaged 1.85  $\mu\text{g}$  ( $\pm 2.06$ , 95 % C.I.).

**Experiment 4: Determining  $\text{NO}_3^-\text{-N}$  contamination in MgO (MgO- $\text{NO}_3^-\text{-N}$ ) and N contamination in Devarda's alloy (DA-N) with tracer dilution and using variable amounts of MgO and Devarda's alloy.** When assessing the reagent- $\text{NO}_3^-\text{-N}$  amount in nitrate diffusions we quantified the increasing dilution of tracer  $\text{NO}_3^-\text{-N}$  by increasing the amounts of reagents added. The conversion of tracer dilution to total reagent-N (Eq. [1]) and subsequent subtraction of known contributions of reagent- $\text{NH}_4^+\text{-N}$  and KCl- $\text{NO}_3^-\text{-N}$  yielded the  $\text{NO}_3^-\text{-N}$  contamination in MgO and Devarda's alloy (Figure 1). In Figure 1, the means and 95 % C.I. of slope and y-intercept for the MgO regression are  $2.22 \pm 0.2$  and  $-0.09 \pm 0.35$ , respectively. The means and 95 % C.I. of slope and y-intercept for the Devarda's alloy regression are  $0.38 \pm 0.18$  and  $0.38 \pm 0.34$ , respectively. The y-intercepts of the regression lines for the variable reagents give independent estimates for N contamination of the fixed reagents. The y-intercept of the MgO regression indicates that 0.2 g of

Devarda's alloy contain on average 0  $\mu\text{g N}$ ; and the y-intercept of the MgO regression indicates that 0.25 g MgO contains on average 0.38  $\mu\text{g NO}_3^- \text{-N}$ . Therefore, it is expected that 1 g reagent should contain on average 0  $\mu\text{g N}$  and 1.5  $\mu\text{g NO}_3^- \text{-N}$  in Devarda's alloy and MgO, respectively. Based on the regression slopes, however, 1 g reagent contains 0.38  $\mu\text{g N}$  and 2.22  $\mu\text{g NO}_3^- \text{-N}$  for Devarda's alloy and MgO, respectively. Even though estimates from intercepts and slopes are fairly similar, we will work with contamination estimates based on the regression slopes in the remainder of this paper due to their narrower confidence intervals. Thus, the reagent- $\text{NO}_3^- \text{-N}$  contained in the amounts of MgO and Devarda's alloy used in diffusing 50-ml and 120-mL volumes amounts to 1.2  $\mu\text{g}$  and 1.8  $\mu\text{g}$ , respectively.

An alternative approach of assessing MgO- $\text{NO}_3^- \text{-N}$  is provided by the colorimetric analysis of KCl solution containing MgO. Any  $\text{NO}_3^- \text{-N}$  in this solution is derived from contamination in KCl and MgO. After a 6 d period, each of four 50-mL (0.25 g MgO added) and 120-mL (0.5 g MgO added) samples, respectively, contained 0.7 (SD = 0.13)  $\mu\text{g}$  and 1.6 (SD = 0.11)  $\mu\text{g MgO-NO}_3^- \text{-N}$ .

## II. $\delta^{15}\text{N}$ of Contaminants

**Experiment 5: Determining the proportional contribution and  $\delta^{15}\text{N}$  values of contaminants (KCl- $\text{NO}_3^- \text{-N}$ , MgO- $\text{NO}_3^- \text{-N}$ , DA-N) in nitrate diffusions.** When diffusing large amounts of reagents in variable combinations but without added standard- $\text{NO}_3^- \text{-N}$ , the resulting amounts of N in acid traps and  $\delta^{15}\text{N}$  values can be used to determine each reagent's  $\delta^{15}\text{N}$  value. The analog of Exp. 5 was not carried out for ammonium diffusions because there were only very small amounts of reagent- $\text{NH}_4^+ \text{-N}$ . Results for measured total N blanks and their  $\delta^{15}\text{N}$  values in nitrate diffusions are shown in Table 3. These values constitute input parameters ( $M_b$  and  $E_b$ ) for solving Eqs. [2], [3] and [4] for the proportion of N in reagents, the proportional contribution of each reagent's N to the total measured blank ( $M_b$ ), and the  $\delta^{15}\text{N}$  value of each reagent, respectively. The results, i.e., N mass and  $\delta^{15}\text{N}$  values of each reagent and all reagents combined, for 50-mL and 120-mL diffusion volumes are presented in Table 4. The *measured* N blanks of MgO and Devarda's alloy (Exp. 5a/b, Table 4) correspond well with results from *calculated* blanks based on tracer dilutions (Exp. 3 and 4). Thus, N blanks measured in this experiment represent approximately complete recovery so that there is no or minimal  $\text{Error}_{\text{recovery}}$  in the measured blank- $\delta^{15}\text{N}$  values (Table 3).

The *measured* total N blank of the reagent combination in Exp. 5c ( $M_b = 29.5 \mu\text{g}$ , Table 3) matched closely the *expected* total N blank of this reagent contamination (28.1  $\mu\text{g}$  and 29.0  $\mu\text{g}$ ) when inserting N content ( $P_{\text{MgO-NO}_3^- \text{-N}}$ ,  $P_{\text{DA-N}}$ ) derived from Exp. 5a and 5b, respectively, into Eq. [2]. This

indicates the soundness of the values for N content in MgO and Devarda's alloy.

The isotopic value of all contaminants combined was approximately -14 ‰ (Table 4). Therefore, when combined with the amount of contaminant-N, the overall sample- $\delta^{15}\text{N}$  is depleted by 0.8 ‰ for 50-mL diffusion volumes and by 1.6 ‰ for 120-mL diffusion volumes relative to the standard- $\text{NO}_3^-$ -N at -3.45 ‰.

### III. Error in $\delta^{15}\text{N}$ with Incomplete Recovery

**Experiment 6: Diffusion of natural abundance standard-N using variable diffusion durations.** Now, that we have established how much N is contributed from reagents, we can rigorously assess sample recovery and its associated effect (i.e., underestimation) on sample- $\delta^{15}\text{N}$  if recovery is incomplete. Quantifying this error ( $\text{Error}_{\text{recovery}}$ , Eq.[6]) and correcting for it is the final step before calculating the standard (or target)- $\delta^{15}\text{N}$ :

$$\delta^{15}\text{N}_{\text{target}} = (\text{m}_{\text{sample}} * \delta^{15}\text{N}_{\text{sample}} - \text{m}_{\text{reagent}} * \delta^{15}\text{N}_{\text{reagent}}) / \text{m}_{\text{target}} \quad [7]$$

where  $\text{m}_{\text{sample}} = \text{m}_{\text{reagent}} + \text{m}_{\text{target}}$ ,  $\text{m}_{\text{reagent}}$  is the mass of N contributed from all reagents (from Exp. 4),  $\text{m}_{\text{target}}$  is the mass of target-N (from colorimetric analysis),  $\delta^{15}\text{N}_{\text{reagent}}$  is the overall isotopic value of reagent-N (from Exp. 5a), and  $\delta^{15}\text{N}_{\text{sample}}$  is the measured isotopic value of diffused sample (i.e., observed sample- $\delta^{15}\text{N}$ ) corrected for incomplete recovery (i.e., actual sample- $\delta^{15}\text{N}$ ).

The absolute error between sample- $\delta^{15}\text{N}$  observed at incomplete recovery and the actual sample- $\delta^{15}\text{N}$  ( $\text{Error}_{\text{recovery}}$ ) is shown in Figures 2 and 3. With both ammonium and nitrate diffusions the error in  $\delta^{15}\text{N}_{\text{sample}}$  is a linear function of recovery for recoveries > 43 % (Figure 2). Because data of Set 2 were erratic only Set 1 and 3 were used to derive the linear equations.

With ammonium diffusions, the linear equation for recoveries > 43 % is  $\text{Error}_{\text{recovery}} = 0.186 * [\% \text{ recovery}] - 19.3$  (Figure 2).  $\text{Error}_{\text{recovery}}$  has to be subtracted from the observed sample- $\delta^{15}\text{N}$  to yield the actual sample- $\delta^{15}\text{N}$ . Due to the negligibility of reagent- $\text{NH}_4^+$ -N in our ammonium diffusions, the actual  $\delta^{15}\text{N}_{\text{sample}}$  is nearly identical to  $\delta^{15}\text{N}_{\text{target}}$  and, thus, corrections of  $\delta^{15}\text{N}_{\text{sample}}$  for  $\delta^{15}\text{N}_{\text{reagent}}$  using Eq. [7] were not applied.

With nitrate diffusions, recovery and sample- $\delta^{15}\text{N}$  explicitly incorporate the contributions of reagent-N. Surprisingly, recoveries of [standard + reagent]-N in 50-mL volumes were higher than 100 % (Set 1: 116 %, Set 2:  $\approx$  107 %, Set 3: 103 %) after 6-10 d diffusion periods (Figure 3). Because

it is meaningless to derive an equation for  $\text{Error}_{\text{recovery}}$  when there are  $> 100\%$  recoveries, we also excluded Set 1. Thus, using only Set 3 and recoveries  $> 43\%$ , the equation for error with incomplete  $\text{NO}_3^-$ -N recovery is:  $\text{Error}_{\text{recovery}} = 0.257 * [\% \text{ recovery}] - 27.9$  (Figure 3). Parameter estimates of this equation represent only an approximate solution because the values for the slope and intercept of the equation do not exactly match as a consequence of recoveries being slightly  $> 100\%$ . Matching values of parameter estimates are required in order to achieve  $0\%$   $\text{Error}_{\text{recovery}}$  at  $100\%$  recovery. However, once incomplete recovery has been correctly accounted for, target- $\delta^{15}\text{N}$  can be calculated using Eq [7].

## DISCUSSION

### Success of Experiments

When assessing the amount of N contamination in reagents by Exp. 1 to 4, we found that estimates derived from different experiments generally agreed well. For example, estimates of  $\text{MgO-NH}_4^+$ -N from Exp. 1 (0-0.15  $\mu\text{g}$  per 0.25 g  $\text{MgO}$ ) agreed with those of Exp. 2 (0.06  $\mu\text{g}$  per 0.25g  $\text{MgO}$ ). Because recovery in Exp. 2 is unknown, the measured  $\text{MgO-NH}_4^+$ -N could be underestimated. Even if that was the case,  $\text{NH}_4^+$ -N contamination in  $\text{MgO}$  would still be negligible. Estimates of  $\text{NO}_3^-$ -N contamination in the amounts in  $\text{MgO}$  and Devarda's alloy typically used for 50-ml diffusion volumes from Exp. 3 (1.85  $\mu\text{g}$ ) and Exp. 4 (1.2  $\mu\text{g}$ ) agreed as well. Furthermore, Exp. 4 provided two independent estimates of  $\text{MgO-NO}_3^-$ -N and DA-N through the slopes and intercepts of two regression lines (Figure 2). When the 95 % C.I. are taken into account, the two independent estimates agreed; i.e., 2.02 to 2.42  $\mu\text{g}$   $\text{NO}_3^-$ -N per 1 g  $\text{MgO}$  ( $\text{MgO}$  regression slope)  $\approx$  0.04 to 0.72  $\mu\text{g}$   $\text{NO}_3^-$ -N per 0.25 g  $\text{MgO}$  (Devarda's alloy regression intercept), and 0.2 to 0.56  $\mu\text{g}$  N per 1 g Devarda's alloy (Devarda's alloy regression slope)  $\approx$  -0.44 to 0.26  $\mu\text{g}$  N per 0.2 g Devarda's alloy ( $\text{MgO}$  regression intercept). In addition, the colorimetric analysis of  $\text{KCl-MgO}$  solution for  $\text{MgO-NO}_3^-$ -N (0.7  $\mu\text{g}$  in 0.25 g  $\text{MgO}$  and 1.6  $\mu\text{g}$  in 0.5 g  $\text{MgO}$ ) corroborated the estimates based on regression slope and intercept (Exp. 4). Our results for  $\text{NO}_3^-$ -N contamination of  $\text{MgO}$  and Devarda's alloy are similar to those reported by Stark and Hart (1996). Sigman et al. (1997), however, found considerably (6-19 times) higher amounts of N from Devarda's alloy. Both studies quantified contamination by measuring blanks; because of this, the actual recovery is unknown and contamination could be underestimated by an unknown degree. The tracer dilution method is therefore preferable because it

does not require complete recovery for accurate blank size determination (Stark and Hart 1996). However, the tracer dilution method does require accurate measurements of the tracer-N isotopic enrichment and of the amount tracer-N added. Overall, we are confident that our estimates of N contamination in reagents are reasonably accurate.

Experiments 5a and 5b were conducted to identify reagents'  $\delta^{15}\text{N}$  values. Since the individual reagent- $\delta^{15}\text{N}$  values were very similar and the overall  $\delta^{15}\text{N}$  value of all reagents combined was nearly identical between Exp. 5a and 5b (Table 4) our estimate of overall reagent- $\delta^{15}\text{N}$  appears to be sound.

In Exp. 6 we established the relationship between incomplete recovery and error in sample- $\delta^{15}\text{N}$ . This implies that recovery itself has to be accurately estimated. However, variability occurred with the estimates of the amount of target-N (Table 2) and reagent-N (e.g., Exp. 4 vs. Exp. 5) assumed to be diffused. Some error with respect to the amount of target-N assumed to be diffused is likely introduced by pipetting very small amounts of standard-N onto filter discs (proxy for amount of diffused N) and into diffusion vessels. Thus, colorimetric analysis of an aliquot of the solution in diffusion vessels might have provided a better way of establishing target-N amount in diffusion vessels.

N recoveries with ammonium diffusions were generally high (Figure 2). Averaged for 6-10 day diffusion durations, recoveries were 99 % (50 mL, Set 1), 84.5 % (120 mL, Set 1), 93.7 % (50 mL, Set 3), and 92.1 % (120 mL, Set 3). We speculate that the lower recovery from the 120-mL samples in Set 1 was due to small headspace in 150-mL vessels. In Set 3, recovery is equal for both sample volumes that we attribute to the use of 240-mL diffusion vessels which provide a larger headspace for 120-mL samples. The seemingly lower recovery in Set 3 relative to 50-mL samples of Set 1 could be an artifact of the comparatively high N amount assumed to be diffused (Table 2). The erratic data from Set 2 for both ammonium and nitrate diffusions is difficult to explain. One potential cause could be residual contamination from the previously used diffusion vessels (vessels from Set 1 and 3 were brand new). However, vessels had been thoroughly acid washed and rinsed. With nitrate diffusions sample-N recoveries (incorporating reagent-N) from 50-mL volumes after 6-10 d diffusion durations were well above 100 %, especially for Sets 1 and 2 (Figure 3). Thus, the seemingly complete recoveries in 120-mL samples are likely too high as well. These high recovery values indicate inaccurate mean estimates for reagent-N and/or errors in estimating the amount of diffused standard-N by using non-diffused standards. Variability in those mean estimates has been shown (Exp. 4 and Table 2). Alternatively, contamination might also have been introduced post-diffusion; this seems likely for Set 1 given the large amount of unexplained N. Even though we used our 'cleanest' data set for deriving the relationship between incomplete recovery and error in sample- $\delta^{15}\text{N}$  (Set 3, maximal recovery in 50-mL samples  $\approx$  103 %) the exact relationship needs further investigation.

### Ammonium Diffusions

The reagents used in ammonium diffusions did not contain significant amounts of contaminant- $\text{NH}_4^+$ -N relative to the 50  $\mu\text{g}$  target- $\text{NH}_4^+$ -N. The  $\text{NH}_4^+$ -N in the KCl lot used for the majority of the experiments constituted 0.8 % and 2 % of the sample-N in 50-mL and 120-mL samples, respectively. The resulting difference between sample- $\delta^{15}\text{N}$  and target- $\delta^{15}\text{N}$  caused by these low levels of KCl contamination with  $\text{NH}_4^+$ -N (i.e., > 98 % sample purity) would be negligible (< 1 ‰) in most cases unless the isotopic difference between reagent-N and the target-N was > 50 ‰ (see Fig. 4 in Robinson 2001). Johnston et al. (2003) have found large amounts of  $\text{NH}_4^+$ -N contamination in KCl and that ashing KCl prior to use can reduce it. An alternative to ashing is testing several KCl lots (e.g., colorimetrically) and choosing those with acceptably low N contaminations. The quality of DI water can also be an issue; DI water has been found contaminated with  $\text{NH}_4^+$ -N in a study by Pritchard and Guy (2005).

Due to the negligibility of reagent- $\text{NH}_4^+$ -N in our ammonium diffusions the only complicating factor is error due to incomplete recovery. This might be a minor issue with small diffusion volumes as recovery has been found to be very high or complete in these cases (Stark and Hart 1996; Mulvaney et al. 1997). High recovery from larger volumes, however, requires longer diffusion periods (Mulvaney et al. 1997) and possibly larger diffusion vessels for providing sufficient headspace. If recovery is incomplete, a simple correction for the underestimation of the true sample- $\delta^{15}\text{N}$  is possible and imperative. The empirical relationship between recovery and error of our study is nearly identical to the one found by Holmes et al. (1998). Thus, as this relationship appears to be constant in a range of diffusion conditions, there is no need to establish it separately for every study.

### Nitrate Diffusions

Tests of our reagents revealed that there was significant N contamination relative to 50  $\mu\text{g}$  target- $\text{NO}_3^-$ -N. KCl in particular could be a major  $\text{NO}_3^-$ -N source; our KCl constituted 4.6 % and 10 % of sample- $\text{NO}_3^-$ -N in 50-ml and 120-mL samples, respectively. N contamination from MgO and Devarda's alloy constituted an additional 2.4 % and 3.4 % of sample- $\text{NO}_3^-$ -N in 50-ml and 120-mL samples, respectively. Blanks occurring with the denitrifier and azide method are consistent and low. The blanks of these two methods are 2.5 to 5 % and 0.1-10 %, respectively (0.5 nmol blank given 10-20 nmol target-N (Sigman et al. 2001); 2 to 3 nmol blank given 25 nmol to 2  $\mu\text{mol}$  target-N (McIlvin and Altabet 2005)). The blank with the derivatization method is < 6 % (< 42  $\mu\text{mol}$  blank with 700  $\mu\text{mol}$  target-N) (Johnston et al. 1999).

Having a relatively large proportion of reagent-N in the sample necessitates the investigation of the reagents' isotopic signature. We found depleted reagent-N (mainly owing to KCl at -16 ‰) which will alter the sample- $\delta^{15}\text{N}$  by ca 0.8 ‰ (50-mL samples) or 1.6 ‰ (120-mL samples), given that our reagent- $\delta^{15}\text{N}$  and target- $\delta^{15}\text{N}$  differed by 10 ‰. The alteration of sample- $\delta^{15}\text{N}$  will increase proportionally with increasing amounts of reagent-N and with increasing difference between  $\delta^{15}\text{N}$  of reagents and target-N. Whether this alteration is acceptable can only be assessed once reagent- $\delta^{15}\text{N}$  is known (see also Robinson (2001) for more error analysis). Another complicating factor is that extracts of undisturbed forest soil often contain very little  $\text{NO}_3^-$ -N. Diffusing larger volumes of these samples requires larger amounts of reagents which will increase the proportion of reagent-N relative to target-N. Given an inherent error in estimating reagent- $\delta^{15}\text{N}$ , the propagated error in the corrected target- $\delta^{15}\text{N}$  will be larger for larger volume samples than for smaller volume samples. Frequently, soil extracts contain so little  $\text{NO}_3^-$ -N that the diffusion method is not usable. Then, the denitrifier method (Sigman et al. 2001) or the azide method (McIlvin and Altabet 2005) are the only analytical alternatives.

The final important issue associated with reagent-N is assessment of recovery. If reagent-N is not explicitly considered, recovery of target-N will be overestimated and error in  $\delta^{15}\text{N}$  due to incomplete recovery will be underestimated. Once recovery of sample-N (i.e., [target-N + reagent-N]) is known, a linear equation analogous to the one for ammonium diffusions can be applied to correct the underestimation of sample- $\delta^{15}\text{N}$  with incomplete recovery. The equation we established for nitrate diffusions has a lower intercept and, resulting from the fixed endpoint of [100 % recovery, 0 ‰ error], a steeper slope than the equation for ammonium diffusions (Figures 2 and 3). This would be consistent with an additional fractionating step associated with the reduction of nitrate, so that with a given sample-N recovery the error in  $\delta^{15}\text{N}$  will be slightly larger for nitrate diffusions than for ammonium diffusions. Our equation for nitrate diffusions, however, needs further validation because of the inconsistencies associated with our higher than 100 % sample-N recoveries.

### **Interference from breakdown of organic N in soil extracts**

In ammonium diffusions, breakdown of labile organic N apparently is a minor problem for 6-day and shorter diffusion periods at room temperature using the mild alkali MgO. Mulvaney et al. (1997) found no or little decomposition of experimentally added 300  $\mu\text{g}$  N of glucosamine and glutamine (representing alkali-labile organic N compounds) under those conditions. However, they found that decomposition increased at higher temperatures and longer diffusion periods. For example, 100-mL

sample volumes required a 9 to 11 d diffusion period for complete recovery. At this volume, and at 20 °C, 8 % and 6 % of the glucosamine and glutamine, respectively, had decomposed. At 25 °C, the liberated  $\text{NH}_4^+$ -N from the organic N breakdown increased to 22 % and 15 %, respectively. Thus, if there is concern about interference from organic N, diffusions should be terminated after 6 (or less) days even with larger than 50-mL diffusion volumes and error in sample- $\delta^{15}\text{N}$  due to incomplete recovery should be corrected with the equation we established.

In nitrate diffusions, higher rates of organic N decomposition have been observed in the presence of Devarda's alloy even with short diffusion periods (Saghir et al. 1993a); e.g., roughly one third of the 300  $\mu\text{g}$  of N added as glucosamine or glutamine decomposed when 50-mL samples were diffused for 3 d at 25 °C under constant shaking. In a subsequent study (Mulvaney et al. 1997), diffusions for 5 d without shaking under otherwise equal conditions resulted in breakdown of 8.5 % of the added organic N. In the two studies, breakdown of ca 18 % and 3 %, respectively, of the organic N occurred even at very short diffusion durations (18-44 h).

In an additional study with the goal of modifying the diffusion method for organic N laden samples, Mulvaney and Khan (1999) diffused a test mixture containing 10 labile amino-compounds (8 amino acids, 2 amino sugars). Samples of 10, 20, and 50 mL of 4 M KCl containing each 10 mg of N from the test mixture were diffused for 22 to 28 h, 39 to 56 h, and 4 to 6 d, respectively (variation in diffusion period for each sample volume depended on diffusion temperature, i.e., 30, 25, 20 °C). Liberated  $\text{NH}_4^+$ -N from the test mixture amounted to 0.1 to 0.5 % (22-28 h), 0.4 to 0.6 % (39-56 h) and 1.3 to 3.5 % (4-6 d). The authors concluded that in order to minimize interference from organic N they would have to diffuse small volumes (10 mL) for short periods of time (18 h at 25 °C). Mulvaney and colleagues work with agricultural soils, where organic amendments (manure) and high inorganic N concentrations are common so that small diffusion volume are feasible and such precautions against organic N breakdown are necessary.

There are also a few studies in non-agricultural systems that have demonstrated organic N breakdown. With a variety of extracts (ion exchange resin, water and KCl extracts) of highly organic arctic tundra soils, recoveries of inorganic N were higher than 110 % in the majority of extracts, often exceeding 200 % and in extreme cases even 4000 % when diffusing samples of 100 mL or more at 40 °C for 7 d or longer (Y. Yano Personal Communication 2006).

Sigman et al. (1997) found some DON (dissolved organic N) breakdown to ammonia when samples of concentrated Woods Hole seawater (with salt concentration similar to soil extracts) were incubated at higher temperatures (4 d, 65 °C) followed by 7 d at room temperature. The DON breakdown amounted to 2.2 % of the total DON of 7 to 10  $\mu\text{M}$  when diffusing for ammonium (i.e., no Devarda's alloy was added). If Devarda's alloy was added, about 6.5 % of the DON was released as

ammonia. This also indicates the facilitation of some organic N breakdown by Devarda's alloy. Sigman et al. (1997) recommend an open period with MgO and at higher temperatures so that some of the labile organic N is removed prior to the actual nitrate diffusion.

The high concentrations of soluble organic N content observed in amended agricultural or highly organic soils are unlikely to occur in most non-agricultural and mineral soils. Consequently, precautions for avoiding organic N breakdown as suggested by Mulvaney and Khan (1999) (low volumes + short diffusion duration) might not be necessary for such samples. Additionally, the low inorganic N concentrations of non-amended mineral soils would not allow for the diffusion of small volumes. Unfortunately, in diffusions of soil extracts with relatively low soluble organic N content the potential breakdown of organic N is difficult to detect let alone quantify. Organic N breakdown, if occurring, could be masked by a sample-N (i.e., target-N + reagent-N) recovery of < 100 % which is sometimes observed in nitrate diffusions (Stark and Hart 1996; K. Stephan Unpublished Data 2006; V. Jin Personal Communication 2006). This could be attributed to the presence of reducible organic functional groups and/or to metal chelation which might necessitate larger additions of Devarda's alloy (Mulvaney and Khan 1999). Breakdown of organic N can be detected only if > 100 % recoveries of sample-N are observed. Higher than expected recoveries of target-N have been noted when reagent-N had not been taken into account (T.D. Hooker Personal Communication 2006). Robinson and Conroy (1999) also did not quantify reagent-N and discarded results that were not within 10 % of the expected target-N. Even though the authors attributed high recoveries to organic N breakdown they could possibly have been explained by reagent-N.

Hannam and Prescott (2003) found that in mineral soils of western British Columbia (Canada) soluble organic N (SON) concentrations were about 10-fold higher than soluble inorganic N concentrations. If 5 % of SON decomposed during diffusion SON-derived  $\text{NH}_4^+$ -N would comprise an unacceptably high proportion (33 %) of sample-N. The authors also found that ca 1-1.5 % of SON are free amino acids. If 5 % of only the free amino acids decomposed, their contribution of  $\text{NH}_4^+$ -N to the sample would be a negligible 0.5 %. Ways to investigate the role of organic N breakdown in diffusions for nitrate are a) 'spiking' soil extracts with tracer nitrate, diffusing them and assessing whether the tracer dilution is larger than expected from soil inorganic [nitrate + ammonium]-N and reagent-N, and b) adding  $^{15}\text{N}$  labeled labile organic N to soil extracts and assessing whether a tracer signal appears in recovered N.

## CONCLUSIONS

Quantifying N contamination and  $\delta^{15}\text{N}$  values of reagents can be tedious and of mixed success yet it is imperative to the applicability of the diffusion method at  $^{15}\text{N}$  natural abundance. Once reagent-N is quantified, error in sample- $\delta^{15}\text{N}$  due to incomplete recovery can be easily corrected for because of the approximately linear relationship between recovery and  $\text{Error}_{\text{recovery}}$ . Subsequently, target- $\delta^{15}\text{N}$  can be calculated by rearranging a mixing model including the amounts and  $\delta^{15}\text{N}$ -values of overall sample-N and reagent-N, and the amount of target-N. Sample-N approximates target-N if N contamination in reagents is low enough so that it only represents a few percent of sample-N. Low reagent-N is feasible for ammonium diffusions.

While the uncertainties related to error associated with incomplete recovery and alteration of target- $\delta^{15}\text{N}$  by N-contamination from reagents can be addressed with various experiments (diffusing standards and blanks), the final uncertainty, breakdown of organic N, needs to be addressed with actual soil samples. More research is necessary to determine to what extent organic N decomposition is occurring during diffusions of mineral soil extracts. From current evidence, interference from organic N seems to be more of an issue for nitrate diffusions. Additionally, commonly found low nitrate concentrations in natural soils exacerbate the effects of potentially decomposing organic N.

Furthermore, we and others (Stark and Hart 1996; Sigman et al. 1997) occasionally observed erratic results with diffusions. Therefore, enough soil extract should be collected so that samples can be diffused in replicates. Diffusions of extracts of ion exchange resins, that integrate soil N isotopic values over a longer time period, as opposed to the point measurements with soil extracts, would require the same corrections as outlined for soil extracts, with the additional quantification of resin contamination.

We conclude that the diffusion method can be an appropriate tool for analyzing the  $^{15}\text{N}$  concentration at natural abundance of soil ammonium. The denitrifier and azide methods potentially represent better alternatives for nitrate- $\delta^{15}\text{N}$ . Because these methods have been developed for the analysis of fresh and ocean water their performance with soil extracts remains to be tested.

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**Table 1.** Diffusion conditions and reagent amounts per diffusion vessel in diffusions of reagent- $\text{NO}_3^-$ -N without standard- $\text{NO}_3^-$ -N additions of Experiment 5. Diffusion conditions were: 5a) 50 mL of 2 M KCl, no open period, 34°C, 6-day diffusion period; 5b) 50 mL of 2 M KCl, no open period, room temperature, 6-day diffusion period; 5c) 120 mL of 4 M KCl, MgO- $\text{NH}_4^+$ -N trapped for 6 days prior to 6-day diffusion period at 34°C. DA, Devarda's alloy; n, number of diffusion vessels; † two replicates combined for IRMS analysis, thus, effective n = 3 or 4.

| Experiment           | Reagent amount (g) |      |    |
|----------------------|--------------------|------|----|
|                      | DA                 | MgO  | n  |
| 5a                   |                    |      |    |
| I Low MgO/ high DA   | 2                  | 0.25 | 3  |
| II High MgO/ high DA | 2                  | 2    | 3  |
| 5b                   |                    |      |    |
| I High MgO/ low DA   | 0.5                | 3    | 6† |
| II High MgO/ high DA | 2                  | 2    | 6† |
| 5c                   |                    |      |    |
| High KCl             | 0.4                | 0.75 | 8† |

**Table 2.** Means (standard deviation, n) of  $\delta^{15}\text{N}$  values and N amounts in non-diffused standards (Experiment 6) based on several (n) IRMS runs. Values from each IRMS run, in turn, represent the averages of replicates within a run, except for Set 2 which only had replicates between runs.  $\delta^{15}\text{N}$  values of directly combusted salts, from which standard solutions were made, are also given;  $\delta^{15}\text{N}$  of salts did not differ from non-diffused standards ( $p > 0.05$ ). Underlined values are used as the expected values of target-N in diffused standards and, thus, are one of the components (besides reagent-N) for calculating N recovery (Eq.[5]) and error in  $\delta^{15}\text{N}$  due to incomplete recovery (Eq. [6]). Values in italics denote the mean of that particular subset of non-diffused standards that was analyzed in the same IRMS run as the diffused standards.

† Set 2 and 3 had new standard solutions, standard- $\text{NH}_4^+$ - $\delta^{15}\text{N}$  of Sets 2&3 differs slightly from that of Set 1 ( $p = 0.024$ ), even though the salt for both standard solutions came from the same source.

‡ Mass spectrometric analysis of N amounts in non-diffused standards (Set 1, 3) yielded means that were not different ( $P > 0.05$ ) from those derived by colorimetric analysis of standard solutions that were to be diffused. The standard deviations can be considerable both with non-diffused standards (see Table) and colorimetric analyses (not shown).

§ Presumably a pipette error led to less than targeted 50  $\mu\text{g}$  of standard-N in Set 2.

| Set | Standard-N  |                                       |
|-----|---|---------------------------------------|
|     | $(\text{NH}_4)_2\text{SO}_4$                      | $\text{KNO}_3$                        |
|     | Salt $\delta^{15}\text{N}$ (‰)                    |                                       |
| 1   | 0.81 (0.17, 4)                                    | -3.39 (0.46, 6)                       |
| 2&3 | 1.0 (0.15, 4)                                     | -3.32 (0.16, 4)                       |
| All | 0.9 (0.17, 8)                                     | -3.36 (0.36, 10)                      |
|     | Non-diffused standard $\delta^{15}\text{N}$ (‰)   |                                       |
| 1   | <u>0.80</u> (0.15, 4)                             | -3.47 (0.08, 4)                       |
| 2&3 | <u>1.11</u> † (0.18, 6)                           | -3.43† (0.52, 6)                      |
| All | 0.99 (0.23)                                       | <u>-3.45</u> (0.45, 10)               |
|     | Non-diffused standard-N amount‡ ( $\mu\text{g}$ ) |                                       |
| 1   | 50.7 (2.74, 4) ( <i>47.5</i> )                    | 47.5 (0.93, 4) ( <i>46.5</i> )        |
| 2§  | <u>33.5</u> ¶ (0.94, 3) ( <i>32</i> )             | <u>39.0</u> ¶ (1.11, 3) ( <i>38</i> ) |
| 3   | 46.7 (4.13, 3) ( <i>51</i> )                      | 48.0 (2.11, 3) ( <i>49.5</i> )        |

**Table 3.** Measured total N blanks ( ) and their  $\delta^{15}\text{N}$  values when diffusing for  $\text{NO}_3^-$ -N contained in large amounts of reagents (Experiment 5, see Table 1 for experimental design). Values in Experiments 5a and 5b include a small signal from MgO- and KCl- $\text{NH}_4^+$ -N because there was no open period. Values in parentheses represent the standard deviation.

† 24.1  $\mu\text{g}$   $\text{NO}_3^-$ -N are expected from KCl alone based on colorimetric analysis of KCl.

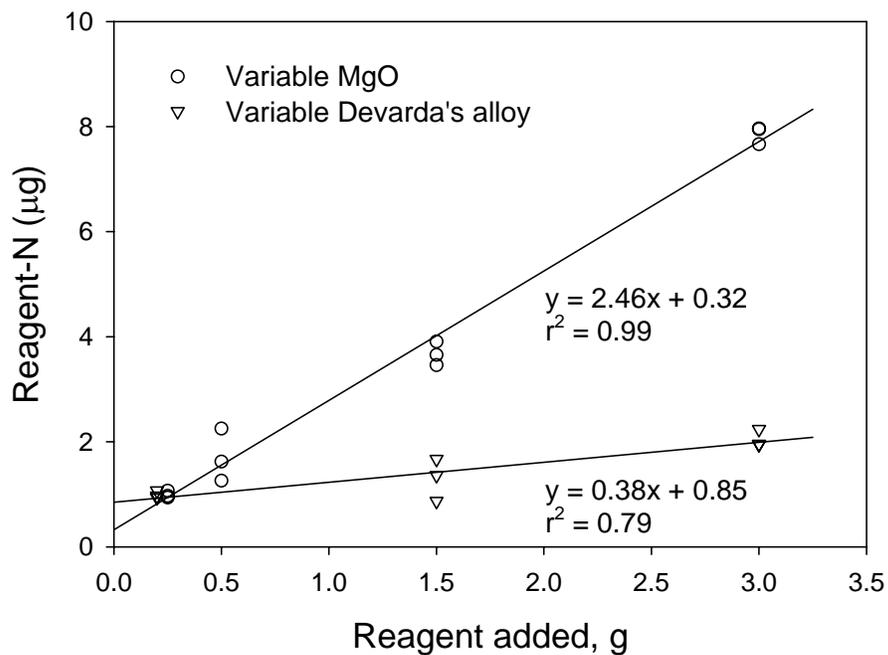
| Experiment           | Blank $\delta^{15}\text{N}$ (‰) | Blank N mass ( $\mu\text{g}$ ) |
|----------------------|---------------------------------|--------------------------------|
| <u>5a</u>            |                                 |                                |
| I Low MgO/ high DA   | -10.11 (1.85)                   | 4.6 (0.10)                     |
| II High MgO/ high DA | -9.65 (0.60)                    | 8.6 (0.18)                     |
| <u>5b</u>            |                                 |                                |
| I High MgO/ low DA   | -9.47 (0.31)                    | 24.8 (2.73)                    |
| II High MgO/ high DA | -8.83(0.28)                     | 17.8 (0.31)                    |
| <u>5c</u>            |                                 |                                |
| High KCl             | -15.2 (0.52)                    | 29.5† (0.46)                   |

**Table 4.** Mass of reagent-N and N isotopic values of each reagent and all reagents combined for 50-ml (120-mL) diffusions (Experiment 5, solutions to Eq. [2] and [4]). Small amounts of MgO- and KCl-NH<sub>4</sub><sup>+</sup>-N are contained in the estimates (0.12 (0.18)  $\mu\text{g}$  from MgO, 0.4 (1)  $\mu\text{g}$  from KCl) because there was no open period. The effect of NH<sub>4</sub><sup>+</sup>-N on the overall contaminant- $\delta^{15}\text{N}$  was assumed to be negligible. Error denotes the deviation of [standard + reagent]- $\delta^{15}\text{N}$  from standard- $\delta^{15}\text{N}$  at -3.45 ‰ given 48  $\mu\text{g}$  of standard-NO<sub>3</sub><sup>-</sup>-N. DA, Devarda's alloy.

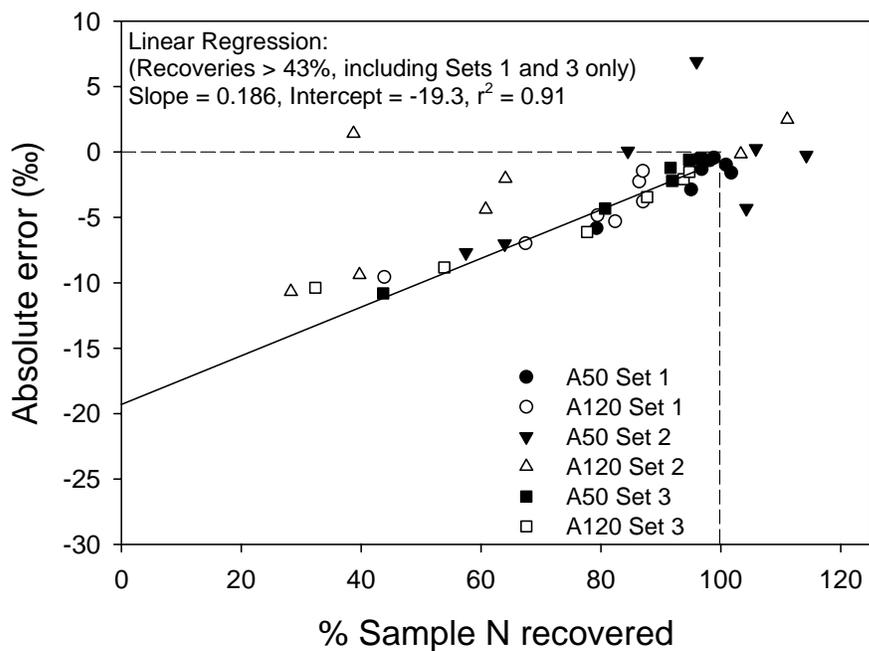
† The amount of N from Devarda's alloy was estimated to be negative using Experiment 5b. Therefore, we used the results for reagent- $\delta^{15}\text{N}$  from Experiment 5a in order to estimate error with incomplete recovery (Experiment 6, Eq.[6]).

|         | Reagent-N in 50 (120) mL ( $\mu\text{g}$ ) |           |           |              | Reagent- $\delta^{15}\text{N}$ (‰) |      |      |               | Error(‰)    |
|---------|--|-----------|-----------|--------------|------------------------------------|------|------|---------------|-------------|
|         | KCl  | MgO       | DA        | all reagents | KCl                                | MgO  | DA   | all reagents  |             |
| Exp. 5a | 2.5 (6.0)                                  | 1.1 (1.7) | 0.2 (0.3) | 3.8 (8.0)    | -16.5                              | -8.9 | -0.2 | -13.5 (-14.2) | -0.8 (-1.6) |
| Exp. 5b | 2.5 (6.0)                                  | 1.7 (2.5) | 0†        | 4.2 (8.5)    | -16.8                              | -7.6 | NA   | -13.1 (-14.1) | -0.8 (-1.6) |

**Figure 1.** Amount of  $\text{NO}_3^-$ -N contamination from MgO and Devarda's alloy (Experiment 4). The regression slopes (both statistically significant,  $P < 0.05$ ) denote the  $\text{NO}_3^-$ -N contamination in each 1 g of MgO and Devarda's alloy, respectively; the corresponding intercept denotes the  $\text{NO}_3^-$ -N contribution of 0.2 g Devarda's alloy and 0.25 g MgO, respectively. The contributions from MgO- $\text{NH}_4^+$ -N (known from Exp. 2) and KCl- $\text{NO}_3^-$  and - $\text{NH}_4^+$ -N (known from colorimetric analysis) have been subtracted.



**Figure 2.** Absolute error between observed and actual sample- $\delta^{15}\text{N}$  as a function of incomplete recovery ( $\text{Error}_{\text{recovery}}$ , Eq.[6]) in ammonium (A) diffusions using diffusion volumes of 50 mL and 120 mL. Actual sample- $\delta^{15}\text{N}$  is nearly identical to the standard- $\delta^{15}\text{N}$  in this case because reagent- $\text{NH}_4^+\text{-N}$  contamination is negligible.



**Figure 3.** Absolute error between observed and actual sample- $\delta^{15}\text{N}$  as a function of incomplete recovery ( $\text{Error}_{\text{recovery}}$ , Eq.[6]) in nitrate (N) diffusions using diffusion volumes of 50 mL and 120 mL. Actual sample- $\delta^{15}\text{N}$  is the weighted average of standard- $\delta^{15}\text{N}$  (Table 2) and reagent- $\delta^{15}\text{N}$  (Table 4, Experiment 5a). Percent N recovery is based on expected [standard + reagent]-N. Maximum recoveries of Sets 1 and 2 were significantly higher than 100 % so that only Set 3 was used to derive the linear equation.

