Carbon and nitrogen cycling in watersheds of contrasting vegetation types in the Fernow Experimental Forest, West Virginia

Charlene Nicole Kelly

Dissertation submitted to the faculty of the
Virginia Polytechnic Institute and State University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY
in
FORESTRY

Stephen H. Schoenholtz, chair
Mary Beth Adams
Jackson R. Webster
Duane F. Berry
James A. Burger

1 April 2010
Blacksburg, Virginia

Carbon and nitrogen cycling in watersheds of contrasting vegetation types in the Fernow Experimental Forest, West Virginia

Charlene N. Kelly

ABSTRACT

Increased anthropogenic deposition of nitrogen (N) and land-use changes associated with planted forests have important implications for sustainable forest management and associated water quality. The purpose of the research for this dissertation was to explore how N deposition will affect the long-term health, productivity, and carbon (C) and N sequestration of conifer and hardwood forest types by examining the mechanisms controlling N cycling and NO_3-N production in two watersheds with contrasting vegetation at the Fernow Experimental Forest (FEF), West Virginia. I utilized watershed C and N budgets to account for differences in stream export of NO_3-N from streams draining adjacent watersheds containing (i) planted Norway spruce (*Picea abies*) and (ii) native Appalachian hardwoods. I also investigated spatial and temporal patterns of dissolved C and N across both watersheds and identified key soil properties associated with NO_3-N in soil solution and streamwater. In a third study, I performed a soil inoculation and incubation experiment, which utilized soil from both watersheds, mixed in ratios in order to create a gradient of soil chemical and biotic characteristics. Important differences in biogeochemical cycling of C and N were documented in the watersheds after nearly 40 years of influence by contrasting vegetation. Total C and N pools were 28% and 35% lower in the spruce watershed than the hardwood watershed, respectively. Results also identify vegetation-mediated differences in soil characteristics, with lower soil pH and base cations, and higher extractable aluminum and C:N ratios measured in the spruce soil as compared to the native hardwood soil. Establishment of a spruce monoculture at the FEF significantly altered N cycling, depleted N stores, increased soil acidity, and altered organic matter dynamics, thus leading to low net nitrification rates. Carbon and N properties and processes in the soil profile should be taken into consideration in forests managed for ecosystem services including C sequestration and improvement or maintenance of water quality through alleviation of N inputs into aquatic ecosystems.

ACKNOWLEDGEMENTS

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

Carbon and nitrogen cycling in watersheds of contrasting vegetation types in the Fernow Experimental Forest, West Virginia

Chapter 1. Introduction

Nitrogen saturation – causes and consequences

Anthropogenic activities have nearly doubled the amount of available nitrogen (N) in the environment, which alters biogeochemical cycles in both terrestrial and aquatic ecosystems (Vitousek et al 1997; Galloway et al 2004). Most of the increases in available N are a result of agricultural production (cultivation of legumes and rice), energy production (burning of fossil fuels), and the development of the Haber-Bosch process to form ammonia (NH_3) from N_2 and H_2 (food and ammunition production) (Galloway et al 2004). Because the N cycle is often considered to be the most complex of all the biologically processed elements, there is currently a limited understanding of the fate of this increasing available N throughout the environment (Galloway 1998). Consequently, there is limited understanding of how to predict how different ecosystems may be negatively impacted by N pollution.

Most temperate forests are N-limited, although an increasing number of forests exhibit characteristics suggesting presence of N in excess of demand of the system (Fenn et al 1998; Aber et al 1998). Sulfur deposition has declined following the Federal Clean Air Act of 1990, yet N deposition patterns have remained relatively constant in the northeastern US and are increasing in the western US (Schlesinger and Hartley 1992; Fenn et al. 1998; DeWalle 2006). This anthropogenic deposition of N may result in accumulation of soil N, leading to a condition described as N saturation within certain forested watersheds (Agren and Bosatta 1988, Aber et al 1989, Stoddard 1994; Peterjohn et al. 1996, Fitzhugh et al 2003).

Nitrogen in excess of demand in a forest can disrupt plant-soil relations, increase soil acidification and phytotoxic Al^{3+} mobility, lead to leaching of important base cations (Ca, Mg) from the soil, increase emissions of greenhouse N gases, reduce methane storage in

the soil, and decrease water quality (Fenn et al 1998, Christ et al 2002). High NO_3-N levels in streams can lead to excessive algal growth and eutrophication in aquatic systems, thus decreasing dissolved oxygen levels (Martin et al 1999). Nitrogen saturation theory suggests that increases in N deposition add to soil N pools and may increase N mineralization (Aber et al 1989; Aber et al 1998). When N becomes excessive, the capacity of a watershed to retain N compounds, especially NO_3-N in water and gaseous N_2O, becomes low (Fenn et al. 1998). Thus, an increase in streamwater NO_3-N and base cations, low retention of inorganic N, increased nitrification rates, and decreased seasonal variation in stream NO_3-N become evident (Christ et al 2002).

Many studies have investigated this ecosystem response described as N-saturation (e.g. Agren and Bosatta 1988, Aber et al 1989, Peterjohn et al. 1996, Fitzhugh et al 2003). However, predicting the propensity of an ecosystem to become N-saturated is difficult, as a multitude of environmental factors play major roles in determining N pathways.

Mechanisms of nitrogen retention

Nitrate is the most mobile form of soil N and once formed, uptake or transformation of this anion must occur in order to promote retention of N in an ecosystem. Transformation occurs through several mechanisms. Nitrogen mineralization is the breakdown of N-containing organic compounds into plant-accessible forms (OM to NH_4^+ or NH_3). Nitrification of NH_3 occurs through microbial oxidation to NO_2^- followed by the oxidation of NO_2^- into NO_3^-. Denitrification is the microbial/bacterial reduction of NO_3^- and NO_2^- that results in release of N to the air. Nitrogen is also incorporated into organic compounds (dissolved or solid organic N) that are held in the soil complexes or in plant tissue following uptake.

Organic nitrogen constitutes a large pool of N in most soils, but its role in nutrient cycling is poorly understood (Brookshire 2005). Recent studies of Scots pine (*Pinus sylvestris*) have provided evidence that dissolved organic N (DON) in the form of amino acids) may be taken up directly by some vegetation and dissolved N is not necessarily required in inorganic forms of NH_4^+ or NO_3^- for uptake, as previously thought (Ohlund

and Nasholm 2004). Furthermore, fractions of N (specific amino acids) have also been shown to vary in availability and some may be preferentially sorbed to mineral soil (Yu et al 2002), whereas other DON compounds may be easily leached from the soil (Neff et al 2003).

Nitrogen is retained in a watershed through many mechanisms. These include NH_4^+ immobilization, fixation of N compounds onto clay particles, low mineralization rates, NO_3^- reduction to NH_4^+ or NO_2^- followed by incorporation into OM, plant uptake, N reduction below the rooting zone, or an overall lack of water flow through the soil profile (Vitousek et al 1982). Microbial assimilation may also be an important retention mechanism of NO_3^- (Fenn et al 1998; Schimel and Firestone 1989; Zak et al 1990), although rapid N immobilization can occur without CO_2^- production, indicating possible abiotic mechanisms of NO_3^- immobilization (Aber et al 1998) and a reduction of NO_3^- to NO_2^- (Dail et al 2001). Rapid formation of organic N in both sterile and control soils also indicates an abiotic mechanism of NO_2^- immobilization (Dail et al 2001), although it is still unclear how NO_2^- is converted to DON through abiotic pathways.

Riparian and stream influences on nitrogen cycling

Riparian soils and in-stream processes may also aid in NO_3^- retention in watersheds (Cooper 1990; Hill et al 2000; Bernhardt et al 2003; Valett et al 2002). Mechanisms contributing to reduction of NO_3^- in riparian areas include sediment trapping, plant uptake, increased microbial activity, and denitrification (Martin et al 1999). Cooper (1990) demonstrated that 56-100% of NO_3^- transformation occurred in the riparian soil of a New Zealand headwater stream. The riparian soil was anoxic and high in denitrifying enzymes and available C. Denitrification in the riparian zone may also be a product of longer retention time and local patches of high C at depth (Hill et al 2000).

High NO_3^- in streamwater following forest disturbance in a watershed at the Hubbard Brook Experimental Forest in New Hampshire was rapidly transformed by in-stream mechanisms (Bernhardt et al 2003), resulting in in-stream uptake of inorganic NO_3^- being greater than export. The mechanisms of NO_3^- decline have not been determined and may

be transformation (e.g., denitrification) or retention (e.g., biotic assimilation) and subsequent export of organic forms of N or gaseous N_2, which may be explained through stream nutrient spiraling theory (Webster and Patten 1979).

Stream nutrient spiraling incorporates the use, transformation, and retention of nutrients within the stream (Webster and Patten 1979; Newbold et al 1983; Valett et al 2002). A full spiral is complete when a nutrient enters the water in mineral form, is bound into organic form, and finally is released back to solution following mineralization (Valett et al 2002). Spiral length varies among streams and depends on residence time in water, size of the stream, temperature, and leaf litter chemistry. There appears to be significant transformation of N in forested headwater streams prior to export downstream and benthic demand plays a large role in the cycling of DON (Brookshire et al 2005). It has been demonstrated that N cycling may be quite variable within short distances, although the mechanisms controlling the variation are still under debate and the mechanisms may depend on the site and subsequent vegetation differences (Christ et al 2002).

Vegetation influences on carbon and nitrogen dynamics

Large vegetation-mediated variation in N cycling has been documented by many studies (Robertson 1982; Finzi et al 1998; Stark and Hart 1997; SteMarie and Pare 1999; Fitzhugh et al 2003; Lovett et al 2004; Christenson et al 2009). These differences have been shown with variation in N mineralization and nitrification processes, C:N ratios, and foliage and litter N content. One of the primary influences that trees have on soils is through inputs of organic matter, both above- and below-ground, which varies with species in quantity and chemical composition (Melillo et al 1983; Binkely 1995; Lovett et al 2004). This organic matter is the main driver for microbial and/or sorption processes by which nutrient bioavailability and/or storage are determined. Small changes in soil labile C concentrations may be responsible for large changes in N retention in watersheds. Stream NO_3^-N concentrations have exhibited large increases in association with decreasing soil and surface water DOC (Goodale et al 2005). Thus, vegetation influences on soil C are closely linked to N dynamics.

Differences in mycorrhizal fungal associations with different vegetation types may also play a large role in ecosystem N cycling (Michelsen et al 1996; Gilliam et al 2001). Tree species with different types of mycorrhizal fungi associations have access to different sources of N within the soil (Michelsen et al 1996). There is evidence that both ectomycorrhizal and ericoid mycorrhizal plants access organic forms of N in soils with high organic matter (Ohlund and Nasholm 2004). There is also evidence that mycorrhizal fungi may directly influence N cycling in soil. For example, ericoid mycorrhizae secrete organic acids that inhibit nitrifying microbes and incorporate N compounds into organic complexes that are then unavailable for biotic processing (Straker 1996; Read and Perez-Moreno 2003).

Tree species also alter physical and micro-climatic properties of soils through differences in canopy and rooting architecture. Canopy density can influence interception and evapotranspiration rates, resulting in differences in temperature and moisture regimes. These changes in temperature and moisture then regulate microbially driven decomposition and nitrification. Canopy architecture also can result in differences in quantity and chemical composition of throughfall and stemflow, resulting in differences of atmospheric inputs into the ecosystem (Mina 1967). Root architecture can influence soil C and N storage and cycling. Deeper rooting systems increase subsurface soil oxygen and organic matter, both recalcitrant and labile, through soil penetration, root exudates, root sloughing, and turnover (Farrell 1990; Rhoades 1997) and increase surface nutrients by redistributing subsurface nutrients (Virginia 1986).

Planted forests

Planted forests are a key component of production forestry involving cultivation of forest ecosystems established mainly for the increased production of wood biomass, but planted forests may also be utilized in soil and water conservation efforts (Carnus et al 2006). As of 2001, the total area of plantation forest worldwide was 187 million ha, or 5% of the global forest cover (Food and Agriculture Organization 2001). However, with increasing demands for energy and wood products the use of planted forests is rapidly expanding, mostly using coniferous species, (Carnus et al 2006). Within the US, 11% of forest land

is planted (22 million ha), with the greatest proportion of this occurring in the South (67%; Stanturf and Zhang 2003). The area of intensively managed planted forests is also projected to increase in the US (Haynes 2001; Prestemom and Abt 2002).

Planted forests provides many benefits, including alleviation of harvesting pressure from native forests (Sedjo 2001), by meeting wood demands of an increasing population through advances in biomass yield associated with improvements in genetics and nutrition management (Fox et al 2004). Also, C sequestration in tree biomass may provide a significant sink for atmospheric CO_2^-. However, planted forests can also be associated with negative consequences of soil erosion and declines in site quality when sites are not managed properly. Also, conversion of native vegetation to monocultures of conifer may disrupt biogeochemical cycling of C and N (Guo and Gifford 2002).

Significant ecosystem losses of C and N following conversion to conifer from hardwood vegetation has been shown by Kasel and Bennett (2007), who documented a 30% decrease in soil C content with conversion of native broadleaf forest to planted pine after 37 years in Australia. Guo and Gifford (2002) also observed this pattern in a meta-analysis of land-use change. A 12-15% loss of soil C was documented when native broadleaf forests were converted to conifer plantings, whereas no changes in soil C and N were observed upon recovery to native broadleaf forest. This loss of soil C and N may be attributed to both disturbance and changes in amount and composition of plant material returned to the soil via litter, root processes, and mycorrhizal fungi (Lugo and Brown 1993; Chapela et al 2001).

In summary, atmospheric N pollution is expected to increase, vegetation-mediated differences in soil C and N cycling and storage have been well documented, and establishment of planted forests is expected to increase in landscape importance. Thus, understanding the processes controlling soil C and N cycling within contrasting forest types must be enhanced to advance management practices. It is still unknown how the continuing chronic deposition of anthropogenically derived N may alter the composition, health, and biogeochemistry of different forests types over time. This information is

necessary in order to establish management regimes that will maintain productive forests and protect associated aquatic systems. Vegetative species selection, both within forests and in riparian zones, may become imperative for maintaining forest productivity and addressing water quality issues in areas of increasing N deposition.

Research context

Two watersheds within the Fernow Experimental Forest, West Virginia provide an excellent opportunity for research to address how vegetation type may determine the propensity of N saturation in a forest and how forest vegetation type may influence C and N cycling. This region of West Virginia receives approximately 7.2 kg N through atmospheric deposition annually (DeWalle et al 2006), which is among the highest in the US (National Atmospheric Deposition Program/National Trends Network 2008) and precipitation in this region has a pH of approximately 4.4 (National Atmospheric Deposition Program/National Trends Network 2004).

The watersheds utilized in this study are nearly adjacent, with similar site factors and management histories, differing primarily in vegetation cover. Nearly 40 yr following clear-cut logging of these watersheds, one has regenerated to native hardwood (WS 7), while the other was planted to a monoculture of Norway spruce (*Picea abies*) (WS 6). These watersheds exhibit divergent NO_3-N concentrations in streamwater at their respective weirs, where WS 7 is exporting relatively high amounts of NO_3-N and WS 6 has very low stream NO_3-N (Fig. 1; data courtesy MB Adams, USFS). Long-term stream NO_3-N values have been used as an indicator of N saturation (Aber et al 1989). Thus the hardwood forest in WS 7 is considered to be N saturated, and the spruce forest in WS 6 is considered to be accumulating N. This divergent pattern in stream NO_3-N export was the impetus for the following studies described within this dissertation.

The paired-watershed approach has been instrumental to our current understanding of the effects of anthropogenic-induced alterations in water and nutrient cycling in forested ecosystems (Hewlett and Helvey 1970; Swank and Douglass 1975; Likens 1985; Adams 1993). In particular, paired-watershed studies have improved our knowledge of the

impacts that acid deposition has upon biogeochemical cycling of C, N and base cations, and in furthering our understanding of the ecological consequences of N-saturation (Aber et al 1989, 2002; Kahl et al 1993; Adams et al 1993; Campbell et al 2000).

The purpose of the research for this dissertation was to explore how N deposition will affect the long-term health, productivity, and C and N sequestration of conifer and hardwood forest types by examining the mechanisms controlling N cycling and NO_3-N production in the two watersheds with contrasting vegetation. The results of this research will aid in the investigation of the ecological effects and possible ecosystem values of conifer species conversion as N deposition continues to alter forest ecosystem composition and processes.

Research objectives and hypotheses

The overall objective of my research was to determine factors controlling the ecosystem fate of N within the spruce watershed (WS6) described above, because ecosystem losses as stream NO_3-N were always much lower than the adjacent native hardwood watershed (WS 7). Additionally, I aimed to determine the biogeochemical mechanisms whereby the influence of spruce vegetation led to minimal ecosystem N losses. Each of the main research chapters (Ch. 2-4) was written with the intent to submit to peer-reviewed journals as presented in this dissertation.

Chapter 2 addresses the following specific research objectives: 1) determine selected pool sizes of C and N within each watershed to ascertain if significant differences in these pools occurred after nearly 40 years of influences from contrasting forest vegetation and 2) measure rates of net N mineralization to determine if this flux was associated with differences in size of selected C and N pools in the two watersheds. It was hypothesized that a slower mineralization flux occurs in the spruce soils, leading to larger C and N pool sizes of forest floor and mineral soil.

Chapter 3 addresses the following specific research objectives: 1) determine specific soil properties that may have been altered by the influence of the spruce vegetation relative to

the native Appalachian hardwood soils occurring in association with a regenerating hardwood stands and 2) analyze how these soil properties may be related to soil solution and in-stream fluxes of C and N spatially and temporally across the two watersheds with contrasting vegetation. It was hypothesized that properties associated with lower soil pH and differences in C quality (e.g., higher C:N ratio) would be the factors most strongly correlated to low concentrations of NO_3-N in soil solution and streamwater.

In Chapter 4, a soil inoculation and incubation study was performed under controlled laboratory conditions to 1) determine if the variation in net NO_3-N production in these soils can be attributed to varying (i) presence of nitrifying microbes, (ii) quality and amount of soil C substrate, or (iii) incorporation of N compounds into organic substrate; and to 2) identify soil properties that are associated with the divergent rates of NO_3-N production exhibited in these watersheds. It was hypothesized that differing C compounds associated with the hardwood and spruce systems that vary in degradability are involved in the mechanism of the differing rates of net NO_3-N production observed in these watersheds.

Chapter 5 serves as a synthesis for the results and conclusions of Chapters 2-4. The objective of this chapter was to summarize the key findings of this dissertation and to suggest implications for forest management practices regarding tree species selection in management efforts concerning N pollution and C sequestration.

WORKS CITED

Aber JD, Nadelhoffer KH, Steudler P, and JM Melillo (1989) Nitrogen saturation in northern forest ecosystems. BioScience. 39(6): 378-386.

Aber J, McDowell W, Naldelhoffer K, Magill A, Bernson G, Kamakea M, McNulty S, Currie W, Rustad L, and I Fernandez (1998) Nitrogen saturation in temperate forest ecosystems. Bioscience. 48(11):921-934.

Adams MB, Edwards PJ, Wood F, and JN Kochenderfer (1993) Artificial watershed acidification on the Fernow Experimental Forest, USA. Journal of Hydrology. 150: 505-519

Agren GI and E Bosatta (1988) Nitrogen saturation of terrestrial ecosystems. Environmental Pollution. 54(3-4):185-197.

Bernhardt ES, Likens GE, Busco DC, and CT Driscoll (2003) In-stream uptake dampens effects of major forest disturbance on watershed nitrogen export. PNAS. 100(18):10304-10308.

Binkley D (1995) The influence of tree species on forest soils: Processes and patterns. In: Mead DJ and IS Cornforth, Eds. (1995) Proceedings of the trees and soil workshop, Lincoln University 28 February-2 March 1994. Agronomy Society of New Zealand Special Publication No. 10. Lincoln University Press, Canterbury.

Brookshire ENJ, Valett HM, Thomas SA, and JR Webster (2005) Coupled cycling of dissolved organic nitrogen and carbon in a forest stream. Ecology. 86(9):2487-2496.

Campbell JL, Hornbeck JW, McDowell WH, Buso DC, Shanley JB, and GE Likens (2000) Dissolved organic budgets for upland, forested ecosystems in New England. Biogeochemistry. 49(2): 123-142.

Carnus JM, Parrotta J, Brockerhoff E, Arbez M, Jactel H, Kremer A, Lamb D, O'Hara K, and B Walters (2006) Planted forests and biodiversity. Journal of Forestry. 104(2): 65-77.

Chapela IH, Osher LJ, Horton TR, and MR Henn (2001) Ectomycorrhizal fungi introduced with exotic pine plantations induce soil carbon depletion. Soil Biology and Biochemistry. 33: 1733-1740.

Christ MJ, Peterjohn WT, Cumming JR, and Adams MB (2002) Nitrification potentials and landscape, soil and vegetation characteristics in two Central Appalachian watersheds differing in NO_3^- export. Forest Ecology and Management. 159:145-158.

Christenson LM, Lovett, GM, Weathers KC and MA Arthur (2009) The influence of tree species, nitrogen fertilization, and soil C to N ratio on gross soil nitrogen transformations. Soil Science Society of America Journal. 73(2): 638-646.

Cooper BA (1990) Nitrate depletion in the riparian zone and stream channel of a small headwater catchment. Hydrobiologia. 202(1-2).

Dail DB, Davidson EA, and J Chorover (2001) Rapid abiotic transformation of nitrate in an acid forest soil. Biogeochemistry. 54:131-146.

DeWalle DR, Buda AR, MacDougal SE, and BR Swistock (2006) Possible linkages between trends in atmospheric deposition and stream chemistry in Appalachian Plateau hardwood forests. EOS Trans. AGU. 87(52). Fall meeting abstract B24B-07.

Farrell J (1990) The inclue nce of trees in selected agroecosystems in Mexico. In: Gliessman SR, Ed. Agroecology: Researching the ecological basis for sustainable agriculture. 167-183. Springer-Verlag, New York.

Fenn (1998) Nitrogen excess in North American ecosystems: Predisposing factors, ecosystem responses, and management strategies. Ecological Applications. 8(3):706-733.

Finzi AC, Van Breeman N, and CD Canham (1998) Canopy-tree interactions within temperate forests: Species effects on soil carbon and nitrogen. Ecological Applications. 8(2): 440-446.

Fitzhugh RD, Lovett GM, and RT Venterea (2003) Biotic and abiotic immobilization of ammonium, nitrite, and nitrate in soils developed under different tree species in the Catskill Mountains, New York, USA. Global Change Biology. 9: 1591-1601.

Fox TR, Jokela EJ, and LH Allen (2004) The evolution of pine plantation silviculture in the Southern United States. In: General Technical Report. SRS 75. Asheville, NC: US Department of Agriculture, Forest Service, Southern Research Station. Chapter 8: 63-82.

Galloway JN (1998) The global nitrogen cycle: changes and consequences. Environmental Pollution. 102(1,1): 15-24.

Galloway JN, Dentener FJ, Capone DG, Boyer EW, Howarth RW, Seitzinger SP, Asner GP, Cleveland CC, Green PA, Holland EA, Karl DM, Micheals AF, Porter JH, Townsend AR, and CJ Voosmarty (2004) Nitrogen cycles: Past, present, and future. Biogeochemistry. 70(2): 153-226.

Gilliam FS, Yurish BM, and MB Adams (2001) Temporal and spatial variation of nitrogen transformations in nitrogen-saturated soils of a central Appalachian hardwood forest. Canadian Journal of Forest Restoration. 31:1768-1785.

Goodale CL, Aber JD, Vitousek PM, and WH McDowell (2005) Long-term decreases in stream nitrate: Successional causes unlikely; Possible links to DOC? Ecosystems. 8:334-337.

Guo LB and RM Gifford (2002) Soil carbon stocks and land use change: a meta analysis. Global Change Biology. 8(4): 345-360.

Haynes JP (2001) Biodiversity implications of transgenic plantations. In: SH Strauss and HD Bradshaw, Eds. Proceedings of the First International Symposium on Ecological and Societal Aspects of Transgenic Plantations, College of Forestry, Oregon State University, OR, USA. 168-175.

Hewlett JD and JD Helvey (1970) Effects of forest clear-felling on the storm hydrograph. Water Resources Research. 6(3): 768-782.

Hill AR (1996) Nitrate removal in stream riparian zones. Journal of Environmental Quality. 25:743–55.

Kahl JS, Norton SA, Fernandez IJ, Nadelhoffer KJ, Driscoll CT, and JD Aber (1993) Experimental inducement of nitrogen saturation at the watershed scale. Environmental Science and Technology. 27(3): 565-568.

Kasel S and LT Bennett (2007) Land-use history, forest conversion, and soil organic carbon in pine plantations and native forests of south eastern Australia. Geoderma. 137(3-4): 401-413.

Likens GE (1985) An experimental approach for the study of ecosystems: The fifth Tansley lecture. British Ecological Society. 73(2): 381-396.

Lovett GM, Weathers KC, Arthur MA, and JC Schultz (2004) Nitrogen cycling in a northern hardwood forest: Do species matter? Biogeochemistry. 67(3):289-308.

Lugo AE and S Brown (1993) Management of tropical soils as sinks or sources of atmospheric carbon. Plant and Soil. 149(1): 27-41.

Martin TL, Kaushik NK, Trevors JT, and HR Whiteley (1999) Review: Denitrification in temperate climate riparian zones. Water, Air, and Soil Pollution. 111: 171-186.

Melillo JM, Naimen RJ, Aber JD, and KN Eschleman (1983) The influence of substrate quality and stream size on wood decomposition dynamics. Oecologia. 58(3): 281-285.

Michelsen A, Schmidt IK, Jonasson S, Quarmby C, and D Sleep (1996) Leaf 15N abundance of subarctic plants provides field evidence that ericoid, ectomycorrhizal, and non- and arbuscular mycorrhizal species access different sources of soil nitrogen. Oecologia. 105(1): 53-63.

Mina VN (1967) Influence of stemflow on soil. Soviet Soil Science. 7:1321-1329.

Neff JC, Chapin FS, and PM Vitousek (1993) Breaks in the cycle: dissolved organic nitrogen in terrestrial ecosystems. Frontiers in Ecology and the Environment. 1(4): 205-211.

Newbold JD, Elwood JW, O'Neill RV, and AL Sheldon (1983) Phosphorus dynamics in a woodland stream ecosystem: A study of nutrient spiraling. Ecology. 64(5): 1249-1265.

Peterjohn WP, Adams MB, and FS Gilliam (1996) Symptoms of nitrogen saturation in two central Appalachian hardwood forest ecosystems. Biogeochemistry. 35(3):507-522.

Prestemon JP and RC Abt (2002) Timber products supply and demand. Pg. 299-325, Ch. 13. In: DN Wear and JG Greis, Eds. Southern Forest Resource Assessment. General

Technical Report SRS-53. Asheville, NC: US Department of Agriculture Forest Service, Southern Research Station.

Ohlund J and T Nasholm (2004) Regulation of organic and inorganic nitrogen uptake in Scots pine (*Pinus sylvestris*) seedlings. Tree Physiology. 24:1397-1402.

Read DJ and J Perez-Moreno (2003) Mycorrhizas and nutrient cycling in ecosystems—a journey towards relevance. New Phytologist 157:475–492.

Rhoades CC (1996) Single-tree influences on soil properties in agroforestry: lessons from natural forest and savanna ecosystems. Agroforestry Systems. 35(1): 71-94.

Robertson GP (1982) Nitrification in forested ecosystems. Philosophical Transactions of the Royal Society of London. 296(1082): 445-457.

Schimel JP and MK Firestone (1989) Inorganic N incorporation by coniferous forest floor material. Soil Biology and Biochemistry. 21(1):41-46.

Schlesinger WH and AE Hartley (1992) A global budget for atmospheric ammonia. Biogeochemistry. 15(3):191-211.

Sedjo RA (2001) From foraging to cropping: the transition to plantation forestry, and implications for wood supply and demand. Unasylva. 52(204).

Stanturf JA and D Zhang (2003) Plantation Forests in the United States of America: Past, present, and future. XII World Forestry Congress, 2003. Quebec City, Canada.

Stark JM and SC Hart (1997) High rates of nitrification and nitrate turnover in undisturbed coniferous forests. Nature. 385:61-65.

Ste-Marie C and D Pare (1999) Soil, pH and N availability effects on net nitrification in the forest floors of a range of boreal forest stands. Soil Biology and Biochemistry. 1579-1589.

Stoddard JL (1994) Long-term changes in watershed retention of nitrogen: Its causes and consequences. Pages 223-282. In: Baker LA, ed. Environmental Chemistry of Lakes and Reservoirs. Washington DC: American Chemical Society.

Straker, C.J. 1996. Ericoid mycorrhiza: Ecological and host specificity. Mycorrhiza 6:215–225.

Swank WT and Douglass JE (1975) Nutrient flux in undisturbed and manipulated forest ecosystems in the southern Appalachian mountains. Tokyo Symposium (December 1975).

Valett HM, Crenshaw CL, and PF Wagner. (2002). Stream nutrient uptake, forest succession, and biogeochemical theory. Ecology. 83(10):2888-2901.

Virginia RA (1986) Soil development under legume tree canopies. Forest Ecological Management. 16:69-79.

Vitousek PM, Gosz JR, Grier CC, Melillo JM, and WA Reiners (1982) A comparative analysis of potential nitrification and nitrate mobility in forest ecosystems. Ecological Monographs. 52(2):155-177.

Vitousek PM, Aber JD, Howarth RW, Likens GE, Matson PA, Schindler DW, Schlesinger WH and DG Tilman (1997) Human alteration of the global nitrogen cycle: Sources and consequences. Ecological Applications. 7(3): 737-750.

Webster JR and BC Patten (1979) Effects of watershed perturbations on stream potassium and calcium dynamics. Ecological Monographs. 19: 51-72.

Yu Z, Zhang Q, Kraus TEC, Dahlgren RA, Anastasio C, and RJ Zasoski (2002) Contribution of amino compounds to dissolved organic nitrogen in forest soils. Biogeochemistry. 61:173-198.

Zak DR, Groffman PM, Pregitzer KS, Christensen S, and JM Tiedje (1990) The vernal dam: Plant-microbe competition for nitrogen in northern hardwood forests. Ecology. 71(2):651-656.

Chapter 2. Contrasts in carbon and nitrogen ecosystem budgets in adjacent Norway spruce and Appalachian hardwood watersheds in the Fernow Experimental Forest, West Virginia

ABSTRACT

Paired-watershed studies, along with the ecosystem mass-balance budget approach, provide important insight into the effects of altering forested ecosystems on biogeochemical cycles. One common anthropogenic-induced alteration of forests is the conversion of vegetation type through the establishment of planted forests using exotic species, which may alter nutrient cycling characteristics from those of native vegetation. This research utilizes construction of watershed mass-balance budgets of carbon (C) and nitrogen (N) and the measurement of seasonal net N mineralization in an attempt to account for nearly 40 years of large discrepancies in stream NO_3-N export in two nearly adjacent, gauged watersheds at the USDA Forest Service Fernow Experimental Forest in West Virginia. These watersheds have identical management histories, varying primarily by vegetation cover, where one watershed is a monoculture of Norway spruce (*Picea abies*) and the other has regenerated to native Appalachian hardwood. Long-term stream chemistry indicates that the hardwood watershed has approached N-saturation, with relatively high stream export of N (15 kg NO_3^--N/ha), whereas the spruce watershed exhibits virtually no export of N in streamwater. Results of this study indicate that we were unable to account for long-term differences in NO_3-N export via streamflow by estimating the pool size of C and N within the forest floor, mineral soil, above-ground tree biomass, and below-ground tree root biomass in the two watersheds. Total C and N pools were lower in the spruce watershed in nearly every compartment measured, as was total N mineralization. Total C and N pools were 28% and 35% lower in the spruce watershed than the hardwood watershed, respectively. Mean annual net N mineralization was 182 and 64 kg N/ha/yr in the hardwood and spruce soils, respectively, with 79% and <1% of mineralization occurring as nitrification in the hardwood and spruce soils, respectively. Though organic C and N were never measured in the long-term stream chemistry, the discrepancy in C and N budgets between the two watersheds suggests that the spruce watershed may have been subjected to a period of large losses of C and organic N from deeper subsurface soils. This suggests that species conversion has the

potential to significantly alter ecosystem C and N budgets, with implications for long-term productivity, C sequestration, and water quality.

INTRODUCTION

The paired-watershed approach has been instrumental to our current understanding of the effects of anthropogenic-induced alterations to water and nutrient cycling in forested ecosystems (e.g., Hewlett and Helvey 1970; Swank and Douglass 1975; Likens 1985; Adams et al 1993). Stream chemistry at the watershed outlet weirs integrates ecosystem functions (chemical, biological, and physical) and displays responses of the total watershed to alteration. Studies of nutrient mass-balance budgets have been utilized to account for such differences in stream chemistry (e.g., lower stream nitrate export) and to identify ecosystem processes influencing such differences. For example, in a watershed study in the H.J Andrews Experimental Forest in Oregon, total nitrogen (N) ecosystem inputs exceeded exports by 34% (Triska et al 1984). Through construction of a watershed N budget, they were able to account for most of the difference in N as an accumulation in storage of N in the refractory organic matter pool

One common anthropogenic landscape alteration is the conversion of vegetation type through the establishment of planted forests, aimed at increased crop-tree productivity (Carnus et al 2006). Conversions to coniferous forests are the most common practice (Carnus et al 2006). Conversion of native vegetation to monocultures of conifer may disrupt biogeochemical cycling of carbon (C) and nitrogen N (Guo and Gifford 2002). One of the primary influences that trees have on soils is through inputs of organic matter, via both above- and below-ground litter production, which varies by species in quantity and chemical composition (Melillo et al 1982; Binkely 1995; Lovett et al 2004). This organic matter is the main driver for microbial and/or sorption processes by which nutrient bioavailability and/or storage are determined.

Several studies have shown that decomposition and mineralization rates are correlated to the lignin:N ratio in litter material (Aber and Melillo 1982; Melillo et al 1983). Litter high in lignin produces phenolics that more rapidly incorporate NO_2^- abiotically into soil

organic matter (SOM), such as beneath oak (*Quercus spp.*) and beech (*Fagus sylvatica*) (Fitzhugh et al 2003). Microbial community structure has also been shown to change in the rhizosphere of various tree species (Grayston and Campbell 1996). In a pot study where Scots pine (*Pinus sylvestris*), Norway spruce (*Picea abies*) and silver birch (*Betula pendula*) were planted, the soil microbial biomass C and N and the C mineralization rate were increased in soils associated with roots of pine and birch, but not in soils with spruce roots. The stimulatory effect of pine and birch was attributed to their higher volume of fine roots and mycorrhizal associations compared to spruce.

Tree species also alter physical and micro-climatic properties of soils, as differences in canopy density can alter interception, and evapotranspiration rates, resulting in differences in temperature and moisture regimes. These changes in temperature and moisture can regulate microbially driven decomposition and nitrification. Canopy architecture also can result in differences in quantity and chemical composition of throughfall and stemflow, resulting in different amounts and composition of atmospheric inputs into the ecosystem (Mina 1967). Additionally, root architecture can influence soil C and N storage and cycling, as some species, (e.g. conifers) are more shallow-rooted than associated hardwoods. Deeper rooting systems increase subsurface soil oxygen and organic matter, both recalcitrant and labile, through soil penetration, root exudates, root sloughing, and turnover (Farrell 1990; Rhoades 1997) and increase content of surface nutrients by redistributing subsurface nutrients (Virginia 1986).

Large vegetation-mediated variation in N cycling has been documented by many studies (e.g., Robertson 1982; Lovett et al 2004; Christensen et al 2008; SteMarie and Pare 1999; Stark and Hart 1997; Fitzhugh et al 2003). These differences have been shown through variation in N mineralization and nitrification processes, C:N ratios, and foliage and litter N content. For example, soils beneath sugar maple (*Acer saccharum*) exhibited a greater concentration of extractable NO_3^- and NO_2^- than soils under beech (*Fagus sylvatica)* or red oak (*Quercus rubra*) in the Catskill Mountains in New York (Fitzhugh et al 2003), which was attributed to greater incorporation of NO_2^- into SOM beneath oaks that creates N sinks in forest soils. Also in the Catskill Mountains, soils beneath hemlock (*Tsuga*

canadensis) and red oak were shown to have lower nitrification potential than associated beech, sugar maple, and yellow birch (*Betula alleghaniensis*) (Lovett et al 2004), and hemlock had lower foliar and litter N, high soil C:N, low soil N pools and the lowest NO_3^- leaching. In a Norway spruce stand in Finland, immobilization of mineral N was linked to alleleochemical inhibition through volatile organic compounds (terpenes) exuded by Norway spruce, which directly inhibited nitrification, thus resulting in very low nitrate leaching from the stand (Paavolainen et al 1998). Nitrification was initiated in a similar stand following clear-cutting of Norway spruce, where the soil pH and NH_4^+ availability also increased after the stand was removed (Smolander et al 1998). However, high rates of gross nitrification were reported from ^{15}N studies in Sitka spruce (*Picea sitchensis*) forests in Oregon, though net nitrification was negligible (Stark and Hart 1997), indicating significant microbial processing with rapid subsequent immobilization.

Mechanisms by which individual tree species exert such influence on N cycling are still unclear (Lovett et al 2004). Many studies have attempted to link soil characteristics such as soil C:N ratio, lignin:N ratio, soil pH, and phenol concentrations to N retention in forest soils (e.g., Melillo et al 1983, Ste-Marie and Paré 1999; Ross et al 2004). These results are often highly variable and only weak correlations are often noted across a range of soils (Robertson 1982; Ross et al 2004). This implies that in addition to influences of specific tree species, N cycling and retention processes may be additionally affected by forest composition that is a product of harvest practices, past land use, age of the stand, and/or disease outbreaks (Lovett et al 2004).

Two adjacent watersheds within the USDA Forest Service Fernow Experimental Forest (FEF) in West Virginia provide an excellent opportunity to investigate the specific role of tree species in ecosystem N cycling and retention. A large degree of variation in N cycling has been documented among several watersheds within the FEF (Fig. 1), where mean annual stream NO_3-N export has exceeded 15 kg/ha in some watersheds (Peterjohn et al 1996; Gilliam et al 2001). Important observations from long-term stream chemistry of two adjacent watersheds within the FEF include divergent export of stream NO_3-N at the outlets (Adams, USFS, Fig 2). Mean annual stream NO_3-N exported from an experimental 39-yr-old hardwood stand (Watershed 7) is nearly 15 kg/ha, whereas stream

NO$_3$N exported from a nearby experimental 37-yr-old Norway spruce stand (Watershed 6) has been nearly zero for 20 y (mean= 0.18 kg/ha/yr) (Adams, USFS, Fig 2).

Ecosystem C and N mass-balance budgets derived from paired watershed studies incorporate ecological functions that influence C and N cycling and lead to analysis of how specific alterations to an ecosystem may influence these functions. The present work is an attempt to account for nearly 40 years of large discrepancies in stream NO$_3$-N export in two nearly adjacent, gauged watersheds at the FEF via estimates and comparisons of key components of ecosystem C and N budgets. It was hypothesized that because NO$_3$N export has been negligible from the spruce watershed, and because inputs to the two watersheds from atmospheric deposition are equal, then C and N pools will have accumulated to a greater extent in vegetation, forest floor, and soil horizons in the spruce watershed because of slower decomposition of organic material and slower nutrient cycling, resulting in low NO$_3$N export to the stream.

Our specific objectives were to 1) measure selected pool sizes of C and N within each watershed to ascertain if significant differences in these pools occur after nearly 40 years of influences from contrasting forest vegetation and 2) measure rates of net N mineralization to determine if this flux was associated with differences in size of selected C and N pools in the two watersheds. It was hypothesized that a slower mineralization flux occurs in the spruce soils, leading to larger C and N pool sizes of forest floor and mineral soil.

METHODS

Description of the watersheds

The watersheds used in this study are located within the USDA Forest Service FEF near Parsons, West Virginia, USA. The FEF was established as an experimental forest in 1934 and encompasses 1,900 ha. It is surrounded by the Monongahela National Forest. The FEF lies within the unglaciated Allegheny plateau of the Appalachian Mountain Range (Adams et al 1993), with mean elevation range of 300 to 500 m. Annual precipitation is nearly evenly distributed throughout the year, averaging 1,458 mm

(Kochenderfer 2006). Mean monthly precipitation patterns exhibit maximums in June (144 mm) and minimums in October (97 mm). Mean annual temperature is 9.2° C and mean monthly temperatures exhibit maximums in July (20.6° C) and minimums in January (-18° C) (Kochenderfer 2006). Native vegetation of this region is described as mixed central hardwood forest (Core 1966), with common tree species including yellow-poplar (*Liriodendron tulipifera*), sugar maple, black cherry (*Prunus serotina*), and white and red oak (*Quercus alba, Q. rubra*) found on high-quality sites.

Both Watersheds 6 and 7 (WS6 and WS7) were clearcut-logged in sections, beginning in 1964 and concluding in 1967, and maintained barren with herbicides until 1969. Watershed 6 (22 ha) was planted with Norway spruce in 1973, whereas WS7 (24 ha) was allowed to naturally regenerate beginning in 1970. Herbicide was applied to WS6 in 1977 and again in 1980 to prevent hardwood re-growth (Bates 2000). Initial harvesting and herbicide applications through 1969 were implemented to determine the effects of complete deforestation on water yield, and Norway spruce was planted to investigate how conversion of hardwood stands to conifer stands affects quality and quantity of water yield (Kochenderfer et al 1990). After nearly 40 years of growth, the spruce has a closed canopy and dense stand structure. Mean basal area in this watershed is currently 23 m^2/ha. Water yield and stream flow in WS6 are decreasing and are lower than pre-treatment and control levels (hardwood stand, WS7) (Kochenderfer et al 1990). This has been attributed to more transpiration and interception in conifers (Kochenderfer et al 1990).

Watershed 6 has a channel length of 857 m, which runs south through the watershed (Figure 1). Thus, hillslopes form east/west aspects along the stream. Soils in this watershed are mapped as Calvin series (Calvin channery silt loam; Calvin loamy-skeletal, mixed, active, mesic typic Dystrudept) (Soil Survey Staff, USDA NRCS), derived from shale, siltstone, and sandstone parent material. The Norway spruce stand in this watershed has created a litter layer characteristic of natural conifer stands (mor-type), with a thick horizon of non-decomposed needles above further decomposed organic material of spruce origin. There are few other species in the watershed, with sparse

patches of green briar (*Smilax* sp.) and few individual hardwood species that survived the herbicide treatments including black locust (*Robinia pseudoacacia)*, yellow poplar, red maple (*Acer rubrum)*, and sourwood (*Oxydendron arboreum*). Thus, the landscape is a very homogeneous monoculture of Norway spruce. Mean height of dominant trees at 30 yrs of age was approximately 17 m. The stream bed and riparian area have a thick growth of moss mats that are not present in WS7.

Watershed 7 has a channel length of 550 m, which runs eastward through the watershed (Figure 1), resulting in hillslopes with north/south aspects along the stream. Soils in this watershed are mapped as both Calvin (Calvin channery silt loam) and Dekalb series (Dekalb channery loam and Dekalb extremely stony loam; Dekalb loamy-skeletal, siliceous, active, mesic typic Dystrudept), derived from acidic sandstone parent material (Soil Survey Staff, USDA NRCS). This watershed is dominated by yellow-poplar, red oak , and sugar maple, with an under-story of dogwood (*Cornus floridia*), striped maple (*Acer pennsylvanicum*), magnolia (*Magnolia acuminata*), and several species of fern. Mean basal area in this watershed is currently 17 m^2/ha. Both watersheds are gauged with a 120-degree sharp-crested V-notch weir and FW-1 water level recorder.

Historic stream NO_3-N export and specific conductivity data from the spruce and hardwood watersheds indicate close similarity in ecosystem biogeochemical activity at the time of conversion to a Norway spruce stand (Fig. 3a and b). When analyzed by decade after conversion, patterns of stream NO_3-N values were very similar between the two watersheds (R^2=0.96) (Fig. 3a) during the first decade following treatment (1971-1980). The divergence in this parameter did not occur until after the Norway spruce began to fully occupy the site and canopy closure occurred (R^2=0.20 after 1981 and R^2=0.0005 after 1991).

Atmospheric deposition and stream export of NO_3-N and DON
Data for wet and dry deposition of NO_3-N were attained from annual records from the NADP monitoring site WV18 and the CASTnet monitoring site PAR107 (National Atmospheric Deposition Program). Records are available beginning in 1979 and inputs

prior to 1979 were assumed to be equal to the first year of available data (1979) in order to create a complete budget. Stream flow and weekly NO_3-N concentration data for WS6 and WS7 were attained from the USDA Forest Service Timber and Watershed Lab, Parsons, WV. These data were used to calculate total inputs and exports of NO_3-N from the two watersheds for 1973 through 2009.

In addition to long-term stream export NO_3-N data, total dissolved N was measured from July 2007 to September 2009 in monthly stream samples from both watersheds in order to determine export values of dissolved organic N (DON) that had not previously been measured in these watersheds. Water samples were collected in acid-washed HDPD bottles at the weir outlet and filtered through Whatman 42 filter paper prior to freezing for storage. Total dissolved N was analyzed on a Shimadzu TOC-TN analyzer and DON was determined by subtracting NO_3-N and NH_4-N from total dissolved N.

Potential flux from net N mineralization

To determine the potential flux of inorganic N resulting from N mineralization, intact soil cores (0-10 cm) were collected at 12 sampling sites (Fig. 4) in each watershed in stainless steel cores (25.4 cm length x 10 cm width). Two soil cores were sampled at each soil sampling site, with one immediately taken to the laboratory to measure initial soil N pools, and the other remaining in the field, capped and aerated by holes made in the upper part of the tube for a 4-week *in situ* incubation (Tietema et al 1990). Three replicate soil cores of both initial and incubated samples were taken at each site. Soils were removed from initial and incubated cores, air-dried, and sieved through 2mm mesh. Potassium chloride (2 *M*) extractions were performed to determine concentrations of inorganic N (NH_4-N and NO_3-N). Extracts were analyzed for inorganic N on an auto-analyzer (Bran-Luebbe). Differences in NH_4-N and NO_3-N concentrations between the initial sample and the field-incubated sample were considered as net ammonification, net nitrification or net immobilization occurring during the 4 week incubation. Incubations were performed seasonally (four times a year) for two years to determine temporal differences in net ammonification, nitrification, or immobilization.

Soil C and N pools

In order to estimate C and N pool sizes within surface soil horizons and the forest floor, soil samples were collected in July 2007 from 30 sampling sites within each watershed at each horizon (Fig. 4). Soil horizons are defined in these watersheds as A (0 to 10 cm depth) and B (10 to 46 cm depth). Generally, bedrock occurs at approximately 45-50 cm. A-horizon soils were collected with a 10-cm diameter soil corer from 10 random locations at each sampling site and composited. B-horizon samples were collected from 3 random locations at each sampling site with a 10-cm diameter soil corer and composited. Soils were air-dried and sieved to 2 mm. Coarse fragments within soil samples were cleaned and weighed to determine the percent of the fine soil material at each sampling site. Total N and C were analyzed on a CN elemental analyzer (Elementar VarioMax CNS, Hanau, Germany). Bulk density (BD) was determined at each soil sampling site and horizon using a hammer-driven corer.

Forest floor samples were collected in October 2007 and 2009 and were characterized by determining depth, oven-dry weight (dried at 105° C for 48 hr), and total C and N at each sampling location. Total C and N in forest floor were analyzed on a CN elemental analyzer (Elementar VarioMax CNS). All organic material < 2 cm in diameter was included in forest floor analyses. Freshly deposited litter materials were collected monthly from July 2008 through October 2009 in 1 m x 1 m screened traps (1 mm mesh size) located at 12 sampling locations (Fig. 4) throughout the watersheds. Litter C and N analysis was performed on samples collected in October 2009 on a CN elemental analyzer (Elementar VarioMax CNS).

Aboveground and belowground biomass C and N pools

Diameter at breast height (DBH) of trees was determined by the USDA Forest Service Timber and Watershed Lab in 2003 in each watershed using 0.004 ha plots in the spruce watershed and 0.04 ha plots in the hardwood watershed. Each individual tree was converted to dry-weight biomass (kg) using allometric equations for both above- and below-ground (Table 1). Spruce DBH data were converted to height (m) using the

equation from Huang et al (1992), (species group 5; function number 13) prior to applying biomass allometric equations (Table 1). Above-ground biomass equations from Fehrmann and Kleinn (2006) were applied for the spruce watershed. This function was designed from Norway spruce stands in central Europe and utilizes tree height class in addition to the DBH parameter (Table 1). Below-ground biomass in the spruce watershed was estimated using the equation from Drexhageand Gruber (1999). This function was developed from 40-yr-old Norway spruce stands in Germany. Individual tree biomass was then scaled to kg/ha for each watershed.

An above-ground biomass (kg) equation from Jenkins et al 2003 was used for the hardwood watershed (Table 1). The variables applied in this equation are species-group specific, designed for tree species across the US, and were applied as follows: soft maple/birch equation was used for red maple and sweet birch, hard maple/oak/hickory/beech equation was used for sugar maple, northern red oak, black cherry, yellow poplar, and American beech, and the Mixed hardwood equation was used for the remaining species. Below-ground biomass in the hardwood watershed was estimated using the equation for all northern hardwood species at middle elevation (630-710 m) developed by Vadeboncouer et al (2007; Table 1). This function was developed from data collected at Hubbard Brook Experimental Forest, New Hampshire.

From total above-and below-ground biomass estimates, tree compartment mass and percent C and N (bole wood, bole bark, twigs and branches, foliage, and roots) were estimated using values published by Whittaker et al (1974) for the hardwood watershed and by Feng et al (2008) for the spruce watershed (Table 2). Concentration of C was assumed to be 50% of biomass within each compartment. Concentration of C and N were multiplied by each tree compartment mass (kg) to attain total biomass values of C and N (Whittaker et al 1979; Feng et al 2008).

Data analysis
The experimental design of this paired-watershed study is an example of pseudo-replication, with an effective sample size of one (Gilliam et al 2001). However,

differences occurring in the data collected are assumed to be caused by treatment (vegetation) differences because the soils in both watersheds are commonly classified in the Calvin soil series (Soil Survey Staff, USDA NRCS) and historical data of stream conductivity indicate that differences between watersheds in soil and stream chemistry occurred primarily following the clearcut harvests in 1967 and subsequent planting of Norway spruce. One-way ANOVA was performed to determine differences in C and N pool size within each compartment by watershed. One-way ANOVA was performed to determine differences in *in situ* net nitrification and net ammonification annually and within each season by watershed. All statistical analyses were performed using SAS-JMP version 8.0, and an α=0.05 significance level was used.

RESULTS

Inputs and exports

Atmospheric inputs of NO_3-N via wet and dry deposition since 1973 (year of planting of Norway spruce) are shown in Fig. 2. It was assumed here that atmospheric inputs into each watershed within the FEF are identical. Since 1973, combined wet and dry atmospheric deposition of NO_3-N amounted to 319.83 kg N/ha. Total stream export of NO_3-N from the spruce watershed since 1973 was 45.93 kg N/ha. This was only 13% of the stream export of NO_3-N that occurred during the same time period in the hardwood watershed, which has exported 341.07 kg NO_3-N/ha, exceeding the value of atmospheric deposition by approximately 21 kg N/ha. Monthly measures of total dissolved N in stream export (measured from July 2007-Sept 2009) indicated that annual DON export from the spruce watershed was also very low (mean = 0.477 kg DON/ha/yr). In contrast, annual DON export (mean = 10.03 kg DON/ha/yr) from the hardwood watershed was nearly equal to export of NO_3-N (mean = 10.70 kg NO_3-N/ha/yr) during the July 2007 – Sept. 2009 interval when DON was measured along with NO_3-N.

Potential flux from N mineralization

Total net N mineralization annual flux was approximately three times greater in the hardwood watershed than in the spruce watershed (p=0.0342; Fig. 5) during the 2007-2009 sampling period. Mean annual net N mineralization was 182 and 64 kg N/ha/yr

from the hardwood and spruce soils, respectively. Of the total annual net inorganic N mineralized, a greater proportion of the total N mineralized was from nitrification in the hardwood soils (79%). In the spruce watershed, most of the total net N mineralized was limited to ammonification (nearly 94%). Highest seasonal net mineralization occurred in the summer months in the hardwood watershed (mean in summer = 21.43 kg N/ha/month), and in the spring months in the spruce watershed (mean in spring = 10.83 kg N/ha/month).

Soil and forest floor C and N pools

Carbon and N pools within the mineral soil horizons were significantly lower in the spruce watershed relative to the hardwood watershed (Fig. 6a). Within the A-horizon, spruce soil contained about 20% less C (kg/ha) than the hardwood soil (p= 0.0106; Fig. 6a). Carbon concentration also tended to be lower in the A-horizon of the spruce soil (38.8 g C/kg in spruce soil versus 45.0 g C/kg in hardwood soil; p=0.0887). In addition to lower C concentrations, BD was significantly lower (p=0.0258) in the A-horizon of the spruce soil, (0.91 Mg/cm^3) than in the hardwood soil (1.005 Mg/cm^3). Within the B-horizon, spruce soil contained approximately 30% less C (kg/ha) than the hardwood soil. Carbon concentration was also lower in the B-horizon of the spruce soil (12.1 g C/kg in the spruce versus 17.7 g C/kg in the hardwood soil; p=0.0020). Differences in BD were not evident in the deeper B-horizon soils, with values of 1.24 and 1.19 Mg/cm^3 in spruce and hardwood soils, respectively.

Nitrogen pools in the mineral soil followed the same pattern as C, with A-horizon soil in the spruce watershed containing nearly 35% less N than the hardwood soil (p<0.0001; 2.03 g N /kg in spruce versus 2.93 g N/kg in hardwood) (Fig. 6b). Within the B-horizon pool, spruce soil contained nearly 38% less N content (kg/ha) than the hardwood soil (p=0.0002; 0.73 g N/kg in spruce soil versus 1.17 g N/kg in hardwood soil).

Forest floor biomass in the spruce watershed was 43% greater than in the hardwood watershed (p<0.0001; Fig. 6c). However, this accumulation of biomass in the forest floor material accounted for only a small percentage of the total C and N storage in the spruce

watershed (Fig. 6a and 6b). Forest floor C content (kg/ha) was significantly greater in the spruce watershed than the hardwood watershed (3,813.63 and 2,343.35 kg C/ha in the spruce and hardwood watersheds, respectively; $p<0.0001$) (Fig. 6a). The pool of forest floor N (kg/ha) was significantly greater in the spruce watershed than in the hardwood watershed (132.25 kg N/ha in the spruce watershed and 74.68 kg N/ha in the hardwood watershed; $p<0.0001$) (Fig. 6b). Forest floor N concentration was not different between the two watersheds (14.6 and 14.3 g N/kg in the spruce and hardwood watersheds, respectively).

Monthly litter inputs collected from July 2008 through October 2009 did not differ significantly in terms of biomass between the two watersheds ($p=0.1404$), though litter biomass tended to be greater in the hardwood watershed (Fig. 6c). Hardwood litter inputs were 1,852 kg/ha/month and spruce litter inputs were 1,474 kg/ha/month. Carbon and N content in litter were estimated from samples collected in October 2009 (Fig. 6a and b). Nitrogen content in hardwood litter was significantly higher than the spruce ($p<0.0001$; Fig. 6b; 74.73 kg N/ha/yr in the spruce watershed and 151.72 kg N/ha/yr in the hardwood watershed). Nitrogen concentration in hardwood litter was 12.0 g N/kg, whereas the spruce litter contained only 7.6 g N/kg.

Tree biomass pools
Both above- and below-ground biomass in trees (kg/ha,), as estimated by allometric equations, were higher in the hardwood watershed (Fig. 6c). Above-ground biomass estimates were approximately 30% less in the spruce watershed (116,800 kg/ha) relative to the hardwood watershed (166,000 kg/ha). Below-ground biomass estimates were approximately 45% less in the spruce watershed (21,000 kg/ha) relative to the hardwood watershed (38,000 kg/ha). This higher biomass in the hardwood watershed equated into higher pool sizes of C and N in the hardwood trees than the spruce (Fig. 6a and b). Nitrogen content in the hardwood watershed in above-ground biomass (410 kg N/ha) was approximately 30% greater than the spruce watershed (278 kg N/ha). Estimates of below-ground N content exhibited a similar pattern, with more below-ground biomass N content occurring in the hardwood watershed (267 kg N/ha) than in the spruce watershed

(250 kg N/ha). Though current basal area was slightly greater in the spruce watershed (27.23 and 26.91 m^2/ha in the spruce and hardwood watersheds, respectively), the density of spruce wood is less than that of most hardwoods, especially oak (0.45 and 0.63 g/cm^3 density in spruce and red oak, respectively) (USDA Forest Service Research and Development, Forest Products Lab, Madison, WI).

DISCUSSION

Effects of contrasting vegetation on C and N pools

The goal of this study was to quantify selected ecosystem C and N pools in two watersheds that exhibit large differences in long-term stream export of NO_3-N measured since establishment of contrasting forest types in 1973. This divergence in export of NO_3-N is assumed to be a function of the differences in forest vegetation (i.e. Norway spruce versus native hardwoods). In a review of annual input-output N budgets of 24 watersheds located within the northeastern US from 1979-1998, all but one watershed exhibited net retention of deposited NO_3-N, with a mean annual input-output balance of +2.5 kg NO_3-N/ha (Campbell et al 2004). The one watershed that had net watershed loss of NO_3-N via stream export was also located within the FEF, with an annual input-output balance of -0.7 kg NO_3-N/ha (Watershed 4, vegetative reference). From 1979-1998, the hardwood watershed of our study (WS7) has a mean annual input-output balance of -4.5 kg NO_3-N/ha. Thus, this watershed may not be representative of many hardwood watersheds across the northeastern US in terms of N cycling and may indeed be exhibiting symptoms of N saturation (Campbell et al 2004).

Results of this study indicate that we were unable to account for the differences in NO_3-N export via streamflow (Fig.2) through estimation of the size of C and N pools within the forest floor, mineral soils, above-ground tree biomass, and below-ground tree root biomass in the two watersheds. Total C and N pools were lower in the spruce watershed in nearly every compartment measured (Fig. 6), as was total N mineralization (Fig. 5). Total C pools were 28% less in the spruce and total N pools were 35% less in the spruce as compared to the hardwood watershed. The B-horizon soil compartment exhibited the largest difference in both C and N stores (32% less C and 38% less N in the spruce

28

watershed), followed by the A-horizon soil compartment, where there was nearly 20% less C and 34% less N in the spruce soil than in the hardwood soil. These results were contrary to the hypothesis that soil and forest floor C and N stores would be higher in the spruce watershed, thereby accounting for 40 years of relatively high atmospheric N input and very low stream export of NO_3-N from the spruce watershed.

Total N pools in the mineral soil and in tree biomass of two additional watersheds (Watersheds 4 and 10) within the FEF are shown in Fig. 7. Watersheds 4 and 10 are often used as references because they have been left to natural recovery since being logged in 1905 (Kochenderfer 2006). Comparing the spruce watershed (WS6) to watersheds 4, 7, and 10, which are characterized by native hardwood forests, illustrates that the spruce watershed is relatively deplete of N in the measured pools (Fig. 7). It is also noteworthy that the native hardwood watershed of this study (WS7) has similar estimated N pool sizes in soil and biomass as reference watershed 10 (Fig. 7).

What accounts for differences in C and N pools?

Three possible mechanisms have been identified that could explain why, after 40 years of much lower NO_3-N export and high atmospheric deposition, the spruce watershed does not exhibit patterns of C and N accumulation in comparison to the hardwood watershed. These include: 1) the watersheds are intrinsically different and the spruce watershed has always had much smaller storage of C and N than the surrounding watersheds, 2) the spruce watershed has been losing N via denitrification/gaseous loss at a much greater rate than the hardwood watershed for the past 40 years (Reddy and Patrick 1975), and/or 3) the spruce watershed underwent a phase of soil organic matter degradation when the hardwood stand was replaced by the Norway spruce stand, as this converted vegetation type induced stronger soil acidification, causing a large amount of organic forms of N to be leached from the system (Guggenberger et al 1994; Currie et al 1996; McDowell et al 1998; Campbell et al 2000).

It is unlikely that the spruce watershed is intrinsically different than the surrounding hardwood watersheds within the FEF to the degree observed in the present study (Fig 7).

The soils within all of these watersheds are of the same soil series (Calvin and Dekalb; both are classified as dystrudepts), with similar historic land use and atmospheric inputs (Kochenderfer 2006). Historic stream NO_3-N export and specific conductivity data from the spruce and hardwood watersheds indicate close similarity in ecosystem biogeochemical activity at the time of conversion to a Norway spruce stand (Fig. 3a and b). When analyzed by decade after conversion, it can be seen that in the first decade following treatment (1971-1980), patterns of stream NO_3-N values were very similar between the watersheds (R^2=0.96) (Fig. 3a). The divergence in this parameter did not occur until after the Norway spruce began to occupy the site and canopy closure occurred (R^2=0.20 after 1981 and R^2=0.0005 after 1991).

Large losses of NO_3-N were never detected in the long-term stream chemistry data for the spruce watershed (Adams, USFS, Fig. 2). This suggests that N might have been lost through fluxes in gaseous phase of N_2O or NO (Reddy and Patrick 1975) (gaseous losses not measured), and/or through stream export of DON (Campbell et al 2000) (also not measured). It is unlikely that denitrification processes can totally explain the smaller N pools in the spruce watershed because 1) large fluxes of denitrification usually results in accumulation of C in the organic horizons of soils (Malmer and Wallen 1993; Hernandez and Mitsch 2007), which was not observed in the current study and 2) N losses via denitrification usually account for a small portion of total ecosystem N (Mohn et al 2000; Menyailo et al 2002).

For denitrification to occur, soils must be sufficiently saturated, have sufficient substrate of suitable C and oxidized N, and have moderate pH and temperature (Federer and Klemedtsson 1988). Within the subsurface soils of the spruce watershed, there is evidence of gray mottled soils at some of the sampling sites, indicating anoxic conditions and denitrification processes may explain a small portion of the differences in soil N between the two watersheds. However, denitrification potential rates were reported to be very low within the Long Term Soil Productivity plots in the FEF, especially in recently harvested plots (292 ng N_2O-N/g/hr) (Wallenstein et al 2006). Also, denitrification enzyme activity (DEA) was reported to be 2-3 times lower in soils beneath Norway

spruce relative to adjacent silver birch and Scots pine stands in Finland (Priha and Smolander 1999). These stands were all established 60 years prior to measurement, and were of similar pH to the spruce soils at the FEF (pH 3.8-3.9 in surface mineral soils). In another study, potential denitrification activity in the top 0-10 cm mineral soil was also found to be lowest beneath Norway spruce (6 mg N_2O-N/kg soil/day), compared to 5 other tree species in Russia (Menyailo et al 2002).

In ecosystems that exhibit high rates of denitrification, organic accumulation in soil is common, as seen in wetlands, especially sphagnum and peat bogs (e.g., Malmer and Wallen 1999; Herndandez and Mitsch 2007). Organic matter accumulation is the result of slow decomposition under anaerobic conditions, which is a required condition for denitrification. This organic matter accumulation results in the formation of peat deposits and C storage (Hernandez and Mitsch 2007). No evidence of organic matter accumulation was observed within the spruce watershed in the current study, suggesting that large fluxes of N through denitrification likely have not occurred.

Additionally, total annual denitrification rates are usually a small portion of ecosystem N fluxes. For example, Mohn et al (2000) showed that annual total denitrification flux in a fertilized spruce forest was only 2.9 kg N/ha/yr, compared to denitrification flux of 1.7 kg N/ha/yr in unfertilized plots. This is a small fraction of what is lost through annual stream export of NO_3-N and DON in the hardwood watershed in this study.

It is also possible that soils within the spruce watershed underwent a phase of soil organic matter degradation as the spruce stand became established, inducing a loss of C and N that was not detected in the long-term stream data that only measured losses of NO_3-N, and did not measure DON. The process of soil organic matter decline is mediated by soil acidification, and C inputs into the mineral soil are also slowed as organic matter begins to accumulate in the forest floor as soil acidity increases (Brumme and Khanna 2008). Soil acidification decreases biotic activity and increases the mobility of Al, leading to the degradation of organic matter and losses of soil C. Guggenberger et al (1994) also showed that as podzolization progresses and soil acidification increases, the soil capacity

for retention of dissolved organic C is reduced, resulting in a shifting of illuvial horizons downward, and a larger export of dissolved organic C from the solum.

This concept of soil organic matter degradation is strongly supported by a chronosequence study of soil C stocks beneath red spruce (*Picea rubens*) forests in northeastern North America (Diochon et al 2009). Soils within these forests exhibited increasingly smaller stocks of C from 1-, 15-, and 45-year-old stands, reaching a minimum of approximately 76 Mg C/ha in the soil profile at 45 years. Soil C stocks began to increase after 45 years, and were considerably higher in 80- and 125-year-old stands. Soil C values in the 45-year-old red spruce stand were very similar to the Norway spruce soils in the present study, which contain about 74 Mg C/ha. Carbon loss (decrease in C concentration and content) from young stands in the Diochon et al study (2009) was reported to occur through enhanced mineralization of organic compounds (verified with stable C isotopic analysis), especially in the deeper soil horizons. Similar declines in soil C were also observed in spruce forests of similar age by other authors (Tremblay et al 2002, Parker et al 2001; McLaughlin and Phillips 2006), indicating that this rate of C loss from spruce soils is a common phenomenon.

Forest clearing may result in decreases in soil C, caused by disturbance and changes in microclimate, but C stores generally recover to original levels after several decades, especially if the stand is allowed to regenerate (Harrison et al 1995, Chen et al 2005; Kashian et al 2006). For example, a planned contrast analysis showed a 30% decrease in soil C content with conversion of native broadleaf forest to pine plantation after 37 years in Australia (Kasel and Bennett 2007), with no changes in soil C observed in the absence of conversion to pine and recovery to native broadleaf forest (Guo and Gifford 2002). This loss of soil C can be attributed to both disturbance and changes in amount and composition of plant material returned to the soil via litter and rooting (Lugo and Brown 1993). Additionally, the presence of ectomycorrhizal fungi introduced upon vegetation conversion have previously been documented to induce a 30% soil C depletion within 20 years of establishment of an exotic Radiata pine (*Pinus radiata*) plantation in Ecuador (Chapela et al 2001). This study utilized stable C isotopic tools and radiocarbon dating of

fungal tissue to show that ectomycorrhizal fungi can utilize stable soil C stores as an energy source, thus depleting C from the soil profile.

Additional studies from forests that were allowed to regenerate to native species indicate that soil C generally accumulates at a linear rate from time of harvest. For example, linear rates of C accumulation were noted since year of farm abandonment through 115 years in a chronosequence in soils beneath white pine (*Pinus strobus*) in Rhode Island (Hooker and Compton 2003). Johnson (1992) also reviewed harvesting effects on soil C stocks in several forest types, and reported that most studies show no significant change (± 10%) with harvesting, a few studies show large net losses of soil C, and a few studies show a net gain of soil C following harvesting.

Ecosystem N losses in the form of DON export following forest species conversion have not been reported. However, organic forms of N were shown to comprise 90% and about 75% of the total dissolved N in soil solution in organic and E-horizons, respectively, one year after a whole-tree harvesting of a sitka spruce (*Picea sitchensis*) forest in England (Stevens and Wannop 1987). Additionally, several ^{15}N addition studies have shown a high degree of N incorporation into soil organic pools, primarily through abiotic mechanisms (Schimel and Firestone 1989; Emmett and Quarmby 1991). A large portion of soil organic N is typically incorporated in humin compounds (non-digestible portion in 6*M* HCl) (Cheng and Kurtz 1963) and lignin has an inhibitory effect on decomposition and N mineralization, thereby forming stable organic nitrogenous compounds (Berg et al 1984; Fenn eal al 1998).

Spruce vegetation has high lignin content in litter materials and shallow rooting architecture (Melillo et al 1983). High lignin content results in larger proportions of soil organic N relative to inorganic forms because of slower decomposition and mineralization (Berg et al 1984). Shallow rooting architecture may result in flushing of soil C (and N incorporated in stable organic materials) following decomposition of deep roots of the native hardwood that was present prior to conversion, with little subsequent vegetative uptake or stabilization deep in the spruce soil profile. Thus, the spruce

features of slowly decomposable organic matter and shallow rooting may help explain the apparent large mass losses of soil C and N relative to the native hardwood in this watershed study.

Though losses of DON were never measured in the long-term stream chemistry at these sites, it is likely that the spruce watershed was subjected to a period of large losses of C and organic N from deeper subsurface soils, as several other studies have reported (Diochon et al 2009; Chapela et al 2001). It appears that in addition to species influences, time since harvest must also be taken into account when attempting to assess species-mediated ecosystem C and N processes.

CONCLUSIONS

Results of this study suggest that a significant loss of C and N from ecosystem pools likely occurred following conversion from native hardwoods to a monoculture of Norway spruce in the FEF. The present study demonstrates that a watershed that has developed a characteristic state of nutrient pools and fluxes under native vegetation can change relatively quickly when influenced by harvesting and conversion to a new species, leading to a significantly changed state of nutrient pools and fluxes. Consequently, species selection should be taken into account when managing forests for future C sequestration, for provisions of high-quality water, and for effects of high atmospheric inputs of N, especially when relatively short rotation times are implemented between harvests.

WORKS CITED

Aber JD and JM Melillo (1982) Nitrogen immobilization in decaying hardwood leaf litter as a function of initial nitrogen and lignin content. Canadian Journal of Botany. 60(11): 2263-2269.

Adams MB, Edwards PJ, Wood F, and JN Kochenderfer (1993) Artificial watershed acidification on the Fernow Experimental Forest, USA. Journal of Hydrology. 150: 505-519

Augusto L, Ranger J, Binkley D, and A Rothe (2002) Impact of several common tree species of European temperate forests on soil fertility. Annals of Forest Science. 59(3): 233-253.

Bates NS (2000) Effects of forest harvesting on headwater watersheds in West Virginia. Thesis, BS Engineering. Princeton University.

Berg B and O Theander (1984) Dynamics of some nitrogen fractions in decomposing Scots pine needle litter. Pedobiologia. 27(4): 261-267.

Binkley D (1995) The influence of tree species on forest soils: Processes and patterns. In: Mead DJ and IS Cornforth, Eds. (1995) Proceedings of the trees and soil workshop, Lincoln University 28 February-2 March 1994. Agronomy Society of New Zealand Special Publication No. 10. Lincoln University Press, Canterbury.

Brumme R and PK Khanna (2008) Ecological and site historical aspects of N dynamics and current N status in temperate forests. Global Change Biology. 14(1): 125-141.

Campbell JL, Hornbeck JW, McDowell WH, Buso DC, Shanley JB, and GE Likens (2000) Dissolved organic budgets for upland, forested ecosystems in New England. Biogeochemistry. 49(2): 123-142.

Campbell JL, Hornbeck JW, Mitchell MJ, Adams MB, Castro MS, Driscoll CT, Kahl JS, Kochenderfer JN, Likens GE, Lynch JA, Murdoch PS, Nelson SJ, and JB Shanley (2004) Input-output budgets of inorganic nitrogen for 24 forest watersheds in the northeastern United States: A review. Water, Air, and Soil Pollution. 151: 373-396.

Carnus JM, Parrotta J, Brockerhoff E, Arbez M, Jactel H, Kremer A, Lamb D, O'Hara K, and B Walters (2006) Planted forests and biodiversity. Journal of Forestry. 104(2): 65-77.

Chapela IH, Osher LJ, Horton TR, and MR Henn (2001) Ectomycorrhizal fungi introduced with exotic pine plantations induce soil carbon depletion. Soil Biology and Biochemistry. 33: 1733-1740.

Chen J, Chen W, Liu J, Cihlar J, and S Gray (2000) Annual carbon balance of Canada's forests during 1895-1996. Global Biogeochemical Cycles. 14(3): 839-850.

Cheng HH and Kurtz LT (1963) Chemical distribution of added nitrogen in soils. Soil Science Society of America. 27: 312-316.

Christenson LM, Lovett, GM, Weathers KC and MA Arthur (2009) The influence of tree species, nitrogen fertilization, and soil C to N ratio on gross soil nitrogen transformations. Soil Science Society of America Journal. 73(2): 638-646.

Core EL (1966) Vegetation of West Virginia. McClain Printing Co., Parsons, W.Va., 217 p., illus.

Currie WS, Aber JD, McDowell WH, Boone RD, and AH Magill (1996) Vertical transport of dissolved organic C and N under long-term N amendments in pine and hardwood forests. Biogeochemistry. 35: 471-505.

De Schrijver A, Geudens G, Augusto L, Staelens J, Mertens J, Wuyts K, Gielis, and K Verheyen (2007) The effect of forest type on throughfall deposition and seepage flux: a review. Oecologia. 153(3): 663-674.

Diochon A, Kellman L, and H Beltrami (2009) Looking deeper: An investigation of soil carbon losses following harvesting from a managed northeastern red spruce (*Picea rubens* Sarg.) forest chronosequence. Forest Ecology and Management. 257(2): 413-420.

Drexhage M and F Gruber (1999) Above- and below-stump relationships for Picea abies: Estimating root system biomass from breast-height diameters. Scandinavian Journal of Forest Research. 14(4): 328-333.

Emmett BA and C Quarmby (1991) The effect of harvesting intensity on the fate of applied 15N-ammonium to the organic horizons of a coniferous forest in N. Wales. Biogeochemistry. 15(1): 47-63.

Farrell J (1990) The incluence of trees in selected agroecosystems in Mexico. In: Gliessman SR, Ed. Agroecology: Researching the ecological basis for sustainable agriculture. 167-183. Springer-Verlag, New York.

Federer CA and L Klemedtsson (1988) Some factors limiting denitrification in slurries of acid forest soils. Scandinavian Journal of Forest Research. 3(1-4): 425-435.

Feng Z, Brumme R, Xu Y-J, and N Lamersdorf (2008) Tracing the fate of mineral N compounds under high ambient N deposition in a Norway spruce forest at Solling/Germany. Forest Ecology and Management. 255(7): 2061-2073.

Fenn (1998) Nitrogen excess in North American ecosystems: Predisposing factors, ecosystem responses, and management strategies. Ecological Applications. 8(3):706-733.

Fehrmann L and C Kleinn (2006) General considerations about the use of allometric equations for biomass estimation on the example of Norway spruce in central Europe. Forest Ecology and Management. 236(2-3): 412-421.

Fitzhugh RD, Lovett GM, and RT Venterea (2003) Biotic and abiotic immobilization of ammonium, nitrite, and nitrate in soils developed under different tree species in the Catskill Mountains, New York, USA. Global Change Biology. 9: 1591-1601.

Gilliam FS, Yurish BM, and MB Adams (2001) Temporal and spatial variation of nitrogen transformations in nitrogen-saturated soils of a central Appalachian hardwood forest. Canadian Journal of Forest Restoration. 31:1768-1785.

Grayston SJ and CD Campbell (1996) Functional biodiversity of microbial communities in the rhizospheres of hybrid larch (*Larix eurolepis*) and Sitka spruce *(Picea sitchensis)*. Tree Physiology. 16, 1031-1038.

Guggenberger G, Zech W, and H-R Schulten (1994) Formation and mobilization pathways of dissolved organic matter: evidence from chemical structural studies of organic matter fractions in acid forest floor solutions. Organic Geochemistry. 21(1): 51-66.

Guo LB and RM Gifford (2002) Soil carbon stocks and land use change: a meta analysis. Global Change Biology. 8(4): 345-360.

Harisson KG, Post WM, and DD Richter (1995) Soil carbon turnover in a recovering temperate forest. Global Biogeochemical Cycles. 9(4): 449-454.

Hernandez ME and WJ Mitsch (2007) Denitrification potential and organic matter as affected by vegetation community, wetland age, and plant introduction in created wetlands. Journal of Environmental Quality. 36: 333-342.

Hewlett JD and JD Helvey (1970) Effects of forest clear-felling on the storm hydrograph. Water Resources Research. 6(3): 768-782.

Hooker TD and JE Compton (2003) Forest ecosystem carbon and nitrogen accumulation during the first century after agricultural abandonment. Ecological Applications. 13(2): 299-313.

Huang S, Titus SJ, and DP Wiens (1992) Comparison of nonlinear height-diameter functions for major Alberta tree species. Canadian Journal of Forest Research. 22: 1297-1304.

Jenkins JC, Chojnacky DC, Heath LS, and RA Birdsey (2003) National-scale biomass estimators for United States tree species. Forest Science. 49(1): 12-35.

Johnson DW (1992) Effects of forest management on soil carbon storage. Water, Air, and Soil Pollution. 64(1-2): 83-120.

Kasel S and LT Bennett (2007) Land-use history, forest conversion, and soil organic carbon in pine plantations and native forests of south eastern Australia. Geoderma. 137(3-4): 401-413.

Kashian DM, Romme WH, Tinker DB, Turner MG, and MG Ryan (2006) Carbon storage on landscapes with stand-replacing fires. BioScience. 56(7): 598-606.

Kochenderfer JN, Edwards PJ, and JD Helvey (1990) Land management and water yield in the Appalachians. In: RE Riggins, EB Jones, R Singh, and PA Rechard, Eds.,

Proceedings IR Conference Watershed Management, ID DIV/ASCE, Watershed Planning and Analysis in Action Symposium, Durango, CO, July 9-11, 1990, pp. 523-532, American Society of Civil Engineers, New York.

Kochenderfer JN (2006) Fernow and the Appalachian hardwood region. In: Adams MB, DeWalle DR, and JL Hom (2007). The Fernow Watershed Acidification Study. Environmental Pollution. Springer Netherlands.

Likens GE (1985) An experimental approach for the study of ecosystems: The fifth Tansley lecture. British Ecological Society. 73(2): 381-396.

Lovett GM, Weathers KC, Arthur MA, and JC Schultz (2004) Nitrogen cycling in a northern hardwood forest: Do species matter? Biogeochemistry. 67(3):289-308.

Lugo AE and S Brown (1993) Management of tropical soils as sinks or sources of atmospheric carbon. Plant and Soil. 149(1): 27-41.

Malmer N and B Wallen (1999) The dynamics of peat accumulation on bogs: mass balance of hummocks and hollows and its variation throughout a millennium. Ecography. 22: 736-750.

McDowell WH, Currie WS, Aber JD, and Y Yano (1998) Effects of chronic nitrogen amendments on production of dissolved organic carbon and nitrogen in forest soils. Water, Air, and Soil Pollution. 105: 175-182.

McLaughlin JW and SA Phillips (2006) Soil carbon, nitrogen, and base cation cycling 17 years after whole-tree harvesting in a low-elevation red spruce (*Picea rubens*)-balsam fir (*Abies balsamea*) forested watershed in central Maine, USA.

Melillo JM, Naimen RJ, Aber JD, and KN Eschleman (1983) The influence of substrate quality and stream size on wood decomposition dynamics. Oecologia. 58(3): 281-285.

Menyailo OV, Hungate BA, and W Zech (2002) The effect of single tree species on soil microbial activities related to C and N cycling in the Siberian artificial afforestation experiment. Plant and Soil. 242(2): 183-196.

Mina VN (1967) Influence of stemflow on soil. Soviet Soil Science. 7:1321-1329.

Mohn J, Scurmann A, Hagedorn F, Schleppi P, and R Bachofen (2000) Increased rates of denitrification in nitrogen-treated forest soils. Forest Ecology and Management. 137(1-3): 113-119.

Paavolainen L, Kitunen V, and A Smolander (1998) Inhibition of nitrification in forest soil by monoterpenes. Plant and Soil. 205:147-154.

Parker JL, Fernandez IJ, Rustad LE, and SA Norton (2001) Effects of nitrogen enrichment, wildfire, and harvesting on forest-soil carbon and nitrogen. Soil Science Society of America Journal. 65: 1248-1255.

Peterjohn WP, Adams MB, and FS Gilliam (1996) Symptoms of nitrogen saturation in two central Appalachian hardwood forest ecosystems. Biogeochemistry. 35(3):507-522.

Priha O and A Smolander (1999) Nitrogen transformations in soil under *Pinus sylvestris*, *Picea abies*, and *Betula pendula* at two forest sites. Soil Biology and Biochemistry. 31(7): 965-977.

Reddy KR and WH Patrick, Jr (1975) Effect of alternate aerobic and anaerobic conditions on redox potential, organic matter decomposition and nitrogen loss in a flooded soil. Soil Biology and Biochemistry. 7: 87-94.

Rhoades CC (1996) Single-tree influences on soil properties in agroforestry: lessons from natural forest and savanna ecosystems. Agroforestry Systems. 35(1): 71-94.

Robertson GP (1982) Nitrification in forested ecosystems. Philosophical Transactions of the Royal Society of London. 296(1082): 445-457.

Ross DS, Lawrence GB, and F Fredriksen (2004) Mineralization and nitrification patterns at eight northeastern USA forested research sites. Forest Ecology and Management. 188(1-3): 317-335.

Rothe A, Huber C, Kreutzer K, and W Weis (2002) Deposition and soil leaching in stands of Norway spruce and European Beech: Results from the Hogwald research in comparison with other European case studies.

Schimel JP and MK Firestone (1989) Nitrogen incorporation and flow through a coniferous forest soil profile. Soil Science Society of America. 53: 779-784.

Smolander A, Priha, O, Paavolainen L, Steer J, and E Malkonen (1998) Nitrogen and carbon transformations before and after clear-cutting in repeatedly N-fertilized and limed soil. Soil Biology and Biochemistry. 30(4): 477-490.

Soil Survey Staff, Natural Resources Conservation Service, United States Department of Agriculture. Web Soil Survey. Available online at http://websoilsurvey.nrcs.usda.gov/ accessed [2/10/10].

Stark JM and SC Hart (1997) High rates of nitrification and nitrate turnover in undisturbed coniferous forests. Nature. 385:61-65.

Ste-Marie C and D Pare (1999) Soil, pH and N availability effects on net nitrification in the forest floors of a range of boreal forest stands. Soil Biology and Biochemistry. 1579-1589.

Stevens PA and CP Wannop (1987) Dissolved organic nitrogen and nitrate in an acid forest soil. Plant and Soil. 102(1): 137-139.

Swank WT and Douglass JE (1975) Nutrient flux in undisturbed and manipulated forest ecosystems in the southern Appalachian mountains. Tokyo Symposium (December 1975).

Tietema A, Duysings JJHM, Verstraten JM, and JW Westerveld (1990) Estimation of actual nitrification rates in an acid forest soil. In: Nutrient Cycling in Terrestrial Ecosystems; Field Methods, Application, and Interpretation. Ed. AF Harrison, P Ineson, and OW Heal. Elsevier. New York.

Tremblay S, Ouimet R, and D Houle (2002) Prediction of organic carbon content in upland forest soils of Quebec, Canada. Canadian Journal of Forest Research. 32(5): 903-914.

Triska FJ, Sedell JR, Cromack K, Gregory SV, and FM McCorison (1984) Nitrogen budget for a small coniferous forest stream. Ecological Monographs. 54(1):119-140.

Vadeboncoeur MA, Hamburg SP, and RD Yanai (2007) Validation and refinement of allometric equations for roots of northern hardwoods. Canadian Journal of Forest Research. 37: 1777-1783.

Virginia RA (1986) Soil development under legume tree canopies. Forest Ecological Management. 16:69-79.

Wallenstein MD, Peterjohn WT, and WH Schlesinger (2006) N fertilization effects on denitrificaiton and N cycling in an aggrading forest. Ecological Applications. 16(6): 2168-2176.

Whittaker RH, Bormann FH, Likens GE, and TG Siccama (1974) The Hubbard Brook Ecosystem Study: Forest biomass and production. Ecological Monographs. 44(2):233-254.

Whittaker RH, Likens GE, Bormann FH, Eaton JS, and TG Siccama (1979) The Hubbard Brook Ecosystem Study: Forest nutrient cycling and element behavior. Ecology. 60(1): 203-220.
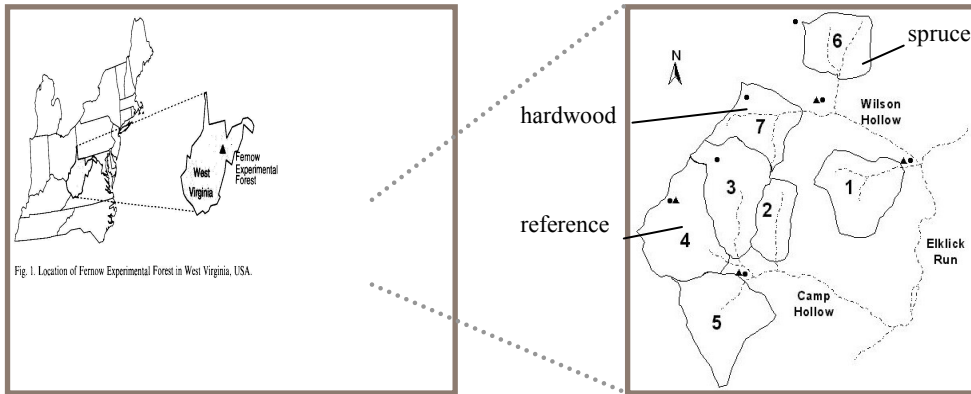
Figure 2.1 Location of the Fernow Experimental Forest, Monongahela National Forest, near Parsons, WV. Expanded region indicates watershed boundaries within the FEF. WS-6 is the Norway spruce monoculture and WS-7 is the native hardwood stand. Figure adapted from Adams et al 1993.
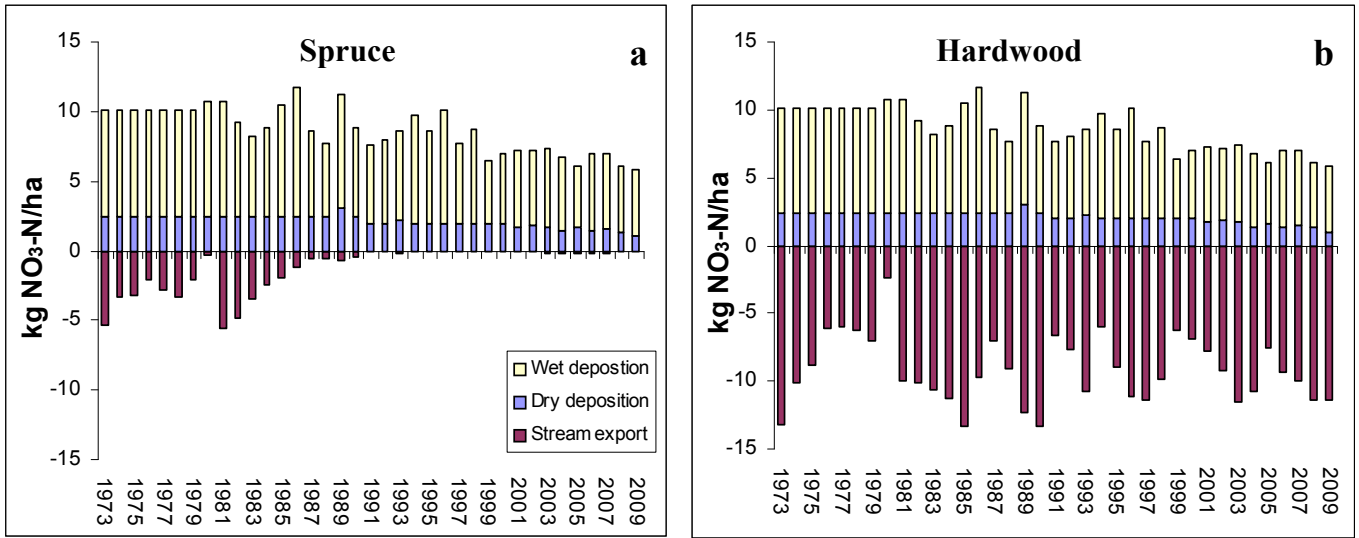
Figure 2.2. Annual atmospheric deposition and stream export of NO$_3$-N from the a) spruce watershed (WS6) and b) hardwood watershed (WS7) at the Fernow Experimental Forest, WV.
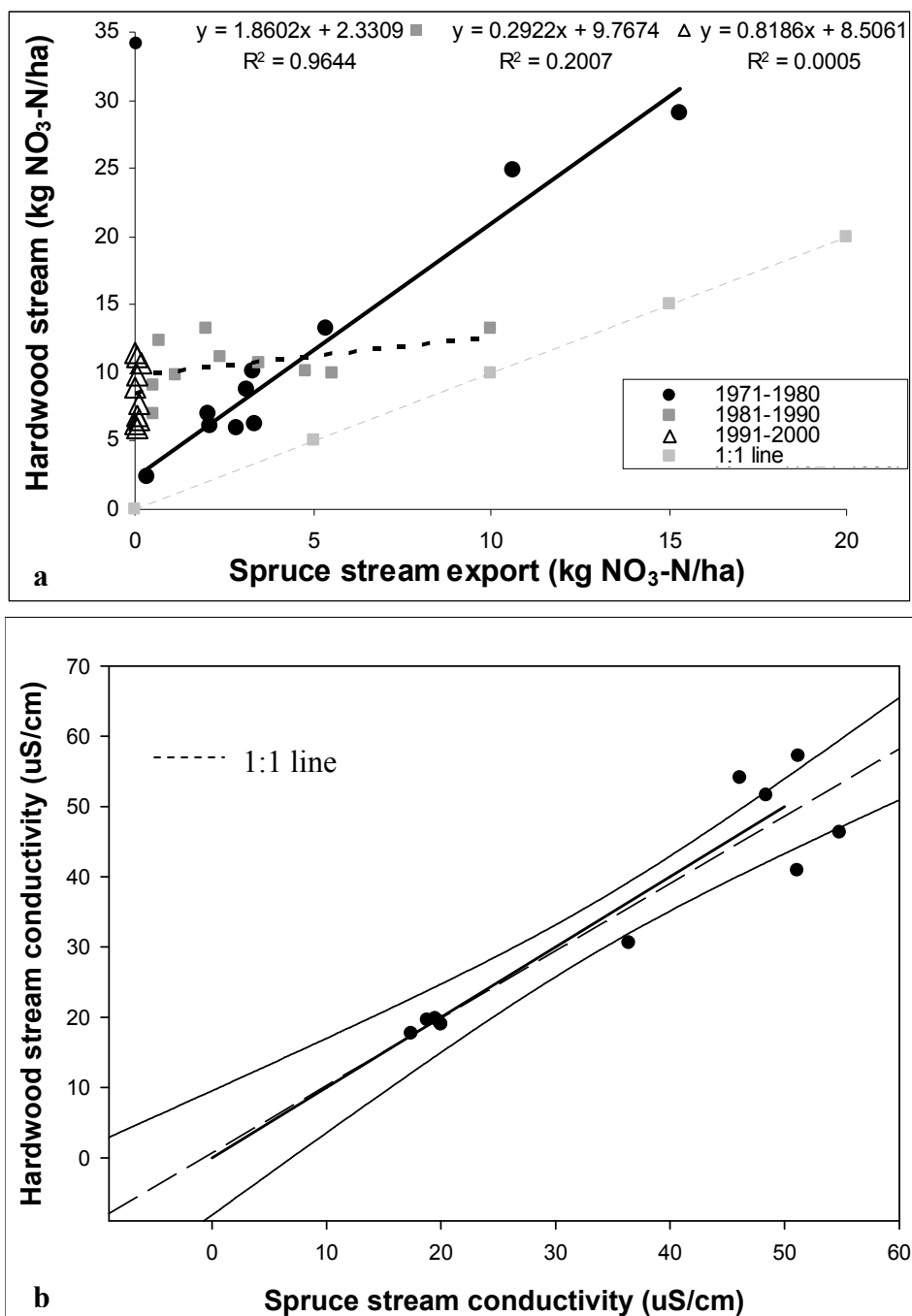
Figure 2.3. Long-term stream chemistry (annual means) depicting relationships in a) in stream NO_3-N export from the spruce and hardwood watersheds and b) the strong relationship in stream conductivity between the two watersheds as measured before treatment from 1959-1967. Nitrate-N relationships are analyzed by decades since treatment, indicating a divergence in NO_3-N export between the two watersheds.
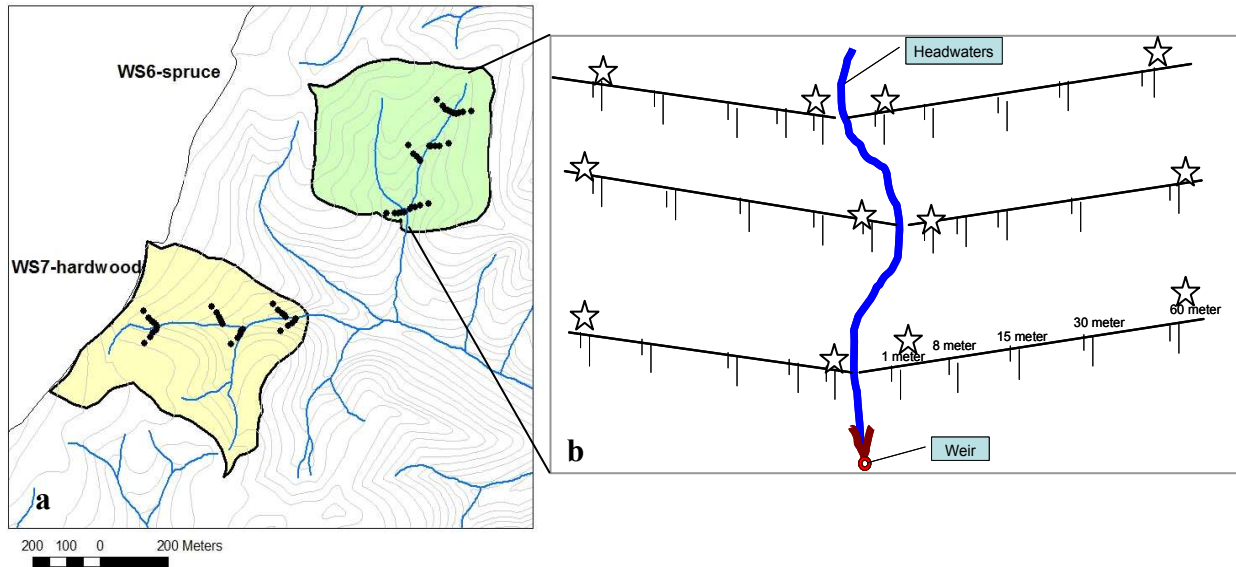
Figure 2.4. (a) Proximity of study watersheds WS6 (Norway spruce) and WS7 (native hardwoods) within the Fernow Experimental Forest, WV and locations of transects and sampling sites within each watershed (indicated by black circle). (b) Sites of soil collections (vertical lines) and N mineralization cores and litter collection (stars) within each transect.

Table 2.1. Equations and variables used for estimations of above-and below-ground biomass pools for the two study watersheds in the FEF.

Compartment	Equation	Variables	Reference
Spruce height H = height (m)	$H = 1.3 + a(1-e^{-bD^c})$	a = 24.5127 b = 0.0308 c = 1.1361 D = DBH (cm) e = exponential function	Huang et al (1992)
Spruce above-ground biomass agb = above-ground biomass (kg)	$\ln(agb) = \ln a + b (\ln D)$	*Tree height class (m):* 4.2-8.0: a = 0.155, b = 2.061 8.0-11.7: a = 0.585; b = 1.643 11.7-15.5: a = 0.194; b = 2.205 15.5-19.3: a = 0.420; b = 1.940 19.3-23.0: a = 1.863; b= 1.519 23.0-26.8: a = 1.229; b = 1.711 26.8-30.6: a = 1.146; b = 1.772 D = DBH (cm)	Fehrmann and Kleinn (2006)
Spruce below-ground biomass bgb = below-ground biomass (kg)	$bgb = \beta_0 * (D)^{\beta_1}$	β_0 = 0.02 β_1 = 2.36 D = DBH (cm)	Drexhage and Gruber (1999)

Hardwood above-ground biomass
agb (kg)
	$agb = Exp(\beta_0 + \beta_1 \ln D)$	*Tree species:*
		Soft maple/birch:
		  β_0 = -1.9123; β_1 = 2.3651
		Hard maple/oak/hickory/beech:
		  β_0 = -2.0127; β_1 = 2.4342
		Mixed hardwood :
		  β_0 = -2.4800; β_1 = 2.4835
		D = DBH (cm)
		Exp = exponential function
			Jenkins et al (2003)

Hardwood below-ground biomass bgb (g)	$bgb = A + B \log(D)$	A = 1.5766 B = 2.3407 D = DBH (cm)	Vadeboncouer et al (2007)

Table 2.2. Values for biomass and nitrogen concentration by tree compartment for *Acer saccharum* and *Picea abies* used for estimations of above- and below-ground N content for the two study watersheds in the FEF.

	Tree compartment	Biomass	N concentration
		(% of total)	*%*
Acer saccharum [1]	Branch and bark wood	31	0.37
	Stem bark	7.5	0.55
	Stem sapwood	59.6	0.098
	Twigs and leaves	1.5	2.19
	Roots	-----	0.71
Picea abies [2]	Needles	5.0	1.30
	Twigs and branches	0.6	0.67
	Bole bark	5.0	0.52
	Bole wood	90.0	0.16
	Roots	-----	1.20

[1]From Whittaker et al (1974 and 1979)
[2]From Feng et al (2008)
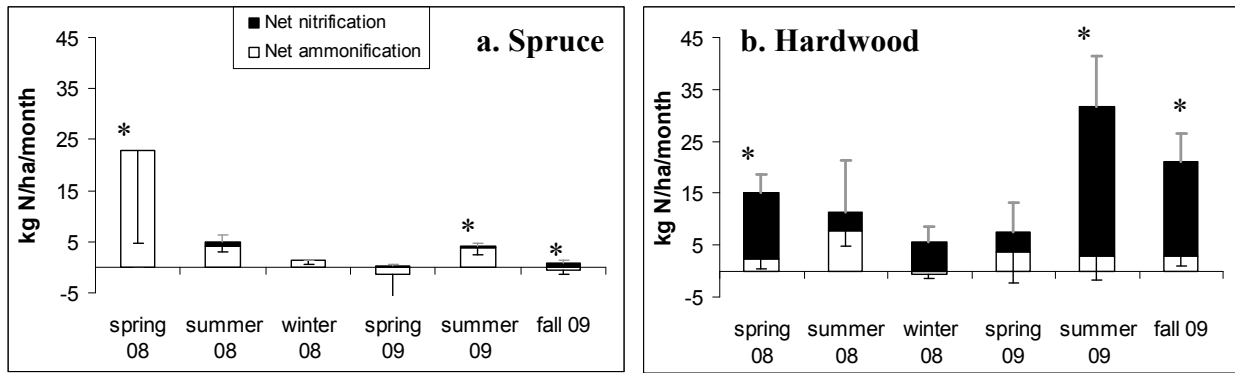
Figure 2.5. Seasonal nitrogen mineralization within A-horizon soils, depicting net nitrification and net ammonification within the a) spruce watershed (WS6) and b) hardwood watershed (WS7). Error bars represent standard error, black line below is error associated with ammonification, gray line above is error associated with nitrification. Asteriks denote seasons with significantly different net nitrification between watersheds at the α=0.05 level.
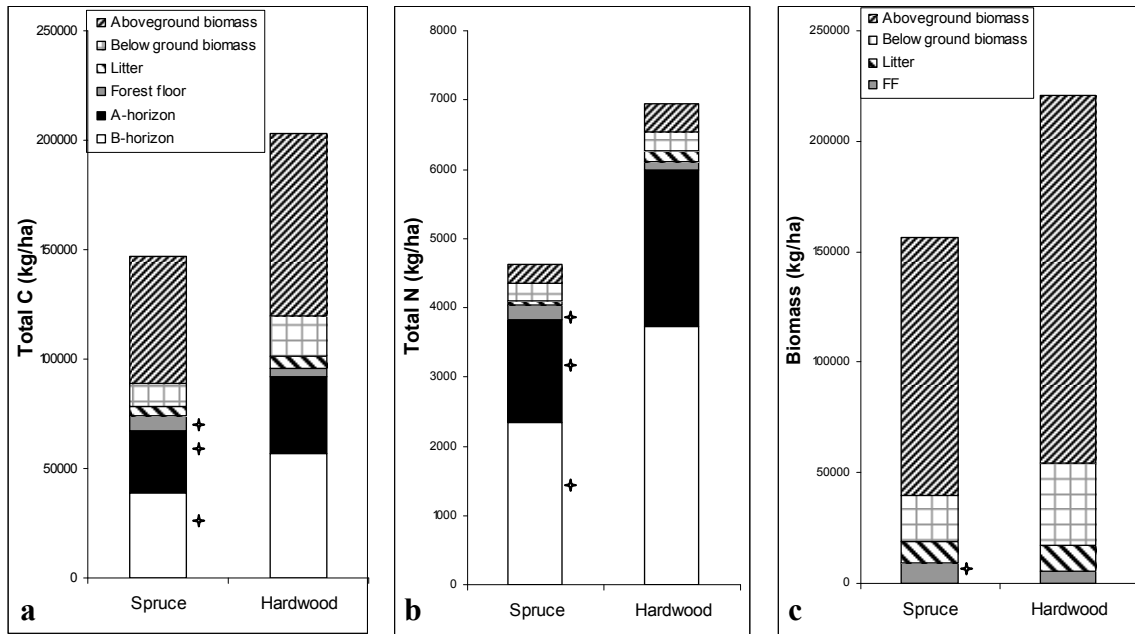
Figure 2.6. Watershed budgets depicting of a) C content, b) N content and c) biomass contained within each soil and biomass compartment in the spruce (WS6) and hardwood watersheds (WS7) in the Fernow Experimental Forest, WV. Asteriks denotes significant differences between spruce and hardwood pools at α=0.05 level.
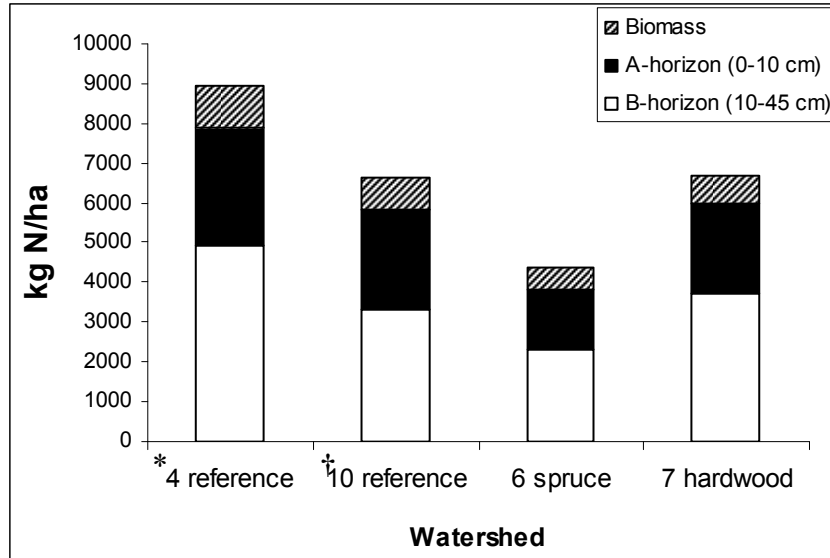
Figure 2.7. Nitrogen contained within A- and B- soil horizons and within total biomass (above- and below-ground) for four watersheds within the Fernow Experimental Forest. [*] Data from Adams et al 2006 (sampled in 2002), [†] Data from Christ et al 2003 (sampled in 1997).

Chapter 3. Spatial and temporal dynamics of dissolved carbon and nitrogen in watersheds of contrasting forest vegetation

ABSTRACT

Anthropogenic sources of nitrogen (N) have altered the global N cycle to such an extent as to nearly double the amount of available N in many terrestrial ecosystems. However, predicting the fate of N inputs has become increasingly difficult, as a multitude of environmental factors play major roles in determining N pathways. I investigated the role of specific vegetation and subsequent soil and forest floor characteristics in the production and export of dissolved carbon (C) and N within two adjacent watersheds of the USDA Forest Service Fernow Experimental Forest in West Virginia. These watersheds have similar management histories, with the exception of contrasting vegetation cover types. One watershed is a monoculture of Norway spruce (*Picea abies*) and the other has regenerated to native Appalachian hardwoods. Long-term stream chemistry indicates that the hardwood stand has approached N-saturation, with a relatively large annual stream export of dissolved N (15 kg NO_3-N/ha), whereas the spruce stand exhibits virtually no export of N to the stream. Results of this study demonstrate vegetation-mediated differences in soil characteristics, with lower soil pH and base cations, and higher extractable aluminum and C:N ratios in the spruce soil as compared to the native hardwood soil. Soil solution NO_3-N (as collected from tension lysimeters) was consistently lower in the spruce watershed (mean soil solution = 0.027 mg NO_3-N/L) than the hardwood watershed (mean = 1.92 mg NO_3-N/L). In addition to much higher NO_3-N concentrations in the hardwood watershed, dissolved organic N (DON) in soil solution and streamwater was higher in the hardwood than the spruce watershed (mean stream DON = 1.02 and 0.15 mg N/L in the hardwood and spruce streams, respectively). Properties most strongly correlated to soil solution NO_3^-N concentrations across both watersheds were mineral soil exchangeable K^+, basal area, and soil N content. Vegetative species composition, through its effect on soil properties, along with basal area stocking, are important predictors of subsequent NO_3^-N leaching.

INTRODUCTION

Nitrogen (N) in excess of biotic demand in forested ecosystems can disrupt plant-soil relations, increase soil acidification and Al^{3+} mobility, accelerate leaching of important base cations (Ca, Mg), increase emissions of greenhouse N gases, reduce methane storage in soil, and impair water quality (Fenn et al 1998, Christ et al 2002). High NO_3-N levels in streams can lead to eutrophication and excessive algal growths in aquatic systems, thus leading to lowered dissolved oxygen levels (Martin et al 1999). Many ecological studies have been executed with goals of determining what properties of a forest ecosystem lead to its degree of N retention capabilities (e.g. Vitousek and Matson 1985; Peterjohn et al 1999; Lovett et al 2002; Goodale and Aber 2001; Christopher et al 2008, Ross et al 2004), though our understanding of mechanisms of N retention is still incomplete. The ability to predict which ecosystems may be predisposed to N saturation and subsequent high N export is important to management efforts aimed to maintain forest productivity and downstream ecosystem water quality.

Nitrogen saturation hypotheses suggest that increases in N deposition add to soil N pools and accelerate N mineralization (Aber et al 1995). This eventually leads to excess available N and elevated NO_3-N leaching (Aber et al 1989; Aber et al 1998). Symptoms of N saturation have been documented in several watersheds within the USDA Forest Service Fernow Experimental Forest (FEF) in West Virginia (Fig. 1), where mean annual stream NO_3-N concentrations have exceeded 15 kg/ha in some watersheds (Peterjohn et al 1996; Gilliam et al 2001). Important observations from long-term stream chemistry of two adjacent watersheds within the FEF include divergent stream NO_3-N export fluxes at the outlets (Adams, unpublished data, USFS, Fig 2). These two watersheds have similar management histories, whereby both were clear-cut logged in sections in 1964-1967. Following clearcut harvests, both watersheds were kept barren for four years with herbicide treatments until 1969. In 1973, one watershed (WS-6, Fig. 1) was planted to Norway spruce (*Picea abies*), and the second watershed (WS-7) was allowed to naturally regenerate as a native hardwood stand dominated by sugar maple (*Acer saccharum)*, tulip-poplar (*Liriodendron tulipifera),* red oak (*Quercus rubra),* and black cherry (*Prunus serotina)*. Both stands are approximately 40-yr old and the only apparent difference

between watersheds is the vegetation, which is considered a "treatment effect". Mean annual stream NO_3-N exported from the hardwood stand is nearly 15 kg/ha, whereas mean annual stream NO_3-N exported from the spruce stand has been nearly zero for 20 y (mean = 0.18 kg NO_3-N/ha/yr) (Adams, USFS, Fig 2). In conjunction with negligible stream NO_3-N from the spruce stand, a decline in stream pH values has been documented since 1971 (mean pH=5.86 from the stream draining the spruce watershed and 6.18 from the stream draining the hardwood watershed) (Adams, USFS, unpublished data).

This pattern of low N export from the spruce watershed was also observed from a Norway spruce stand in Finland, where immobilization of mineral N was linked to alleleochemical inhibition through volatile organic compounds (terpenes) exuded by Norway spruce. These compounds were reported to inhibit nitrification, thus resulting in very low nitrate leaching from the stand (Paavolainen et al 1998). Paradoxically, several spruce forests in Europe exhibit stream N export that greatly exceeds the N export of associated hardwood forests (generally *P. abies* versus European beech, *Fagus sylvatica*; see review by Gundersen et al 2006), which is in contrast to the N export patterns associated with spruce and hardwood stands within the FEF. In addition, watershed fertilization studies at the Harvard Forest in Massachusetts showed that mixed hardwood forests were stronger sinks for N inputs than the conifer (red pine, *Pinus resinosa*) counterpart, and the conifer forest exhibited higher exports of N following fertilization (Currie et al 1996). Thus, the research reported in this paper aims to further explore the seemingly enigmatic role that vegetation type plays in N cycling and retention in forested watersheds.

The purpose of this research was to investigate vegetation-mediated patterns of selected N and C dynamics along transects from stream and riparian zone to upland soils of the two contrasting watersheds in the FEF described above. Specific objectives were to 1) determine specific soil properties that may have been altered by the influence of the spruce vegetation relative to the native Appalachian hardwood soils and 2) analyze how these soil properties are related to soil solution and in-stream fluxes of C and N spatially and temporally within the two watersheds. It was hypothesized that parameters

associated with lower soil pH and differences in C quality (e.g., higher C:N ratio) would be the factors most strongly correlated to low concentrations of soil solution NO_3^-N and subsequent export in streamwater .

I investigated how selected key soil properties that are correlated to soil solution NO_3^-N concentrations vary spatially across the watersheds (riparian/upland), and if these patterns vary with vegetation type.   Riparian soils were hypothesized to have different characteristics than upland soils in both watersheds because of higher OM and increased prevalence of saturation that is often observed in riparian areas, resulting in minimized delivery of N to the stream through increased denitrification and/or incorporation into organic compounds (Cooper 1990, Hill 1996). In addition, areas formed by seeps and localized depressions that favor pooling of water, along with thick moss cover, have formed in the riparian areas of the spruce watershed that are not present in the hardwood watershed, thus likely altering flow paths and increasing retention times of water.

Additionally, I investigated spatial and temporal variability of stoichiometric relationships within the pools of dissolved organic C (DOC) and N (i.e. NO_3-N:total N and dissolved organic N:DOC ratios) throughout the year. I also explored whether these relationships were influenced by vegetation type.   These stoichiometric patterns may provide insight into substrate limitations and possible sources of the measured NO_3-N in streamwater. The dominant forms of dissolved N in the two watersheds were expected to be different, with a higher proportion of dissolved organic N (DON) than dissolved inorganic N (DIN) occurring in the spruce watershed because of the differences in C compounds produced by the two vegetation types (i.e. slower decomposition in spruce), and DIN being dominant in the hardwood system, which is characteristic of systems approaching N saturation (Peterjohn et al 1996).

METHODS

Description of watersheds

The two watersheds used in this study (Watershed 6 and Watershed 7) are located within the FEF near Parsons, West Virginia, USA (Fig. 1). The 1,900-ha FEF was established

as an experimental forest in 1934 and is surrounded by the Monongahela National Forest. The FEF lies within the unglaciated Allegheny Plateau of the Appalachian Mountain Range (Adams et al 1993), with mean elevation range of 300 to 500 m. Annual precipitation is nearly evenly distributed throughout the year, averaging about 1,458 mm (Kochenderfer 2006). Mean monthly precipitation patterns exhibit maximums in June (144 mm) and minimums in October (97 mm). Mean annual temperature is 9.2° C and mean monthly temperatures exhibit maximums in July (20.6° C) and minimums in January (-18° C) (Kochenderfer 2006). Native vegetation of this region is described as mixed central hardwood forest (Core 1966), with common site tree species including yellow-poplar, sugar maple, black cherry, and red and white oak (*Quercus alba*).

Both Watershed 6 (WS6), the spruce watershed, and Watershed 7 (WS7), the hardwood watershed, were clearcut logged in sections, beginning in 1964 and concluding in 1967, and maintained barren with herbicides until 1969. Aerial herbicide was applied to WS6 in 1977 and again in 1980 to prevent hardwood re-growth (Bates 2000). Initial harvesting and herbicide applications through 1969 were implemented to evaluate the effects of complete deforestation on water yield, and Norway spruce was planted to investigate how conifer conversion of hardwood stands affects quality and quantity of water yield (Kochenderfer et al 1990). After nearly 40 years of growth, the spruce watershed has a closed canopy and dense stand structure (basal area 23 m^2/ha). Water yield in WS6 is decreasing and is lower than its pre-treatment yields, as well as current yield levels in WS7 (Fig 3; Kochenderfer et al 1990; Adams, USFS, unpublished data). This has been attributed to greater transpiration and interception in conifers (Kochenderfer et al 1990).

The spruce watershed (22 ha; elevation range 730-830 m) has a channel length of 857 m, which runs south through the watershed (Figure 1). Hillslopes form east/west aspects along the stream. Soils in this watershed are mapped as Calvin series (Calvin channery silt loam; Calvin loamy-skeletal, mixed, active, mesic typic Dystrudept) (Soil Survey Staff USDA NRCS web soil survey 2010), derived from shale, siltstone, and sandstone parent material. The Norway spruce stand in this watershed has created a litter layer

characteristic of natural conifer stands (mor), with a thick horizon of non-decomposed needles above further decomposed organic material of spruce origin. There are few other species in the watershed, with sparse patches of green briar (*Smilax* sp.) and few individual hardwood trees that survived the herbicide treatments including black locust (*Robinia pseudoacacia)*, yellow-poplar; red maple (*Acer rubrum)*, and sourwood (*Oxydendron arboreum*). Thus, the landscape is a homogeneous monoculture of Norway spruce. The stream bed and riparian area have a thick growth of moss mats that are not present in the hardwood watershed.

The hardwood watershed (24 ha; elevation range 730-860 m) has a channel length of 550 m, which runs eastward through the watershed (Figure 1), resulting in hillslopes with north/south aspects along the stream. Soils in this watershed are mapped as both Calvin (Calvin channery silt loam) and Dekalb series (Dekalb channery loam and Dekalb extremely stony loam; Dekalb loamy-skeletal, siliceous, active, mesic typic Dystrudept), derived from acidic sandstone parent material (Soil Survey Staff USDA NRCS web soil survey 2010). This watershed is dominated by yellow-poplar, red oak , and sugar maple, with an understory of dogwood (*Cornus florida*), striped maple (*Acer pensylvanicum*), magnolia (*Magnolia acuminata*), and several species of fern.   Both watersheds are gauged with a 120-degree sharp-crested V-notch weir and FW-1 water level recorder.

Soil and Site Characteristics

In order to determine soil characteristics within each watershed, transects were established running perpendicular to the topographic contour lines from the stream to the upper reaches of the watershed on both sides of the stream (Fig. 4). Three transects were established on both sides of each stream (6 per watershed) to capture possible effects of aspect on soil characteristics. Transects were stratified into 3 zones along the length of the stream channel (low, middle, high) in order to compare transects between watersheds and also to investigate within-watershed variability along the longitudinal stream gradient. Soil samples were collected in July 2007 from 5 sites along each transect and from the A- and B-horizons (0 to 10 cm depth and 10 to 46 cm depth, respectively) at each of the 5 sampling sites within a transect (Fig. 4). Generally, bedrock is encountered

at approximately 45 cm. In order to explore if gradients in the riparian zone influence N retention, soils here were sampled at closer intervals to more fully depict the specific locations likely to favor N retention.

Bulk density was determined at each sampling location and in each horizon using a hammer-driven corer. Soils for chemical analyses were air-dried and sieved through 2-mm mesh. Soil pH was measured from a 2:1 extraction of 0.01 M $CaCl_2$ (Hendershot et al 1993). Total N and C were analyzed on a CN elemental analyzer (Elementar VarioMax CNS, Hanau, Germany). Exchangeable cations (Al, Ca, Mg, K, Na) were analyzed following a 1 N ammonium acetate extraction (Lavkulich 1981) and phosphorus (P) was measured as Mehlich III-extractable P. Exchangeable cations and P were analyzed using ICP spectrometry (Varian, Palo Alto, CA). Results from the ICP analysis were used to report cation concentrations, cation exchange capacity (CEC) and percent base saturation (%BS) of the soils (Thomas 1982). Particle size analysis was assessed by the hydrometer method (Sheldrick and Wang 1993).

Forest floor samples were collected in October 2007 and 2009 and were characterized by determining depth, dry weight, and total C and N at each sampling location. All organic material <2 cm in diameter was included in forest floor analysis. Litter materials were collected monthly from 1 m x 1 m screened traps located at the 1- and 60- m sampling locations. Litter C and N analysis was performed on a CN elemental analyzer (Elementar VarioMax CNS, Hanau, Germany) on samples collected in October 2009. Basal area was determined by variable area plots, using the lysimeter location (see below) as the center point of the plot.

To determine the potential flux from net N mineralization, intact soil cores were collected at 12 sampling sites (Fig. 4) in each watershed in stainless steel cores (25.4 cm length x 10 cm width). Two soil cores were sampled at each site, with one serving to measure initial soil inorganic N pools, which was immediately taken back to the laboratory, and the other remaining in the field for a 4-wk *in situ* incubation (Tietema et al 1990). Three replicate samples were taken at each sampling site. The incubation sample was capped and aerated by holes made in the upper part of the tube. Following incubation, soils were

removed from the cores, air-dried, and sieved through 2mm mesh. Extractions using 1 *M* KCl were performed to determine concentrations of inorganic N (NH_4-N and NO_3^-N) and extracts were analyzed for inorganic N on an auto-analyzer (Bran-Luebbe, Charlotte, NC). Differences in NH_4-N and NO_3-N concentrations from the initial sample relative to the field-incubated sample were considered as net ammonification, nitrification or immobilization occurring during the 4 weeks. These incubations were performed seasonally (four times a year) for two years to determine temporal differences in net ammonification, net nitrification, or net immobilization.

Dissolved N and C in Soil Solution and Streams

Soil tension lysimeters (-50 kPa) were installed along all 6 transects within each watershed to sample soil solution at two depths (below the A-horizon at 10 cm and below the primary rooting zone, B-horizon at 46 cm). Lysimeters were placed at each of the 5 sites along each transect at each horizon (Fig. 4). Lysimeters were installed in July 2007 and soil solution was collected under -50 kPa tension monthly beginning in November 2007. Soil solution from lysimeters was collected in acid-washed HDPD plastic bottles and stored at -4° C until analyzed for NO_3^-N and NH_4-N on an auto-analyzer (Bran-Luebbe). When sufficient volume of solution was collected, DOC and total N (TN) were analyzed on a TOC/TN analyzer (Shimadzu TOC) and dissolved organic N (DON) was estimated as DON = TN - (NH_4-N + NO_3-N).

In order to better understand dissolved N patterns as it is transported downstream, water samples were also collected at monthly intervals along the length of the streams and also at the weir located at each watershed outlet (Fig. 4). Streamwater was sampled at 10 sites along the main stream length in each watershed, including at the location of each of the 6 transects established for measurement of soil solution, soil characterization, and N mineralization fluxes. Water from streams was collected in acid-washed HDPD plastic bottles and rinsed three times with streamwater before samples were retrieved. Samples were filtered through Whatman 42 filter paper prior to storage at -4° C. In order to calculate nutrient loads (kg N/ha/yr), flow velocity was determined at each sampling point using a flow meter, and depth and width of the stream were measured to determine

discharge. Water samples were analyzed monthly for DOC, DON, TN, and DIN (NO_3-N, NH_4-N) as described for soil solution analyses.

Data Analyses

The experimental design of this paired-watershed study is an example of pseudo-replication, with an effective sample size of one per treatment level (Gilliam 2001). However, differences occurring in the data collected were assumed to be caused by treatment (i.e., contrasting forest vegetation) effects because the soil series were previously classified to be similar (Soil Survey Staff, NRCS, USDA 2010) and historical data of stream conductivity and export of NO_3-N indicate that differences between watersheds in soil and stream chemistry occurred primarily following the clearcut harvesting in 1967 and subsequent establishment of Norway spruce in WS6 (Adams, USFS, unpublished data).

Two-way ANOVA followed by Tukey's HSD was performed to determine if significant differences occurred in water and soil measures caused by horizon of soil (A, B), zone of each transect along the stream length (low, middle, high), landscape position (riparian, upland), or distance upstream (from weir to headwater). Landscape position was defined as either riparian (1, 8, and 15 m from the streambank) or upland (30 and 60 m from the streambank). One-way ANOVA was used to determine significant differences in soil characteristics and subsequent N forms that may occur between watersheds. Simple linear regressions were performed to determine significant relationships between dissolved C and N in water.

To test for significant associations between soil solution concentrations of NO_3-N and the suite of soil and site properties, correlations were estimated using the Pairwise method to obtain Pearson's correlation coefficients. Significance of these correlations was determined by regressing each parameter individually against mean NO_3-N concentrations from each sampling site (Berger et al 2002). Significantly correlated terms were then used to perform stepwise regressions to select the most influential properties relative to soil solution NO_3-N. Both Pearson's and Stepwise were performed

for both watersheds together, and also for each watershed individually to determine if influential properties varied by vegetation type. This analysis was performed using only A-horizon data because this horizon exhibited the strongest feedback effects on soil properties from stand composition.

RESULTS

Soil and site characteristics

Selected soil characteristics are summarized in Table 1. In the spruce watershed, forest floor mass of 9,179 kg/ha was nearly two times greater than in the hardwood, which had a forest floor mass of 5,238 kg/ha (p<0.001). The slower decomposition rate of spruce litter was also reflected in the lower stores of C and N in both the A- and B-horizons of the mineral soil relative to the hardwood (A-horizon: p<0.0001 and 0.0284 for C and N, respectively; B-horizon: p=0.0003 and 0.0002 for C and N, respectively). Soil acidity also increased beneath the spruce vegetation, and this was reflected in significantly lower measures of CEC, %BS, soil pH, and extractable P, and with higher amounts of extractable Al^{3+} (Table 1). Net N mineralization was significantly lower in the A-horizon of the spruce soil relative to the hardwood soil, with mean monthly fluxes of net N mineralization at 5.33 and 15.21 kg N/ha/month for the spruce and hardwood soils, respectively (p=0.0342) (Table 1). Litter N measured from October 2009 inputs was twice as high in the hardwood watershed, with approximately 23 kg N/ha/month in the hardwood compared with 11 kg N/ha/month in the spruce (Table 1). This was a function of both greater mass of litter inputs (mean mass = 1,810 kg/ha/month in the hardwood and 1,430 kg/ha/month in the spruce), and also a result of higher N concentration of litter in the hardwood (mean %N = 1.42 for hardwood litter and 0.76 for spruce litter).

Basal area stocking within the spruce watershed was approximately 25% greater than the adjacent hardwood (23 and 17 m^2/ha, respectively; p=0.0007). Basal area within the hardwood stand had greater variance around the mean than the planted spruce (std dev=7.53 for hardwood, 5.33 for spruce), indicating presence of gaps with relatively low basal area in the hardwood watershed (minimum = 7 m^2/ha).

Dissolved N and C in soil solution

Soil solution NO_3-N concentration was consistently lower in the spruce watershed, and was near detection limit for both the A- and B-horizons. Mean NO_3-N concentrations of 0.02 and 0.03 mg N/L, respectively (Table 1) were significantly lower ($p<0.0001$) than the soil solution NO_3-N concentrations in the hardwood watershed of 1.83 and 2.00 mg N/L for the A- and B-horizons, respectively. No differences were found in soil solution NO_3-N concentrations between A- and B-horizons within a given watershed ($p=0.5797$) (Table 1).

Organic forms of N comprised approximately 90% of the total dissolved N measured in the spruce soil solution, whereas organic forms represented approximately 40% of the total dissolved N measured in the hardwood soil solution (Fig. 5). Within the inorganic fraction, NO_3-N and NH_4-N comprised about 1% and 9%, respectively, of the total dissolved N in spruce soil solution (all NH_4-N were near detection limit and are not shown). Of total dissolved N in the hardwood soil solution, NO_3-N was the dominant fraction, comprising about 60%, whereas NH_4-N comprised <1% of the total dissolved N (Fig. 5).

Soil solution DOC concentrations were consistent throughout the year, ranging from 1.39 to 3.45 mg C/L in soil solution from the spruce watershed and from 2.88 to 6.10 mg C/L in soil solution from the hardwood watershed, and no significant differences in DOC in soil solution were detected by month within either watershed (data not shown). Mean concentrations of dissolved organic C were significantly lower in spruce soil solution than in hardwood soil solution (Fig 5; $p<0.0004$). Mean DOC in soil solution was 2.87 mg C/L in the spruce watershed, compared to 5.02 mg C/L in soil solution of the hardwood watershed. No significant differences in DOC by horizon were detected within either watershed ($p=0.2916$). Dissolved organic C values did vary significantly by landscape position, and this effect varied by watershed ($p=0.0002$ for watershed x landscape interaction). Dissolved organic C concentrations were lowest in the B-horizon riparian samples in the spruce watershed, with mean values of 1.29 mg C/L in B-horizon riparian soil solution and 5.75 mg C/L in B-horizon upland soil solution (Fig. 5b).

In general, C and N patterns within soil solution (Fig. 6) and streamwater (Fig. 7) were more tightly coupled within the spruce watershed than in the hardwood watershed. The strongest association between dissolved C and N in soil solution was detected in the positive linear DON and DOC association in the spruce watershed (Fig. 6a; R^2=0.49; p<0.0001). This relationship was not significant in soil solution from the hardwood watershed (Fig. 6b; R^2=0.08; p=0.2316), nor was the association between NO_3-N and DOC significant in the hardwood watershed (R^2=0.01; p=0.7097) (Fig. 6b).

Linking soil solution NO_3-N to soil and site properties

Soil solution NO_3-N was regressed against the soil and site properties described above in order to identify those properties most strongly correlated to the concentration of NO_3-N as identified by Pearson's correlation coefficients. This analysis was performed on data sets contained in each watershed individually and for both watersheds combined (Table 2). Because soil solution NO_3-N concentrations were consistently low across the spruce watershed, no properties were significantly correlated to NO_3-N in this watershed. In contrast, NO_3-N concentrations varied significantly across the landscape within the hardwood watershed and two properties accounted for 74% of the variation in the stepwise model (Table 3). Nitrate-N concentrations in the hardwood watershed were positively associated with litter N (kg/ha) (p=0.0861) and with %BS (p=0.0623).

All properties listed in Table 2 were significantly correlated to soil solution NO_3-N concentrations when both watersheds were evaluated together (p<0.01). When these properties were used to create a stepwise regression model, three terms remained in the model (R^2 value of 0.44; P<0.0001) (Table 3). Properties most strongly correlated to soil solution NO_3-N concentrations across both watersheds were mineral soil exchangeable K^+ (p<0.0001), plot basal area (p=0.0013), and soil N (kg/ha; p= 0.0635) (Fig. 8).

Soil solution NO_3-N and soil properties by landscape position

Soil solution NO_3-N concentrations were relatively low and similar across the landscape and between soil horizons in the spruce watershed (p=0.1019 for landscape term;

p=0.4665 for soil horizon term) (Fig. 5). In the hardwood watershed, there was a significant landscape position x soil horizon interaction (p=0.0094) with concentrations of NO_3-N being significantly higher in the riparian position in the A-horizon samples (2.40 mg NO_3-N/L in the riparian position versus 0.50 mg NO_3-N/L in the upland position) (Fig. 5). This landscape effect was not evident in the deeper B-horizon soil solution.

Soil properties identified as significantly correlated to soil solution NO_3-N concentrations in the hardwood watershed (%BS, p=0.0311; and litter N content, p=0.0862) also were significantly higher in the riparian position, indicating potential landscape controls on dissolved N concentrations. Although these patterns of significant correlation between soil solution NO_3-N and %BS and litter N were evident in the spruce watershed, riparian values of these two parameters were not significantly different than the upland landscape position in the spruce watershed (p>0.10) (data not shown).

There was a significant watershed x zone (low, middle, high) interaction (p<0.0001). Soil solution NO_3-N concentrations varied by watershed zone within the hardwood watershed, demonstrating significant spatial variation from the weir to the headwaters. Soil solution NO_3-N concentrations in the hardwood watershed were highest near the headwaters (high zone), especially in the B-horizon (data not shown). This pattern was not evident in soil solution NO_3-N across zones in the spruce watershed.

Dissolved N and C in streams

Nitrate-N and NH_4-N concentrations in the streams were similar to those in soil solutions in both watersheds (Fig. 9). Mean NO_3-N concentrations were significantly higher when averaged across all monthly samples and across all sample locations in the hardwood stream than in the spruce stream (Fig. 9; p<0.0001). Mean streamwater NO_3-N concentrations were 0.03 and 1.20 mg NO_3-N/L in the spruce and hardwood streams, respectively. No differences in NH_4-N concentrations in the streams were evident between the watersheds (p=0.0952), with NH_4-N concentration averaging 0.04 and 0.05

mg NH_4-N/L across all monthly samples in the spruce and hardwood streams, respectively (Fig. 9).

From the headwaters to the weir in the stream draining the spruce watershed, there were no significant longitudinal differences among concentrations of all forms of dissolved N ($p > 0.05$ for NO_3-N, NH_4-N and DON) (Fig. 9a). However, concentrations of all forms of dissolved N were highest at the three sampling locations at the upper reaches of the stream draining the hardwood watershed (Fig. 9b). Mean NO_3-N concentrations were 2.17 mg N/L at the headwater position, and streamwater NO_3-N concentrations generally decreased progressively towards the weir, where mean concentrations were 0.98 mg NO_3-N/L ($p < 0.0001$) (Fig. 9b).

Organic N was the dominant fraction of dissolved N in streamwater draining the spruce watershed (68% of the total N on an annual basis) (Fig. 10a). Little variation in N fractions occurred throughout the year in the spruce stream, and N concentrations in streamwater were always low (mean TN= 0.22 mg N/L), regardless of form. Alternatively, NO_3-N was usually the dominant fraction of N in streamwater draining the hardwood watershed (53% of total N on an annual basis) (Fig. 10a). Throughout the year, DON represented about 45% of the total N in hardwood streamwater, with mean TN of 2.27 mg N/L. However, in the fall months (Sep, Oct, Nov), the DON fraction dominated the total N pool in hardwood streamwater, though NO_3-N concentrations only slightly decreased (and not significantly) during these months (Fig. 10a).

Mean annual concentration of DOC in the stream draining the spruce watershed was slightly greater than in the hardwood watershed, though this trend was not statistically significant ($p=0.1235$) (Fig. 9). However, because stream flow was annually 18-20 cm less in the spruce watershed (Fig. 3), mean annual DOC exports were 12 kg DOC/ha/yr from the spruce watershed and 45 kg DOC/ha/yr from the hardwood watershed. Dissolved organic C concentrations were similar along the longitudinal gradient from headwater to weir in both watersheds (Fig. 9).

Regression relationships between DOC and dissolved N in streams are shown in Fig. 7. These associations were similar to those exhibited from soil solution samples, though the strength of the relationships was diminished in streamwater (highest R^2=0.10 for DOC vs DON in the stream draining the spruce watershed; p=0.0007). Maximum concentrations of stream DOC from both watersheds occurred in April (spruce maximum = 2.18 mg C/L and hardwood maximum = 1.88 mg C/L; data not shown). The ratio of DOC:DON in streamwater was also higher in the spruce watershed than in the hardwood (mean streamwater DOC:DON was 12.66 and 1.79 in spruce and hardwood, respectively) (p<0.0001).

DISCUSSION

Contrasting N retention between watersheds

This study identified key soil and site properties associated with ecosystem N retention, and depicted how these properties were mediated by vegetation type. Perhaps more importantly, this study showed how large intra-watershed variation in measured properties may regulate the large differences in stream N export documented in the long-term stream export data (Adams, USFS, Fig. 2) in watersheds in the FEF. This heterogeneity in patterns of N cycling has been portrayed in other watersheds in the FEF (Peterjohn et al 1999; Gilliam et al 2001; Christ et al 2002). In these studies, reference watersheds receiving no experimental N fertilizer exhibited symptoms of N saturation and a high degree of variation in soil solution NO_3-N and rates of mineralization and nitrification. Differences in soil NO_3-N concentrations within watershed were hypothesized to be associated with aspect and elevation (Peterjohn et al 1999). However, similarities in field and lab soil incubations performed by Gilliam et al (2005) support the theory that spatial variations in N cycling are more attributable to soil variations than physical differences such as aspect or elevation.

As expected, important differences in soil characteristics between the two watersheds have been documented in the current study, especially in higher acidity measures (lower pH and base cations, higher extractable Al^{3+}) and lower C quality (higher soil C:N ratio, more forest floor mass in the spruce watershed) (Table 1). However, these properties

were not significantly correlated to soil solution NO_3-N across both vegetation types (Table 2). Similar patterns of forest floor mass, lower C and N in mineral soil, and lower pH and %BS beneath spruce were also documented in a European study that investigated mechanisms of N retention in Norway spruce, European beech, and Scots pine (*Pinus sylvestris*) forests (Brumme and Khanna 2008). They reported that as the forest floor converted to a mor-type, humus degradation in the mineral soil occurred and was associated with the low percent C and N in the mineral soil.

We propose a mechanism to account for the large discrepancies in NO_3-N export from these two watersheds with contrasting vegetation. The hardwood watershed contained areas of low basal area caused by gap formation in the canopy, which were not present in the dense monoculture of spruce (lowest plot basal area in the hardwood watershed was 6.88 m^2/ha and lowest in the spruce watershed was 13.78 m^2/ha). Within these areas of low basal area in the hardwood watershed, the soil solution concentrations of mobile NO_3-N were the highest (negative correlation between basal area and NO_3-N concentration: R^2=0.36; p=0.0018; data not shown). This association was likely caused by higher net nitrification rates resulting from possible soil disturbance from fallen trees, higher soil moisture with less vegetative uptake, and higher temperatures with more sunlight reaching the forest floor (Ritter and Vesterdal 2006). Increases in soil N availability can lead to luxury uptake by trees and result in higher foliar N (Imo and Timmer 2001) (negative correlation between litter N and basal area: R^2=0.51; p=0.0089; data not shown). Thus, a positive feedback loop may persist, maintaining high soil N status in plots of low basal area. Kohlpaintner et al (2009) also recently showed that vegetative cover and tree size could account for 40% of the variation in soil solution NO_3-N concentrations in a mature Norway spruce stand in Germany, where N deposition was approximately 30 kg N/ha/yr and soil solution NO_3-N concentrations ranged from 9.5-15.8 mg NO_3-N/L.

In forests that are not impacted by high rates of N deposition, areas of high N mineralization would be offset by areas that act as sinks in competition for limited N (Gilliam et al 2001), thus maintaining a low net ecosystem export of N. Data from the

current study provide support for this hypothesis because plots with the highest basal area corresponded to soil solution NO_3-N concentrations near zero in the hardwood watershed. However, in this hardwood watershed, where wet deposition of NO_3-N was nearly 15 kg N/ha/yr, strong N limitation was not likely to occur, and the sink strength was lessened, resulting in net export of N flushing from the system. Although soil solution NO_3-N concentrations may be lower in plots with high basal area, vegetation and microbial populations in the hardwood watershed may not be limited by N, which was supported by the persistence of high stream NO_3-N export throughout the year.

This proposed mechanism was also supported by results from the spruce watershed, where soil solution and stream NO_3-N concentrations were consistently very low. Soil solution NO_3-N concentrations were negatively associated with plot basal area (R^2=0.137; p=0.0687; data not shown), although variation of this parameter was small, resulting in poor model strength and insignificant R^2 values.

Soil solution NO₃-N and soil properties by landscape position

Soil solution NO_3-N and soil properties by landscape position

Surprisingly, soil solution NO_3-N was significantly higher in samples from the riparian landscape positions relative to other landscape positions in the A horizon of the hardwood watershed (Fig 5). It was hypothesized that NO_3-N concentrations would be lower in the riparian position, because of adsorption to DOC compounds and denitrification in anaerobic microsites (Cooper 1990, Hill 1996, Bernhardt et al 2003; Valett et al 2002). Soil N status (concentration of soil N and litter N) also tended to be higher in the riparian position in the hardwood watershed, along with significantly higher riparian soil %BS (p=0.0311). High base saturation was also shown to be significantly correlated to net nitrification potential (R^2=0.51) in another study on reference watersheds 4 and 10 at the FEF (Christ et al 2002). These parameters of high soil N status and high %BS may be indicative of the presence of species that have faster nutrient cycling, such as sugar maple, in the riparian position as opposed to species with slower nutrient cycling, such as oak or beech, in the upland position (Christ et al 2002). Species with fast nutrient cycling produce litter with more labile (lower molecular mass) C

compounds, which are more accessible for microbial cycling, including nitrifiers (Brierley et al 2001, Templer et al 2003, Lovett et al 2004).

The species-specific differences in the lability of C compounds in litter may also cause differences in abiotic retention of inorganic N. Studies using inorganic N isotopes enriched in ^{15}N have demonstrated that incorporation into organic matter is the dominant fate of N additions to vegetated ecosystems (Nadelhoffer 1999; Barrett et al 2002; Tietema 1998), and that the strength of this sink may vary by tree species (Fitzhugh et al 2003). A long-term study in Solling, Germany showed that in Norway spruce stands, the accrual of forest floor mass over 30 years of inventories accounted for an annual increment of 42 kg N/ha in the forest floor material alone. European beech stands in this study exhibited half of this annual increment at 21 kg N/ha in the forest floor (Meiwes et al 2002, in Brumme and Khanna 2008).

The hypothesis that the riparian features of the spruce watershed (moss mats, high OM, longer retention times) would function as a barrier to N export to the stream (e.g. Pinay et al 1995; Triska et al 1989) was not supported. Evidence from this study suggests that net NO_3-N production across the spruce watershed is very minimal (Fig 5), regardless of landscape position, and a strong N limitation is imposed by the densely growing trees, microbes, and/or abiotic adsorption of N to forest floor material.

Dissolved C and N relationships

In both soil solution and streamwater draining the spruce watershed, significant correlations between DOC and DON were documented. This relationship was not nearly as strong in either soil water or streamwater from the hardwood watershed (R^2=0.060 and 0.044, respectively). The weaker relationships between streamwater DOC and dissolved N from the hardwood watershed indicate an uncoupling of the C and N biogeochemical cycles (Fig. 6 and 7). Tight coupling of DOC and DON has been described in N limited streams at the USDA Forest Service Coweeta Hydrologic Labortory in North Carolina (Brookshire et al 2005), and this correlation suggests that C and N in streamwater come from similar sources or flowpaths (Petrone 2005). Brookshire et al (2005) suggest that

uptake of DON and DOC in N-limited streams is a function of strong biotic demand for labile C, and that C demand was also mediated by inorganic N availability. The result is strong linkage of C and N in streamwater. In areas of high N availability, tight coupling is not noted, possibly due to deposited N bypassing microbial processing, and thus not utilizing C as substrate material. Weak relationships between dissolved C and N were also observed by Petrone (2005), who hypothesized that a proportion of N in the hardwood stream was from direct atmospheric deposition, and not the result of soil microbial processing, though this was not directly verified in this study. Stream NO_3-N resulting from direct atmospheric deposition was documented using stable isotopic analysis in the Catskill Mountains of New York during times of high flow (Burns and Kendall 2002; Campbell et al 2002) and also from streams draining upland forests in Vermont during snowmelt (Sebestyen et al 2008).

Additional evidence in support of this explanation of direct atmospheric NO_3-N in streamwater may be seen in the limited variation of dissolved N forms and concentrations observed throughout the year in the present study, even during periods of high leaf fall and cold temperatures (Fig. 10). Most temperate deciduous forest streams exhibit NO_3-N concentration minima as leaves begin to fall, which increases C substrate availability for biological uptake of N (Tank et al 2000; Mulholland 2004).

Temporal patterns of dissolved N

Little variation was observed throughout the year in the forms or concentrations of dissolved N in the streams. Dissolved N in the spruce streams was dominated by DON, with maxima occurring in May (0.21 mg N/L). No patterns of NO_3-N flushing were apparent in the stream from the spruce watershed, with the greatest mean stream NO_3-N concentrations measured in May at only 0.088 mg NO_3-N/L (Fig. 10).

Mean NO_3^-N concentrations in the hardwood stream ranged from a minimum of 0.99 mg NO_3-N/L in October to a maximum of 1.39 mg NO_3-N/L in January (Fig. 10). Dissolved organic N concentrations exhibited nearly opposite patterns in the hardwood stream compared with NO_3-N, with yearly minima occurring in February (0.74 mg N/L), and

maxima occurring in October (1.56 mg N/L). Thus, NO_3-N was usually the dominant form in streamwater from the hardwood watershed, though in the fall DON concentrations slightly increased until December. This pattern of stream N forms has also been documented in the Sleepers River watershed in Vermont (Sebestyen 2007). In that study, stream NO_3-N concentrations decreased at the onset of leaf fall, and DON became the dominant form of dissolved N. Patterns of forms and concentrations of dissolved N from the streams in the current study were also similar to those documented in the soil solution (Fig. 10).

CONCLUSIONS

The aim of this study was to determine key soil and site properties correlated to NO_3-N in soil solution in adjacent watersheds with contrasting vegetation cover of spruce and native hardwood, which exhibit large differences in N stream export of dissolved N. The effect of high N deposition on C and N sequestration of conifer and hardwood forest types was explored by examining some of the mechanisms potentially controlling N cycling and NO_3-N leaching in the two watersheds. Results from this study show that vegetative species composition, along with basal area stocking levels, are important predictors of NO_3-N leaching. This study provides further evidence that the hardwood watershed is N-saturated by documenting 1) relatively high NO_3-N concentrations in soil solution and streamwater that do not vary significantly throughout the year in the hardwood watershed and 2) an uncoupled relationship between DOC and DON in the soil solution and streamwater of the hardwood watershed. Additionally, the riparian soils in the native hardwood watershed had a low capacity to function as a sink for mobile forms of N, thus permitting transport of dissolved N to the stream. In contrast, the soils of the spruce watershed had different N mineralization dynamics, whereby the spruce vegetation is associated with very low amounts of available N across the landscape, and very low amounts of NO_3-N were documented in soil solution and streamwater throughout the year.

Results of this research will aid in the investigation of the ecological effects and possible ecosystem values of conifer species selection as anthropogenic N deposition continues to

alter forest ecosystem composition and processes. It is still largely unknown how the continuing chronic deposition of anthropogenic N may alter the composition, health, and biogeochemistry of different forests types over time.

WORKS CITED

Aber JD, Nadelhoffer KH, Steudler P, and JM Melillo (1989) Nitrogen saturation in northern forest ecosystems. BioScience. 39(6): 378-386.

Aber J, McDowell W, Naldelhoffer K, Magill A, Bernson G, Kamakea M, McNulty S, Currie W, Rustad L, and I Fernandez (1998) Nitrogen saturation in temperate forest ecosystems. Bioscience. 48(11):921-934.

Aber JD, Magill A, McNulty SG, Boone RD, Nadelhoffer KJ, Downs M, and R Hallett (1995) Forest biogeochemistry and primary production altered by nitrogen saturation. Water, Air, and Soil Pollution. 85(3): 1665-1670.

Adams MB, Edwards PJ, Wood F, and JN Kochenderfer (1993) Artificial watershed acidification on the Fernow Experimental Forest, USA. Journal of Hydrology. 150: 505-519

Barrett JE and IC Burke (2002) Nitrogen retention in semiarid ecosystems across a soil organic-matter gradient. Ecological Applications. 12(3):878-890.

Bates NS (2000) Effects of forest harvesting on headwater watersheds in West Virginia. Thesis, BS Engineering. Princeton University.

Berger TW, Neubauer C, and G Glatzel (2002) Factors controlling soil carbon and nitrogen stores in pure stands of Norway spruce (Picea abies) and mixed stands in Austria. Forest Ecology and Management. 159(1-2): 3-14.

Bernhardt ES, Likens GE, Busco DC, and CT Driscoll (2003) In-stream uptake dampens effects of major forest disturbance on watershed nitrogen export. PNAS. 100(18):10304-10308.

Brierley EDR, Wood M, and PJA Shaw (2001) Influence of tree species and ground vegetation on nitrification in an acid forest soil. Plant and Soil. 229(1): 97-104.

Brookshire ENJ, Valett HM, Thomas SA, and JR Webster (2005) Coupled cycling of dissolved organic nitrogen and carbon in a forest stream. Ecology. 86(9):2487-2496.

Brumme R and PK Khanna (2008) Ecological and site historical aspects of N dynamics and current N status in temperate forests. Global Change Biology. 14(1): 125-141.

Burns DA and C Kendall (2002) Analysis of $\delta15N$ and $\delta18O$ to differentiate NO_3^- sources in runoff at two watersheds in the Catskill Mountains of New York. Water Resources Research. 38(5): 9-11.

Campbell DH, Kendall C, Chang CCY, Silva SR, and KA Tonnessen (2002) Pathways for nitrate release from an alpine watershed: Determination using $\delta15N$ and $\delta18O$. Water Resources Research. 38(5): 1-9.

Christ MJ, Peterjohn WT, Cumming JR, and Adams MB (2002) Nitrification potentials and landscape, soil and vegetation characteristics in two Central Appalachian watersheds differing in NO_3^- export. Forest Ecology and Management. 159:145-158.

Christopher SF, Mitchell MJ, McHale MR, Boyer EW, Burns DA, and C Kendall (2008) Factors controlling nitrogen release from two forested catchments with contrasting hydrochemical responses. Hydrological Processes. 22: 46-62.

Cooper BA (1990) Nitrate depletion in the riparian zone and stream channel of a small headwater catchment. Hydrobiologia. 202(1-2).

Core EL (1966) Vegetation of West Virginia. McClain Printing Co., Parsons, W.Va., 217 p., illus.

Currie WS, Aber JD, McDowell WH, Boone RD, and AH Magill (1996) Vertical transport of dissolved organic C and N under long-term N amendments in pine and hardwood forests. Biogeochemistry. 35(3): 471-505.

Fenn (1998) Nitrogen excess in North American ecosystems: Predisposing factors, ecosystem responses, and management strategies. Ecological Applications. 8(3):706-733.

Fitzhugh RD, Lovett GM, and RT Venterea (2003) Biotic and abiotic immobilization of ammonium, nitrite, and nitrate in soils developed under different tree species in the Catskill Mountains, New York, USA. Global Change Biology. 9: 1591-1601.

Gilliam FS, Yurish BM, and MB Adams (2001) Temporal and spatial variation of nitrogen transformations in nitrogen-saturated soils of a central Appalachian hardwood forest. Canadian Journal of Forest Restoration. 31:1768-1785.

Gilliam FS, Lyttle NL, Thomas A, and MB Adams (2005) Soil variability along a nitrogen mineralization and nitrification gradient in a nitrogen-saturated hardwood forest. Soil Science Society of America Journal. 69:247-256.

Goodale CL and JD Aber (2001) The long-term effects of land-use history on nitrogen cycling in northern hardwood forests. Ecological Applications. 11(1): 253-267.

Gundersen P, Schmidt IK, and K Raulund-Rasumssen (2006) Leaching of nitrate from temperate forests- effects of air pollution and forest management. Environmental Review. 14: 1-57.

Hendershot WH, Lalande H, and M Duquette (1984) Soil Reaction and Exchangeable Acidity: In Soil Sampling and Methods of Analysis. Carter, Ed. Canadian Society of Soil Science. 142-145.

Hill AR (1996) Nitrate removal in stream riparian zones. Journal of Environmental Quality. 25:743–55.

Imo M and VT Timmer (2001) Growth and nitrogen retranslocation of nutrient loaded Picea mariana seedling planted on boreal mixedwood sites. Canadian Journal of Forest Research. 31(8): 1357-1366.

Kochenderfer JN, Edwards PJ, and JD Helvey (1990) Land management and water yield in the Appalachians. In: RE Riggins, EB Jones, R Singh, and PA Rechard, Eds., Proceedings IR Conference Watershed Management, ID DIV/ASCE, Watershed Planning and Analysis in Action Symposium, Durango, CO, July 9-11, 1990, pp. 523-532, American Society of Civil Engineers, New York.

Kochenderfer JN (2006) Fernow and the Appalachian hardwood region. In: Adams MB, DeWalle DR, and JL Hom (2007). The Fernow Watershed Acidification Study. Environmental Pollution. Springer Netherlands.

Kohlpainter M, Huber C, Weis W, and A Gottlein (2009) Spatial and temporal variability of nitrate concentration in seepage water under a mature Norway spruce (Picea abies (L.) Karst) stand before and after clear cut. Plant and Soil. 314(1-2): 285-301.

Lavkulich LM (1981) Methods manual, pedology laboratory. Department of Soil Science, University of British Columbia, Vancouver. In Carter, Ed. Soil Sampling and Methods of Analysis. Canadian Society of Soil Science. 173-175.

Lovett GM, Weathers, KC, and MA Arthur (2002) Control of nitrogen losses from forested watersheds by soil carbon:nitrogen ratio and tree species composition. Ecosystems. 5(7):712-718.

Lovett GM, Weathers KC, Arthur MA, and JC Schultz (2004) Nitrogen cycling in a northern hardwood forest: Do species matter? Biogeochemistry. 67(3):289-308.

Martin TL, Kaushik NK, Trevors JT, and HR Whiteley (1999) Review: Denitrification in temperate climate riparian zones. Water, Air, and Soil Pollution. 111: 171-186.

Mulholland PJ, Valett HM, Webster JR, Thomas SA, Cooper LW, Hamilton SK, and BJ Peterson (2004) Stream denitrification and total nitrate uptake rates measured using a field 15N tracer addition approach. Limnology and Oceanography. 49(3): 809-820.

Nadelhoffer KJ, Emmett BA, Gundersen P, Kjonaas OJ, Koopmans CJ, Schleppi P, Tietema A, and RF Wright (1999) Nitrogen deposition makes a minor contribution to carbon sequestration in temperate forests. Nature. 398: 145-148.

Paavolainen L, Kitunen V, and A Smolander (1998) Inhibition of nitrification in forest soil by monoterpenes. Plant and Soil. 205:147-154.

Peterjohn WP, Foster CJ, Christ MJ, and MB Adams (1999) Patterns of nitrogen availability within a forested watershed exhibiting symptoms of nitrogen saturation. Forest Ecology and Management. 119:247-257.

Petrone KC (2005) Export of carbon, nitrogen and major solutes from a boreal forest watershed: The influence of fire and permafrost. Ph.D Dissertation, University of Alaska Fairbanks.

Pinay G, Ruffinoni, and A Fabre (1995) Nitrogen cycling in two riparian forest soils under different geomorphic conditions. Biogeochemistry. 30(1): 9-29.

Ritter E and L Vesterdal (2006) Gap formation in Danish beech (Fagus sylvatica) forests of low management intensity: soil moisture and nitrate in soil solution. European Journal of Forest Research. 125(2): 139-150.

Ross DS, Lawrence GB, and F Fredriksen (2004) Mineralization and nitrification patterns at eight northeastern USA forested research sites. Forest Ecology and Management. 188(1-3): 317-335.

Sebestyen SD (2007) Coupled hydrological and biogeochemical processes that control stream nitrogen and dissolved organic carbon at the Sleepers River Research Watershed. Ph.D Dissertation, State University of New York.

Sebestyen SD, Boyer EW, Shanley JB, Kendall C, Doctor DH, Aiken GR, and N Ohte (2008) Sources, transformations, and hydrological processes that control stream nitrate and dissolved organic matter concentrations during snowmelt in an upland forest. Water Resources Research. 44: 1-14.

Sheldrick BH and C Wang (1993) Particle size distribution. In: MR Carter, Ed. Soil Sampling and Methods of Analysis. Canadian Society of Soil Science. Lewis Publishers, Boca Raton, FL.

Soil Survey Staff, Natural Resources Conservation Service, United States Department of Agriculture. Web Soil Survey. Available online at http://websoilsurvey.nrcs.usda.gov/ accessed [2/10/2010].

Tank JL, Meyer JL, Sanzone DM, Mulholland PJ, Webster JR, Peterson BJ, Wollheim WM, and NE Leonard (2000) Analysis of nitrogen cycling in a forest stream during autumn using a 15N-tracer addition. Limnology and Oceanography. 45(5): 1013-1029.

Templer P, Findlay S, and G Lovett (2003) Soil microbial biomass and nitrogen transformations among five tree species of the Catskill Mountains, New York, USA. 35(4): 607-613.'

Thomas GW (1982) Exchangeable cations. P. 160-161 In: AL Page et al, Eds. Methods of Soil Analysis, Part 2. Chemical and Microbiological Properties, 2nd ed. ASA Pub. No. 9.

Tietema A, Duysings JJHM, Verstraten JM, and JW Westerveld (1990) Estimation of actual nitrification rates in an acid forest soil. In: Nutrient Cycling in Terrestrial Ecosystems; Field Methods, Application, and Interpretation. Ed. AF Harrison, P Ineson, and OW Heal. Elsevier. New York.

Tietema A, Emmett BA, Gundersen P, Kjonaas OJ, and CJ Koopmans (1998) The fate of 15N-labelled nitrogen deposition in coniferous forest ecosystems. Forest Ecology and Management. 101(1-3): 19-27.

Triska FJ, Kennedy VC, Avanzino RJ, Zellweger GW, and KE Bencala(1989) Retention and transport of nutrients in a third-order stream in northwestern California: Hyporheic processes. Ecology. 70(6): 1893-1905.

Valett HM, Crenshaw CL, and PF Wagner. (2002). Stream nutrient uptake, forest succession, and biogeochemical theory. Ecology. 83(10):2888-2901.

Vitousek PM and PA Matson (1985) Disturbance, nitrogen availability, and nitrogen losses in an intensively managed loblolly pine plantation. Ecology. 66(4): 1360-1376.
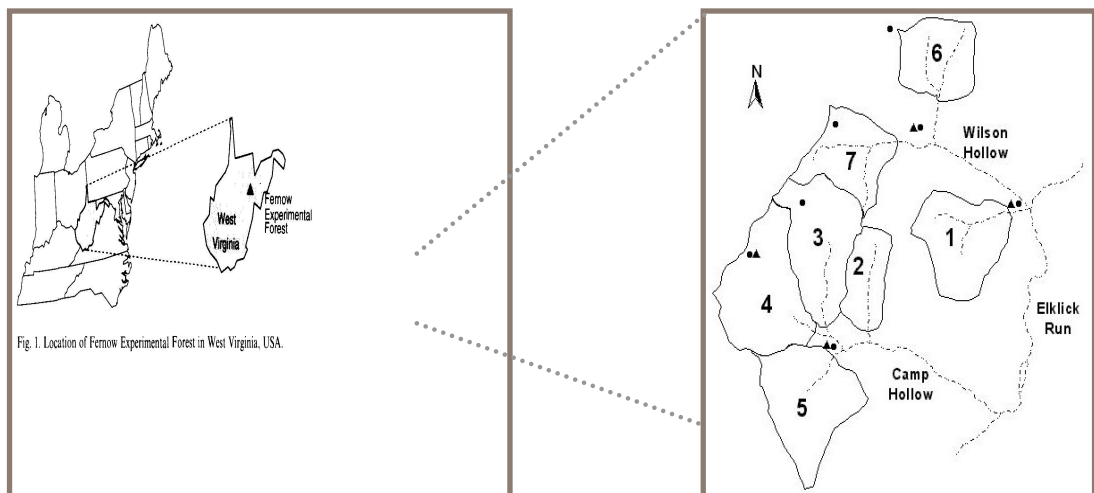
Figure 3.1. Location of the Fernow Experimental Forest, Monongahela National Forest, near Parsons, WV. Expanded region indicates watershed boundaries within the FEF. WS-6 is the Norway spruce monoculture and WS-7 is the native hardwood stand. Figure adapted from Adams et al (1993).
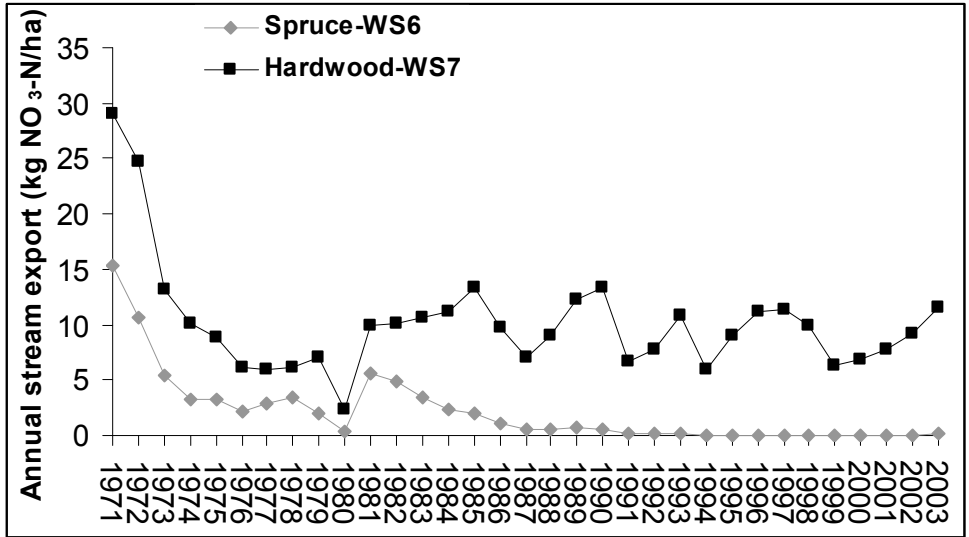
Figure 3.2. Annual stream export of nitrate-N from two nearly adjacent gauged watersheds within the USFS Fernow Experimental Forest in West Virginia, USA. Data courtesy MB Adams, USFS.
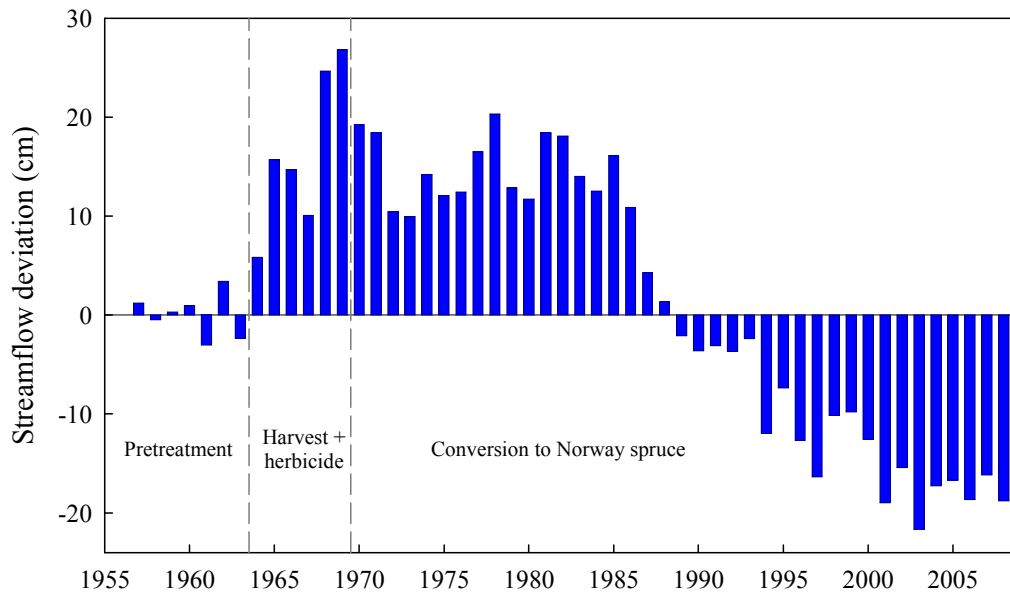
Figure 3.3. Streamflow deviation for the spruce watershed (WS6), as calculated by difference in streamflow from adjacent control hardwood watershed (WS7) since conversion to Norway spruce in 1971 at the Fernow Experimental Forest, WV. Data courtesy MB Adams, USFS.
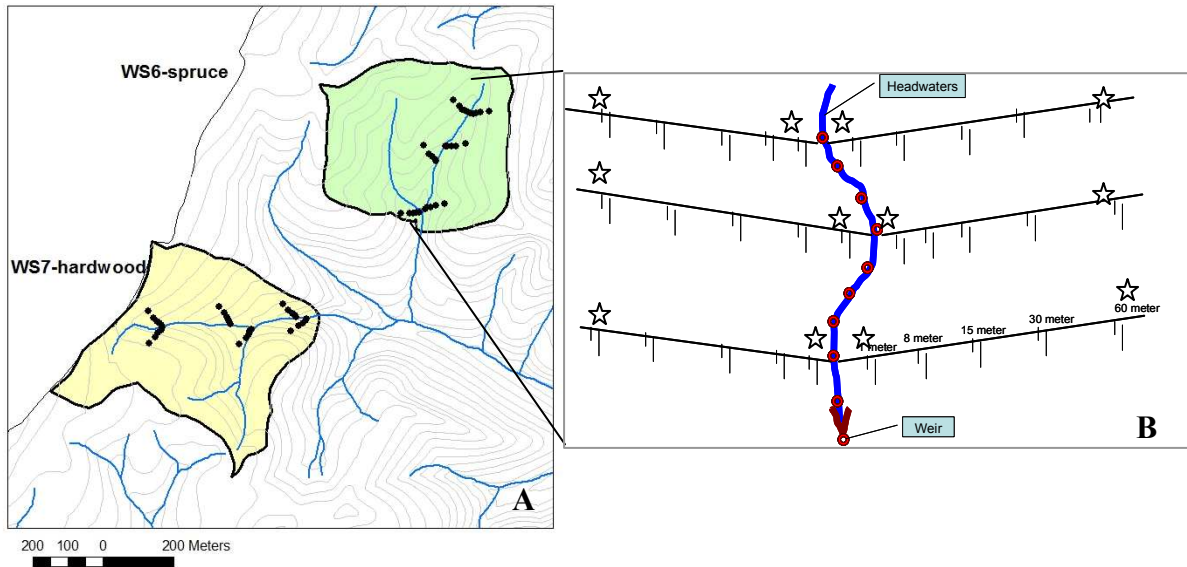
Figure 3.4. (A) Proximity of study watersheds WS6 (Norway spruce) and WS7 (native hardwoods) within the Fernow Experimental Forest, WV and locations of transects and sampling sites within each watershed (indicated by black circles). (B) Sites of soil collections and nested lysimeters (vertical lines), stream sampling sites (circles), and litter traps and N mineralization cores (stars) within each transect.

Table 3.1. Selected soil and site properties within Watershed 6 (spruce) and Watershed 7 (native hardwood) at the Fernow Experimental Forest, WV.

| Property		Soil Horizon A (0-10 cm)		Soil Horizon B (10-45 cm)	
		Spruce	Hardwood	Spruce	Hardwood
Cation exch. capacity	meq$_c$/kg	*6.88	8.20	6.23	6.64
Base Saturation	%	*16.58	25.20	9.62	10.74
pH$_{CaCl2}$	-----	*3.78	3.95	*3.99	4.09
Bulk density	Mg/m3	*0.89	0.99	1.24	1.19
Soil N	kg/ha	*1555	2393	*2336	3732
Soil C	kg/ha	*28673	35478	*38608	56645
Soil C:N	-----	*18.53	15.02	16.48	15.83
Extractable Al^{3+}	kg/ha	*17.36	10.76	19.51	15.32
Extractable Ca^{2+}	kg/ha	*77.40	207.70	48.98	97.07
Extractable K$^+$	kg/ha	*50.08	67.47	50.04	44.94
Extractable P	kg/ha	*7.94	12.13	*2.63	3.43
Basal area	m^2/ha	*23	17	-----	-----
Forest floor mass	kg/ha	*9179	5238	-----	-----
Litter N	kg/ha	*10.54	23.30	-----	-----
Net N mineralization[1]	kg/ha/mo	*64.0	182.50	-----	-----
Soil solution NO$_3^-$N[2]	mg N/L	*0.02	1.83	*0.03	2.00

* significant differences between watersheds within each horizon (p<0.05).
[1] values are means of 6 seasonal sampling events
[2] values are means of 19 monthly sampling events

Table 3.2. Pearson's Pairwise correlation coefficients (r) of associations between soil solution NO_3-N and soil and site properties for both Watershed 6 (spruce) and Watershed 7 (native hardwood) combined, and for each watershed individually.[1]

Watershed	BS[2]	soil N	Ca	K	Ca:Al	Clay fraction	Basal area	Forest floor mass	Litter N	Litter N	Litter C:N
	%	kg/ha	kg/ha	kg/ha	---	%	m^2/ha	kg/ha	kg/ha	%	---
Both spruce and hardwood	0.5000	0.4757	0.5136	0.4943	0.4195	-0.5520	-0.5157	-0.3999	0.6936	0.6115	-0.5334
Spruce only	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Hardwood only	0.3892	NS	0.2564	0.3977	NS	-0.5916	-0.4075	NS	0.2628	NS	NS

[1] Only those soil parameters that are significantly correlated to the mean NO_3^-N concentration (mg NO_3-N/L) of soil solution collected from all A-horizon lysimeters (Nov 2007-Nov 2009) are shown.
[2] BS: base saturation
NS: not significant at $\alpha=0.05$ level

Table 3.3. Significant independent variables to predict soil solution NO$_3$-N concentrations as identified through Stepwise regression models[†].

Dependent variable	Watershed(s)	Model R^2	Independent variables	Coefficients
Soil solution NO$_3^-$N	Hardwood	0.735**	Litter N *(kg/ha)*	0.175*
			Base saturation *(%)*	0.048*
	Spruce and Hardwood	0.436***	Soil N *(kg/ha)*	0.0002*
			Extractable K$^+$ *(kg/ha)*	0.0063**
			Basal area *(m^2/ha)*	-0.072***

[†]A model was constructed for each watershed individually, and also for both watersheds together.
* $p < 0.1$
**$p < 0.005$
***$p < 0.0001$
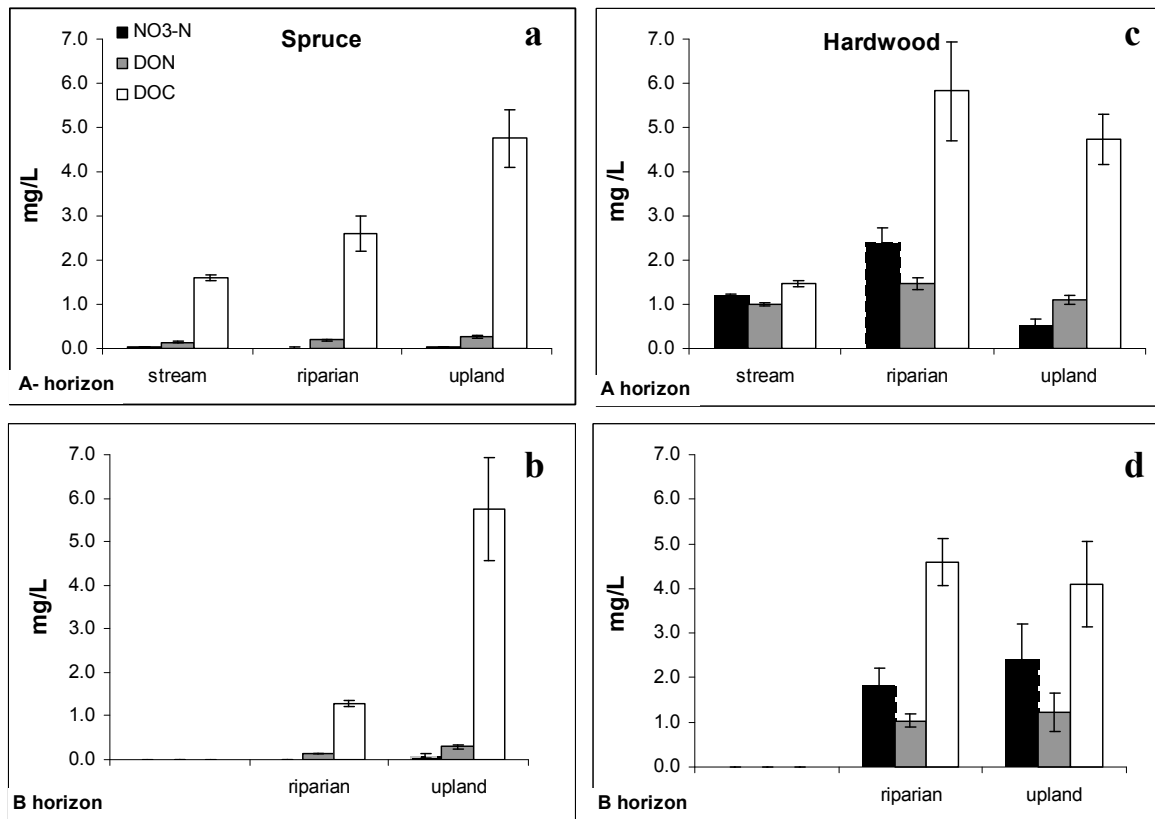
Figure 3.5. Monthly mean concentrations of dissolved C and N in soil solution at two landscape positions and within the stream for Watershed 6 (spruce) a) A-horizon and b) B-horizon and for watershed 7 (native hardwood) c) A-horizon and d) B-horizon. Error bars represent one standard error of the mean from 19 monthly sampling events.
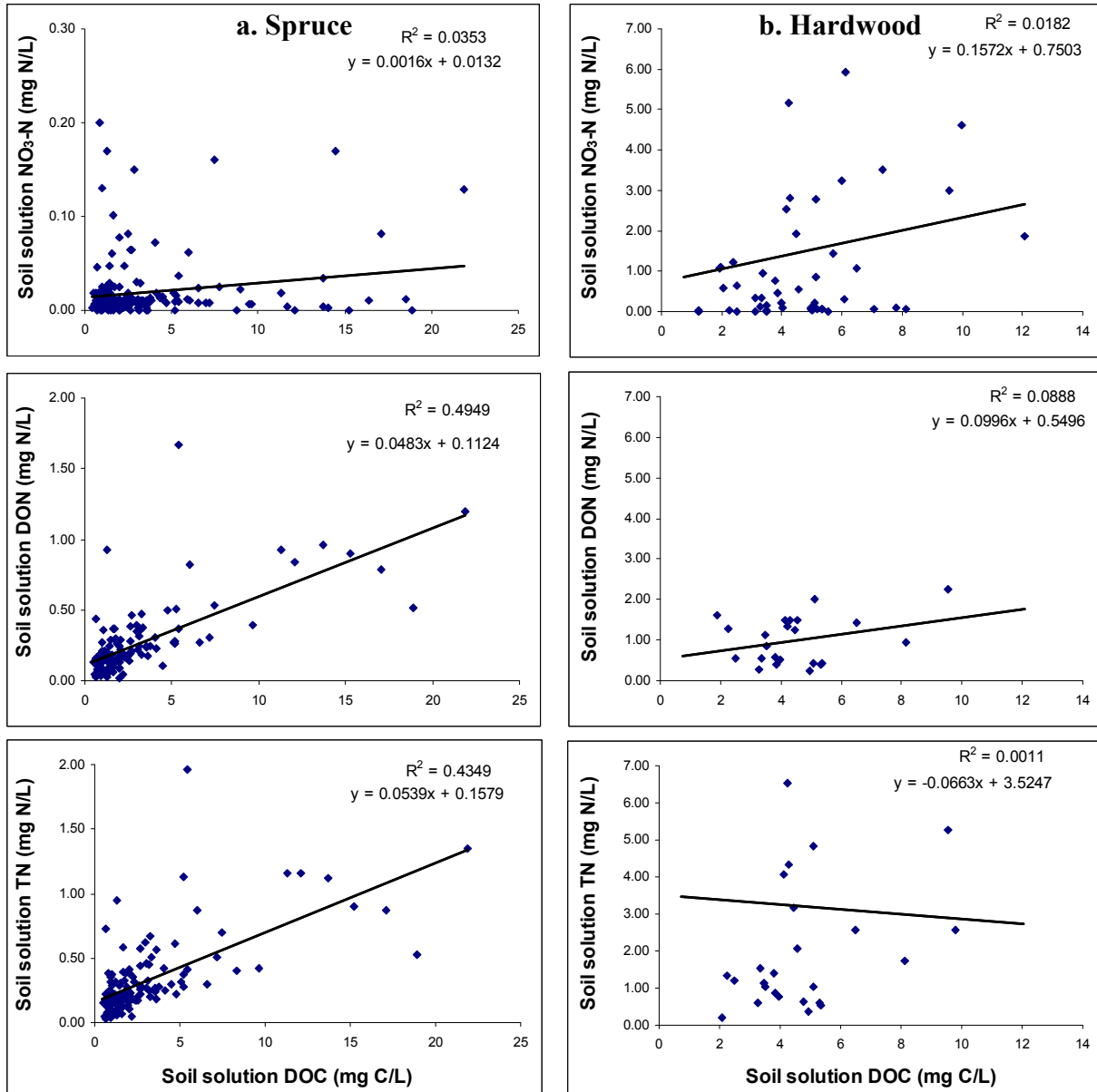
Figure 3.6. Associations between dissolved organic C (DOC) and components of dissolved N (NO$_3$-N, DON, and TN) in soil solution. Data are from A- and B-soil horizons at all sampling sites collected during the entire sampling period (Nov. 2007-Nov. 2009) from a) watershed 6 (spruce) and b) watershed 7 (native hardwood) at the Fernow Experimental Forest.
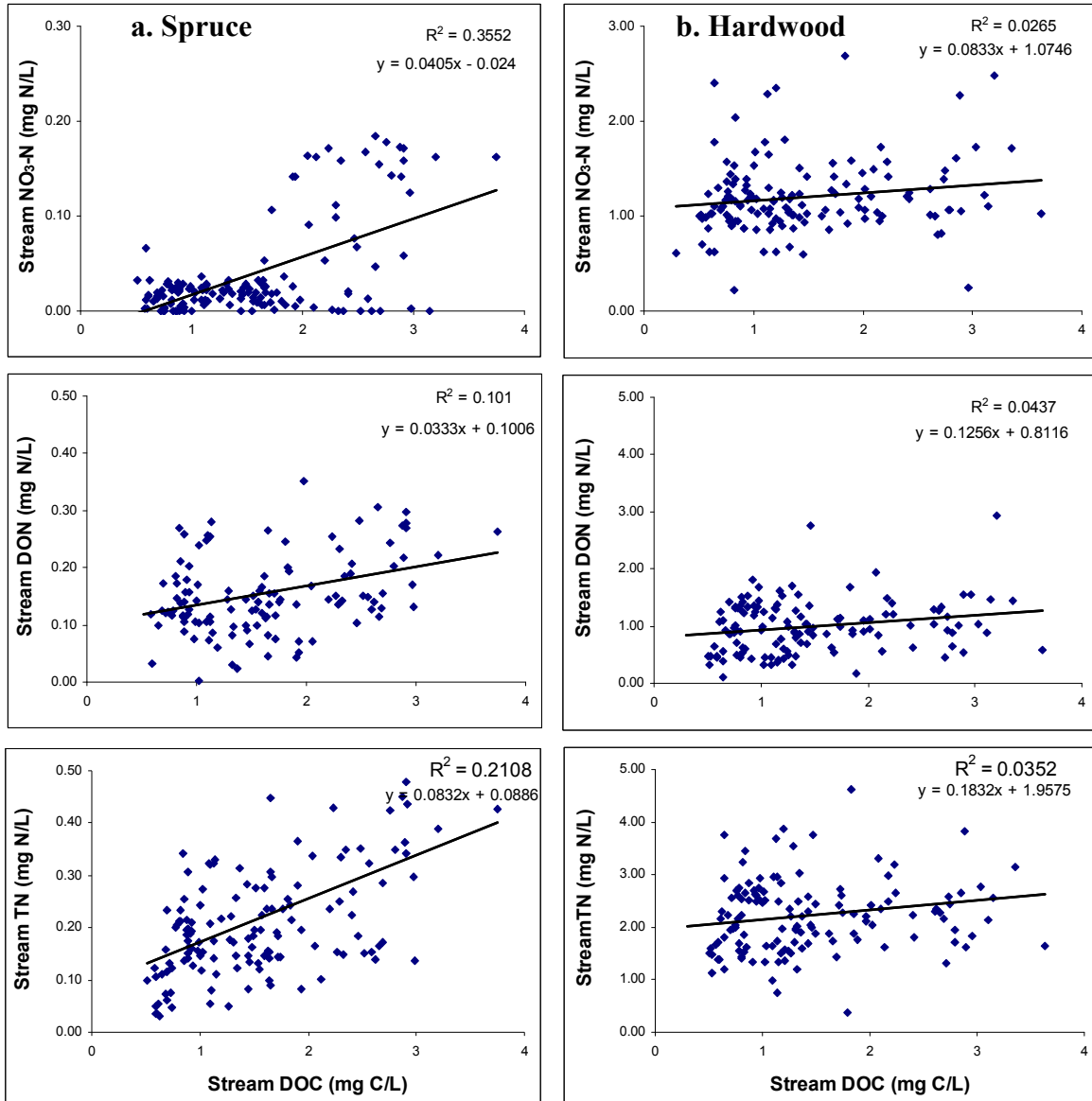
Figure 3.7. Associations of dissolved organic C (DOC) and components of dissolved N in stream water samples (NO_3-N, TN, and DON). Data are from the entire sampling period from all sampling sites from headwater to weir draining the a) watershed 6 (spruce) and b) watershed 7 (native hardwood) at the Fernow Experimental Forest.
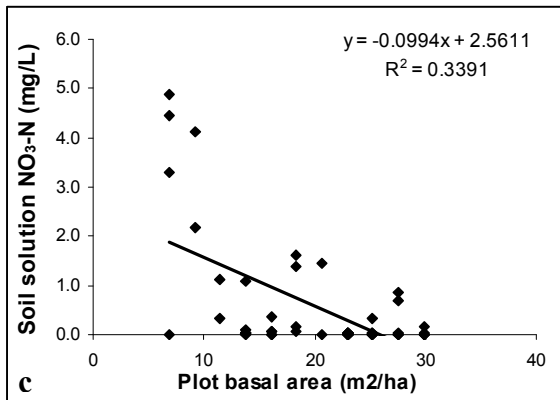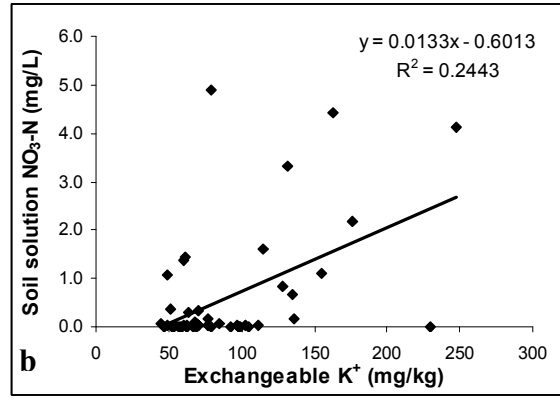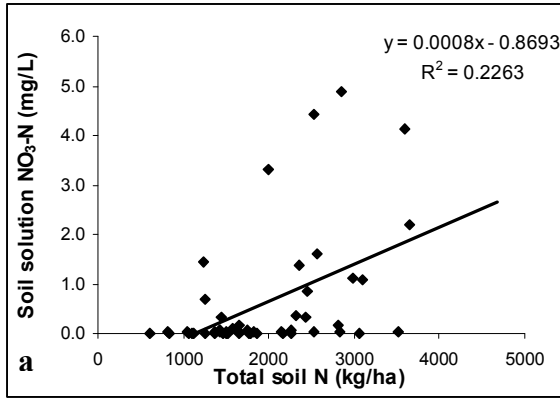
Figure 3.8. Linear relationships of soil solution NO_3-N and significantly correlated properties across both watersheds. Soil water NO_3^-N concentrations are plotted against a) total soil N, b) exchangeable K^+, and c) plot basal area. All relationships are significant at $p < 0.05$.

86
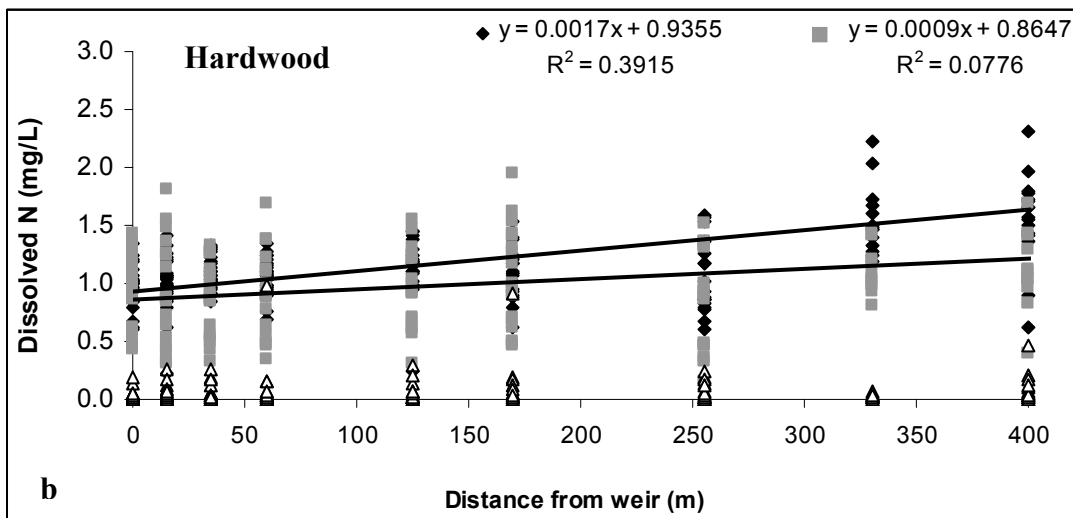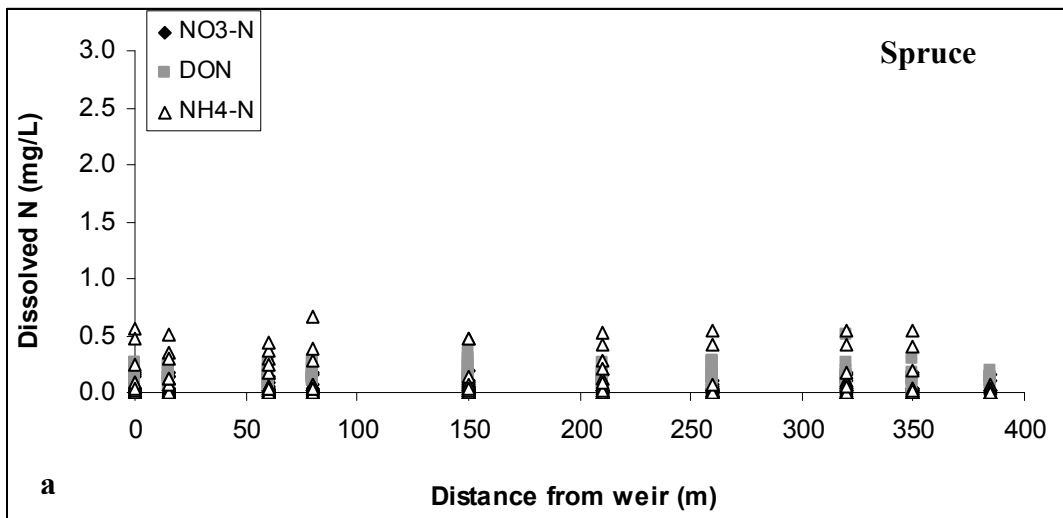
Figure 3.9. Concentrations of dissolved N (NO$_3$-N, NH$_4$-N, and DON) in streams at all sampling sites from weir upstream to headwaters (HW) in a) the stream draining watershed 6 (spruce) and b) the stream draining watershed 7 (native hardwood). Lines represent significant linear trend in NO$_3$-N and DON concentration by stream distance ($p < 0.05$).
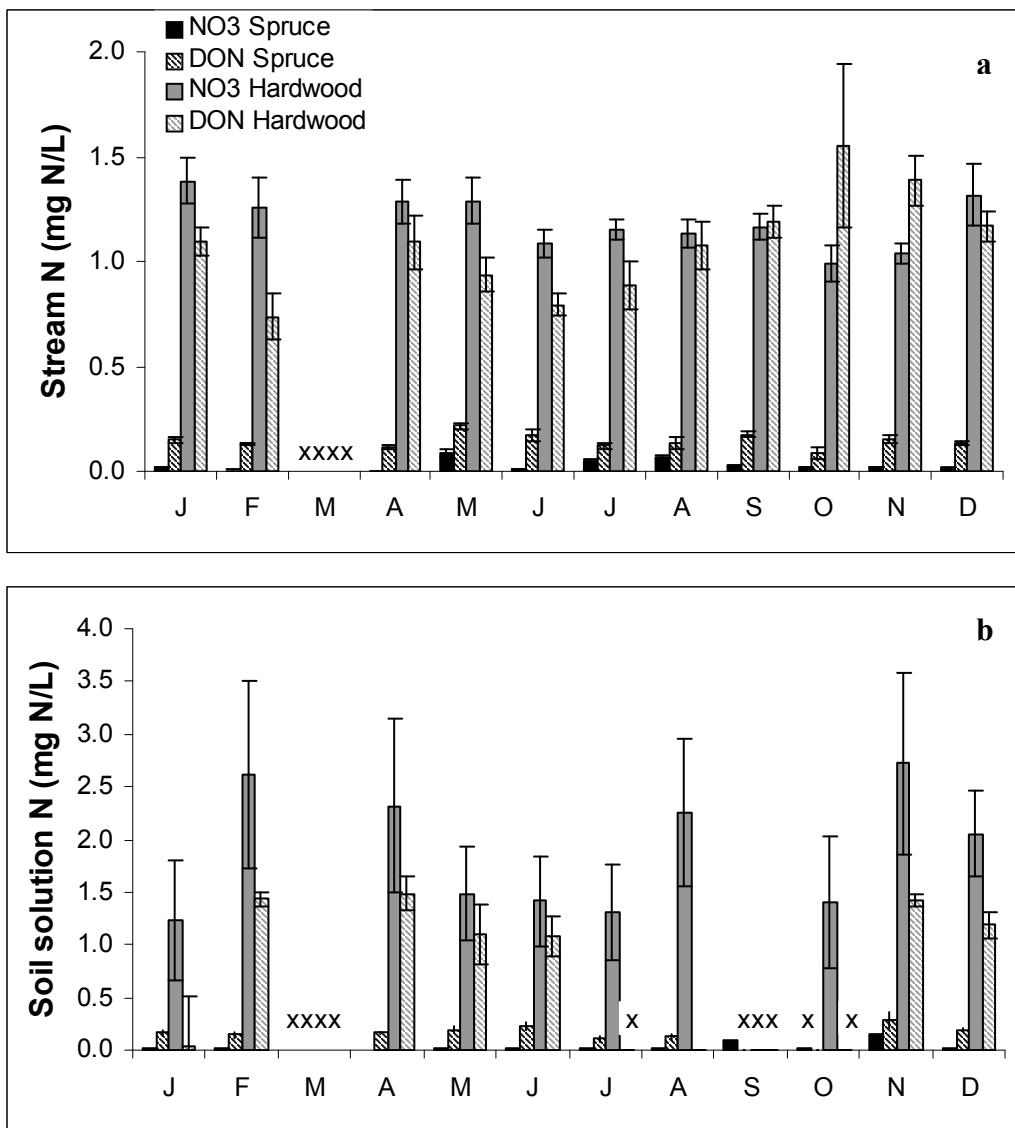
Figure 3.10. Mean monthly concentrations of NO$_3$-N and DON for the spruce and hardwood watersheds from a) stream samples and b) soil solution samples. Values represent means from each individual month as collected from Nov 2007-Nov 2009. Missing data values are represented by x and error bars represent one standard error of the mean from 19 monthly sampling events.

Chapter 4. Soil properties associated with net nitrification in soils influenced by Norway spruce and Appalachian hardwoods in the Fernow Experimental Forest, West Virginia

ABSTRACT

Nitrate (NO_3-N) in soil solution and streamwater can be an important vector of nitrogen (N) loss from forested watersheds, and is associated with negative consequences of soil acidification and eutrophication of aquatic ecosystems. Mobilization of NO_3-N is regulated by many environmental factors, including specific influences of vegetation on the soil environment. It was previously documented that *in situ* potential net nitrification rates were dramatically different in the soils in two nearly adjacent watersheds within the USDA Forest Service Fernow Experimental Forest, WV, characterized by contrasting vegetation type (Norway spruce monoculture versus native Appalachian hardwoods). This research utilizes a soil inoculation and incubation study and measurement of a suite of soil chemical and biological properties to identify key soil properties that may control the potential net nitrification dynamics within two watersheds with contrasting forest vegetation. Soils collected from each watershed were incubated in mixed ratios of hardwood:spruce soils (ranging from pure hardwood soil to 0.75: 0.25, 0.5: 0.5, 0.25: 0.75 mixes, to pure spruce soil) in order to generate a gradient of soil chemical and biological properties. This incubation was performed in order to determine if differences in net nitrification in these soils could be attributed to abiotic retention or biotic inhibition. Results of this study indicate that net NO_3-N production was nine times higher in hardwood soils (mean = 183.51 mg N/kg/28 days) than in the spruce soils (mean = 18.97 mg N/kg/28 days) and differences in net NO_3-N production were attributed to differences in soil substrate quality. Soil properties that were most strongly correlated with NO_3-N production across vegetation types included total soil N, soil C:N ratio, oxalate concentration, and sulfate concentration. Establishment of a spruce monoculture at the FEF significantly altered N cycling, depleted N stores, increased soil acidity, and altered organic matter dynamics, thus leading to low net nitrification rates.

INTRODUCTION

The microbial oxidation process of nitrification plays an important role in nitrogen (N) cycling in forest soils, and high nitrification rates in forests have potentially negative implications for forest health if uptake rates are less than nitrification rates. Nitrate is highly mobile in soil solution and is easily leached to aquatic systems, inducing aquatic eutrophication and leaching of important base cations such as Ca^{2+} and Mg^{2+}. Oxidation of NH_4^+ also produces acidity (H^+ protons), thus decreasing soil pH and increasing mobility of phytotoxic Al^{3+} (Fenn et al 1998, Christ et al 2002).

Many ecological studies have been executed with goals of determining which properties of a forest ecosystem influence the capacity to retain N (e.g. Vitousek and Matson 1985; Peterjohn et al 1999; Lovett et al 2002; Goodale and Aber 2001; Christopher et al 2008, Ross et al 2004), though our understanding of mechanisms of the net production of mobile NO_3^- across sites is still incomplete. Nitrogen can be immobilized through either biotic or abiotic mechanisms (Bengtsson et al 2003), with biotic incorporation into organic matter thought to be the largest component of N immobilization, and clay fixation of NH_4^+ comprising only about 10% of added N (Drury and Beauchamp 1991).

Many ecosystem factors have been identified that may influence net nitrification rates in forest soils, as described in a review by Ste-Marie and Paré (1999). These include temperature, water availability, soil acidity, availability of suitable substrate, nutrient limitations, successional stage of vegetation, and/or alleleopathic inhibition of nitrifier microbial populations. For example, in a Norway spruce (*Picea abies*) stand in Finland, immobilization of mineral N was linked to alleleochemical inhibition through volatile organic compounds (terpenes) exuded by Norway spruce, which directly inhibited nitrification, thus resulting in very low nitrate leaching from the stand (Paavolainen et al 1998). Also, soil pH significantly affects nitrification, with soils of pH <5.3 exhibiting relatively low net nitrification (e.g. Carlyle et al 1990; Ste-Marie and Paré 1999). However, other studies have shown net nitrification to occur even in soils with pH <3.0 (Robertson 1982; DeBouer and Kowalchuk 2001).

Soil C:N ratio is also often cited as a factor that influences nitrification in forest soils, and lower soil C:N was associated with high gross and net nitrification rates in 8 forested sites across the northeastern US (Ross et al 2004). However, other studies have shown that C:N of mineral soil may not be a strong indicator of net nitrification, which may be better explained by temporal and spatial patterns of temperature and moisture (Bengtsson et al 2003). Furthermore, in the NITREX study in Europe, sites with the lowest C:N ratio exhibited the greatest rates of NH_4^+ immobilization (Tietema 1998). This was attributed to effects of a higher mycorrhizal fungi abundance, which upon sieving, released relatively high amounts of inorganic N in the soils with the high C:N ratio (Gundersen et al 1998). Organic matter quality (i.e., as measured by lignin concentration) has also been associated with nitrification (Huang et al 2004), and organic matter quality is often mediated by tree species (Fitzhugh et al 2003). For example, litter high in lignin produces phenolics that more rapidly incorporate NO_2^- abiotically into stable soil organic matter, as observed beneath oak (*Quercus spp.*) and beech (*Fagus sylvatica*) (Fitzhugh et al 2003).

It was previously documented that *in situ* potential net nitrification rates were dramatically different in soils of two nearly adjacent watersheds at the USDA Forest Service Fernow Experimental Forest, West Virginia, USA (FEF). Mean annual net nitrification rates of 144.8 and 4.2 kg NO_3-N/ha/yr were measured in a watershed with native hardwoods (WS7) and a watershed with planted Norway spruce (WS6), respectively (Kelly 2010 Dissertation Chapter 2). These watersheds have nearly identical management histories, soil, and climate, differing primarily by vegetation cover. Additionally, divergent patterns in N export have been documented from these two watersheds. Mean annual stream NO_3-N export from the hardwood watershed is nearly 14 kg/ha, whereas stream NO_3-N exports from the spruce watershed have been nearly zero for 20 y (mean = 0.18 kg/ha/yr) (Adams, unpublished data; Kelly 2010 Dissertation Chapter 2).

The goal of the present research is to identify key soil parameters that influence potential net nitrification dynamics in these watersheds. A soil inoculation and incubation study

was performed under controlled laboratory conditions to 1) determine if the variation in net NO_3-N production in these soils can be attributed to a lack of nitrifying microbes or to incorporation of N compounds into organic substrate within the spruce soils and 2) identify soil properties that are associated with the divergent rates of NO_3-N production exhibited in these watersheds. It was hypothesized that differing soil C compounds associated with the hardwood and spruce systems vary in degradability and are involved in the mechanism causing differing rates of net NO_3-N production noted between these two watersheds.

METHODS

Description of the watersheds

The soils used in this incubation study were collected from two watersheds located within the FEF near Parsons, West Virginia. Soils in both watersheds are mapped mainly as Calvin series (Calvin channery silt loam; Calvin loamy-skeletal, mixed, active, mesic typic Dystrudept) (USDA NRCS web soil survey 2010), derived from shale, siltstone, and sandstone parent material. For a complete description of these nearly adjacent watersheds and management histories, see Kelly (2010 Dissertation Chapter 2). Both watersheds (WS6 and WS7) were clearcut logged in sections, beginning in 1964 and concluding in 1967, and maintained barren using herbicides until 1969.

Watershed 6 (22 ha) was planted with Norway spruce (*Picea abies*) in 1973, whereas WS7 (24 ha) was managed for natural regeneration of the native hardwood forest beginning in 1970. After nearly 40 years of growth, WS6 is now a closed-canopy spruce forest with dense stand structure and a litter layer characteristic of natural conifer stands (mor-type). The forest floor is characterized by a relatively a thick horizon (approximately 2-8 cm) of non-decomposed needles above further decomposed organic material of spruce origin. Mean basal area stocking is 23 m^2/ha. There are few other forest tree species in WS6, with sparse patches of green briar (*Smilax* sp.) and few individual hardwood trees including black locust (*Robinia pseudoacacia)*, yellow poplar (*Liriodendron tulipifera)*, red maple (*Acer rubrum)*, and sourwood (*Oxydendron*

arboreum), that survived the herbicide treatments. Nonetheless, the forest vegetation is a relatively homogeneous monoculture of Norway spruce.

The hardwood watershed (24 ha) is dominated by yellow-poplar, red oak (*Quercus rubra*), and red maple, with an under-story of dogwood (*Cornus florida*), striped maple (*Acer pensylvanicum*), magnolia (*Magnolia acuminata*), and several species of fern. Mean basal area stocking in WS7 is 17 m^2/ha (Kelly Dissertation Chapter 2 2010).

Soil incubation design

During initial field measurements in these watersheds, sampling transects were established perpendicular to the topographic contour lines from the stream towards the upper reaches of the watershed on both sides of the stream (Fig. 1). Three transects were established on both sides of each stream (6 transects per watershed) to capture possible effects of aspect on soil characteristics. For the incubation experiment, soil samples were collected from the A-horizon (0-10 cm) from both WS6 and WS7, at each transect from locations 1 m (riparian) and 60 m (upland) from the stream channel (Fig. 1).

Soil from each watershed at each sampling site was collected in February 2008, and mixed to form a composite sample for each watershed and landscape position. Soils were sieved through 2mm mesh and allowed to equilibrate to 50% water content by weight as measured by percent weight loss at 105° C after 24 hrs. Incubation mixtures were achieved by mixing soils in ratios to form a total equivalent dry weight of 500 g in the following ratios: 1:0, 0.75:0.25, 0.5:0.5, 0.25:0.75, and 0:1 hardwood:spruce (Fig 2; Ste-Marie and Paré 1999). Soil mixtures were placed in sealable plastic bags to minimize moisture loss (Fitzhugh et al 2003) and were aerated daily to prevent development of anaerobic conditions. No significant changes in soil moisture were noted throughout the incubation, as measured weekly by weight. Three replicate incubation mixtures for each landscape position were used in this experiment (n=3 replicates; n=5 soil mixtures, n=2 landscape positions; total N=30).

Soil extractions using $2M$ KCl were performed on a sub-sample of each soil incubation unit immediately upon mixing to determine initial concentrations of extractable inorganic N (NH_4-N and NO_3-N). Extracts were analyzed for inorganic N on an auto-analyzer (Bran-Luebbe, Nordersted, Germany). Soil mixtures were then incubated for 28 days in the dark at 24°C (Paavolainen et al 1998).

Following incubation, soils were again analyzed to determine net mineralization and nitrification rates by comparing values to the initial extractable NH_4-N and NO_3-N for each treatment. Net nitrification was calculated as the difference in extractable NO_3-N between initial and final measurements, net ammonification was calculated as the difference in extractable NH_4-N between initial and final measurements, and total net N mineralization was calculated as the difference in extractable NH_4-N + NO_3-N between initial and final measurements.

Soil chemical properties

It was expected that upon mixing and incubation, the resultant soils would create a gradient of soil chemical and biological properties as indicated in Figure 2. These properties were measured from sub-samples of each mixture at the end of the incubation period. Following the incubation, soils were stored at 4° C prior to processing for the following analyses. Soil pH was measured from a 2:1 extraction of 0.01 M $CaCl_2$ (Hendershot et al 1984). Total N and C were analyzed on a CN elemental analyzer (Elementar VarioMax CNS, Hanau, Germany) (Pella and Columbo 1973). Exchangeable cations (Al, Fe, Ca, Mg, K, Mn) were analyzed following an ammonium chloride extraction (1 N) (Thomas 1982) and phosphorus was measured as Mehlich III-extractable P (Tran and Simard 1993). Exchangeable cations and P were analyzed using ICP spectrometry (Varian, Salt Lake City, Utah). Exchangeable acidity was determined using the KCl method and titration with 1 N NaOH (Thomas 1982).

Organic acids

For analysis of organic acids in each soil mixture, 10 g of incubated soil was extracted with 20 mL of DI water (pH adjusted to 3.8 with HCl). Investigations of organic acids

that are labile at natural soil conditions are often performed with DI water to minimize changes in chemical conditions during extraction (Blum et al 1994; Strobel 2001). Solutions were swirled and allowed to equilibrate for 4 hrs prior to vacuum filtration through Whatman #2 filters. To 8 mL of the extract solution, 1 drop of 1 N NaOH and 0.8 mL of 0.005 M Na$_2$-EDTA were added to chelate Al, which interferes with analysis of organic acids (Klugh-Stewart and Cumming 2009). Solutions were roto-evaporated and stored frozen at -4° C. After solutions were thawed, 20 mL of DI water was used to dissolve residual salts and samples were analyzed with a reverse phase column (Dionex) and assessed for citrate, oxalate, acetate, glycolate, and tartrate, in addition to sulfate.

Carbon fractionation

Dissolved organic matter was fractionated utilizing hydrophobic-retaining DAX-8 resin (Supelite^{TM} Sigma-Aldrich Co., St. Louis, MO) to differentiate operationally defined hydrophobic and hydrophilic fractions as an indicator of solubility (Yu et al 2002). With this method, the hydrophobic fraction consists of humic substances, humic and fulvic acids, tannins, and phenols. The hydrophilic materials not retained on the resin are carbohydrates, carboxylic acids, aromatic amines, and amino sugars, amino acids, and free peptides and proteins. The fractionation procedure involved addition of 100 mL of DI water to 50 g of fresh soil, followed by shaking of solutions for 30 min and equilibration overnight. Following equilibration, solutions were filtered using Whatman #2 filter paper and were split into two subsamples and acidified to pH 2.0 with HCl. Half of the solution was used to determine total values of dissolved organic C (DOC) and total N (TN). The other half of the solution was shaken for 20 minutes with 25 g of DAX-8 resin to retain the hydrophobic fraction of the organic matter, leaving the hydrophilic fraction in solution. Resin was prepared and washed prior to extraction following the protocol of Thurman and Malcolm (1981). Organic C and TN in solution were determined on an Elementar TOC/TN analyzer (Hanau, Germany). The hydrophobic fraction of these parameters was obtained by subtracting the hydrophilic values from the total values.

Soil biotic properties

Microbial communities were assessed to further investigate mechanisms of biotic controls affecting NO_3-N production in the soil mixes (Schmidt et al 2004). Both heterotrophic and autotrophic nitrifier population sizes were determined from the soil mixes using serial dilutions and Most Probable Number (MPN) counts (Woomer 1994; Carter 1993). Heterotrophic nitrifier populations were determined using the protocol of Papen and von Berg (1998). Briefly, 10 g of fresh soil was mixed with 100 mL of sterile 0.9% $NaCl_2$ solution. One mL of each dilution series was added to test tubes containing 9 mL auotoclaved peptone-meat softagar solution (PMSA) medium and vortexed for 10 s. Tubes were stored at 28° C for 14 days and were uncapped and vortexed in a sterile hood daily to supply oxygen for heterotrophic nitrification to occur. After 7 and 14 days, all tubes were tested for the production of NO_3-N and NO_2-N by the addition of colorimetric reagents (Schmidt and Belser 1994) to a 100 µL aliquot of sample in sterile 90-cell well plates. Tubes were scored positive for nitrification if either NO_3-N or NO_2-N was detected.

Autotrophic nitrifer populations were determined following the protocol of Schmidt and Belser (1994). Ten g of fresh soil was added to 95 mL of sterile 0.001 *M* phosphate buffer and shaken for 10 min. Five 10^{-1} serial dilutions of the supernatant were prepared. One mL of each dilution series was added to sterile test tubes containing NH_4^+ oxidizer medium, and no additional C source. Tubes were incubated at 25° C for 21 days, aerated twice weekly, and scored weekly for NO_3^- and NO_2^- production as described above for 6 weeks. Most Probable Number values (# cells/g soil) for both methods were calculated using an MPN calculator (Curiale MPN calculator; Build 23), based on the equation by Hurley and Roscoe (1983).

Microbial biomass C and N were determined using the chloroform-fumigation method (Anderson and Domsch 1978). Microbial cells were lysed by placing soil samples in vacuum-sealed dessicator chambers containing evaporated chloroform for 24 hr. Equivalent soil samples were also placed in vacuum-sealed chambers for 24 hr without

chloroform. All samples were extracted with 0.5 M K_2SO_4 and extracts were analyzed for DOC and TN as described above. Microbial biomass C was calculated as:

$$\text{(Eq. 1)} \qquad MBC = \frac{\text{extractable } C_{fumigated} - \text{extractable } C_{unfumigated}}{0.35}$$

where MBC = microbial biomass C and 0.35 represents the 35% efficiency of chloroform fumigation to kill microbial cells in soils. Microbial biomass N (MBN) was calculated as:

$$\text{(Eq. 2)} \qquad MBN = \frac{\text{extractable } N_{fumigated} - \text{extractable } N_{unfumigated}}{0.35}$$

Data analysis

Predicted values for net nitrification and mineralization were calculated as a weighted value based on ratio of each soil type in the mixture and measured response of each pure soil incubation. Predicted values were compared to observed values of net nitrification (net change in NO_3-N production during incubation period) and net mineralization (net change in NO_3-N + NH_4-N during incubation period) using Wilcoxon two-sample tests (Ste-Marie and Paré 1999). The design was replicated 3 times to achieve lab replication for each of two landscape positions and 5 mixture ratios. Nitrate-N production and soil properties within each landscape position and soil mixture were tested by one-way ANOVA, followed by Tukey's HSD using $\alpha=0.05$ to compare means.

In order to identify relationships among the measured soil properties to measured NO_3-N and NH_4-N production response in the soils, each of the measured soil properties was analyzed from each mixture and values obtained were used to create Spearman's correlation coefficients relating values of soil properties to NO_3-N and NH_4-N production. Non-parametric Spearman's correlation was used because several properties could not be transformed to fit the assumption of normal distribution.

Soil properties that were significantly correlated to NO_3-N production were then used to perform stepwise regression to select the most influential properties affecting soil NO_3-N

production after 28 days. This Stepwise regression procedure was also conducted for both NH_4-N production and total N mineralization. General linear regression was used in this analysis because all significant properties used in the Stepwise model were either normally distributed or could be transformed to fit a normal distribution. Soil properties that were transformed include SO_4^{2-} and total soil N and these were both log-transformed. All statistical analysis was performed using SAS-JMP software version 8.0.

Possible outcomes of the resultant NO_3^- production in the soil mixtures were interpreted in the following manner (Fig 3). If the inhibition of NO_3^- accumulation noted in the spruce soils is caused by persistent alleleochemical compounds or ammonium adsorption (i.e. "abiotic" mechanisms), the addition of fresh spruce soil to hardwood soil would decrease the relative amount of extractable NO_3^- following incubation of the soil mixtures, as demonstrated in a similar inoculation and incubation study of forest floor material (Fig. 3a; Ste-Marie and Paré 1999). If NO_3^- production is related to the inhibition of microbial processes, or lack of nitrifying populations in the spruce soil (i.e. "biotic" mechanisms), addition of hardwood inoculum (and microbial populations therein) to spruce soil would produce more NO_3^- in the mixtures than hardwood soil alone, as indicated in Figure 3b. If NO_3-N production is related to the degree of suitability of substrate in these soils (i.e. C lability), no differences will be seen in observed versus predicted outcomes (Fig. 3c).

RESULTS

Nitrogen fluxes

Observed values of net NO_3-N production in the five soil mixes were not significantly different from predicted values after 28 days incubation ($p>0.05$; Fig. 4). Thus, the "No interaction" theoretical outcome as described in Fig. 3c was observed in the net production of NO_3-N in these soil mixtures.

Net NO_3-N production was highest in 100% hardwood soils (mean = 183.51 mg N/kg/28 days), and declined linearly by a factor of 10 to lowest production in 100% spruce soils (mean = 18.97 mg N/kg/28 days) (Fig. 5a). Net NH_4-N production exhibited the opposite

pattern, with the least production occurring in the 100% hardwood soils (mean = 15.17 mg N/kg/28 days) and increasing linearly to highest production in the 100% spruce soils (mean = 102.00 mg N/kg/28 days) (Fig. 5b). Total N mineralization was only significantly different between the 100% hardwood and 100% spruce soils, with no significant differences detected among the three mixtures of hardwood and spruce soils (soil mixtures 2-4; Fig. 5c). However, total N mineralization was approximately 40% lower in the spruce soil than the hardwood soil (Fig. 5c; 120.9 and 198.7 mg N/kg/28 days in the spruce and hardwood soil, respectively).

Relationships between net nitrification and soil chemical properties

Spearman's correlation coefficients for associations between each measured soil chemical and biotic property and net NO_3-N production are listed in Table 1, with associated p-values. Thirteen soil properties were significantly correlated with net NO_3-N production (p<0.05) with the strongest relationships to net nitrification occurring with total soil N, soil C:N ratio, sulfate, oxalate, exchangeable K^+, and exchangeable Fe (Table 1). Mean values for soil properties that were significantly correlated to net NO_3-N production (p<0.05) in the soil mixtures are also shown for all soil mixtures in Figure 6. Total N concentrations were significantly higher in hardwood soils with steady decreases as more spruce soil was added to the mixtures (p=0.0039; mean= 6.3 and 4.3 g/kg in the hardwood and spruce soils, respectively), and the C:N ratio was significantly lower in the hardwood soil than the spruce soil (p=0.0039) (Fig. 6). Among the dissolved organic matter properties, spruce soils contained a significantly higher fraction of total dissolved C as hydrophobic C than hardwood soils (84% of total dissolved C was in the hydrophobic C fraction in spruce soil and 69% in hardwood soil; p=0.0250) (Fig. 6). The fraction of total dissolved N present as hydrophobic N was similar across all soil mixtures containing spruce soils (15.87% of total N was in the hydrophobic N fraction in spruce and 23.02% in hardwood soils; p>0.05).

Among the organic acid compounds measured, oxalate was the only compound that was significantly different between the spruce and hardwood soils (p<0.0001; mean oxalate concentrations = 0.79 and 0.57 mg/kg in hardwood and spruce soils, respectively; Fig. 6).

Sulfate concentrations were also significantly higher in the spruce soil (p=0.0270; mean sulfate concentrations = 10.22 and 29.78 mg/kg in hardwood and spruce soils, respectively). Of the cations measured, $Fe^{2+/3+}$ concentrations were significantly higher in spruce soils (p=0.0104), and K^+ concentrations tended to be higher in the hardwood soils (p=0.0698; Fig. 6). The sum of bases was almost twice as high in the hardwood soils (mean 1110.04 and 689.32 mg/kg for hardwood and spruce soils, respectively), though the means were not statistically different (p=0.2236).

Relationships between net nitrification and soil biotic parameters

Soil microbial biomass C was the only biotic property measured that was significantly correlated to NO_3-N production (Table 1), and mean microbial biomass C in hardwood soil tended to be higher relative to spruce soil (p=0.0864; mean MBC= 229.65 and 169.38 mg/kg in hardwood and spruce, respectively) (Fig. 6). No significant differences between hardwood and spruce soils were observed in measures of soil microbial biomass N, heterotrophic nitrifier MPN, or autotrophic nitrifier MPN (Table 1).

Landscape influences on net nitrification

Overall, soils collected from the upland landscape position produced significantly more net NO_3-N after 28 days incubation than soils collected from the riparian position, where mean net NO_3-N in upland soils was 124.12 mg/kg/28 days compared to 88.14 mg/kg/28 days in riparian soils (p<0.0001). Effect of landscape position was most pronounced in soil mixtures that contained spruce soil (Fig. 7; soil mixtures 2-4 and S), with only slight and insignificant increases in NO_3-N in the upland 100% hardwood soil mixture compared with the riparian 100% hardwood soil (184.73 and 182.29 mg N/kg/28 days in upland and riparian, respectively) (Fig. 7).

Predicting net nitrification, net ammonification, and total net nitrogen mineralization

Using the variables that were significantly correlated to NO_3-N production from all soil mixtures (Table 1 and Fig. 6), a predictive model was developed using stepwise regression. Four terms remained in the model (R^2 model value of 0.9458; P<0.0001) (Fig. 8). These terms included total soil N (individual R^2= 0.8352; p<0.0001), soil C:N

ratio (individual R^2= 0.7436; p=0.0224), oxalate concentration (individual R^2=0.4801; p=0.0057), and sulfate concentration (individual R^2=0.4285; p=0.0193).   The resultant model can be used to predict net NO_3-N production within these soil mixtures:

(Eq. 3)   Net NO_3-N production (mg/kg/28 d) = -91.08 – 6.63(*soil C:N*) – 26.41 (*log sulfate* mg/kg) + 186.31(*log total soil N* g/kg) + 89.66(*oxalate* mg/kg)

Using the variables that wee significantly correlated to NH_4-N production from all soil mixtures, a predictive model was created using stepwise regression. Three terms remain in the model (R^2 model value of 0.8204; p<0.0001). These terms include total soil N (individual R^2= 0.3224; p=0.0011), total soil C (individual R^2= 0.2501; p=0.0301), and exchangeable Mg^{+2} (individual R^2=0.1572; p=0.0049). The resultant model can be used to predict net NH_4-N production within these soil mixtures:

(Eq. 4)   Net NH_4-N production (mg/kg/28 d) = 196.59 – 207.83(log *total soil N* g/kg) + 1.81(*total soil C* g/kg) + 0.59(*exchangeable Mg^{2+}* mg/kg)

Using the variables that were significantly correlated to total net N mineralization from all soil mixtures, a predictive model was created using stepwise regression. Four terms remain in the model (R^2 model value of 0.92; p<0.0001). These terms include total soil N (individual R^2=0.6212; p<0.0001), soil C:N ratio (individual R^2= 0.7729; p<0.0001), exchangeable Mg^{2+} (individual R^2= 0.3237; p=0.0010), and exchangeable Al^{3+} (individual R^2= 0.1112; p=0.0717). The resultant model can be used to predict total net N mineralization within these soil mixtures:

(Eq. 5)   Net N mineralization (mg/kg/28 d) = 67.06 + 92.45(*total soil N* g/kg) – 10.87(*soil C:N ratio*) + 0.51(*exchangeable Mg^{2+}* mg/kg) + 0.15(*exchangeable Al^{3+}* mg/kg)

Differences by landscape position also occurred in soil properties that remained in the stepwise regression model (Eq. 5) described above (data not shown). Across all soil mixtures, total soil N tended to be higher in the soils collected from the upland landscape position than from the riparian landscape position (p=0.0895; mean = 5.9 and 5.0 g/kg in

the upland riparian and soils, respectively). Soil C:N ratio was significantly higher in all soils collected from the riparian landscape position (p=0.0010; mean C:N ratio= 17.03 and 14.33 in riparian and upland soils respectively).

DISCUSSION

Nitrogen fluxes

Generally, net NO_3-N production during the incubation experiment was highest in the 100% hardwood soil and declined linearly with increasing proportions of spruce soil in the soil incubation mixtures (Fig. 5a). No significant differences occurred between observed and predicted values of net NO_3-N production in any soil mixture (Fig. 4). This suggests that no interaction occurred between the two soil types upon mixing, and the observed pattern of NO_3-N production was assumed to occur as a result of the unsuitability of the spruce soil to provide favorable substrate for use by introduced soil microbes (Fig. 3c). No evidence of persistent alleleochemical inhibition of microbial activity was detected in these soils, because addition of spruce soil to hardwood soil in any ratio did not result in observed NO_3-N production to be less than predicted values (Ste-Marie and Paré 1999).

Lower production of NO_3-N exhibited by spruce soil could be a result of either a low rate of total net N mineralization, or from a low rate of conversion of mineralized N to NO_3-N (Robertson and Vitousek 1981). Total net N mineralization was approximately 40% less in the spruce soil than in the hardwood soil (Fig. 5c; 120.9 and 198.7 mg N/kg/28 d in the spruce and hardwood soil, respectively), and this lower mineralization of N in spruce soil may be partly explained by lower N stores in the spruce soil (Eq. 5). However, data from this study show that a substantial amount of N was mineralized to NH_4-N in spruce soil during the 28-day incubation (Fig. 5b; 102.00 mg NH_4-N/mg/kg/28 days in the 100% spruce soil), and it was the subsequent step of oxidation of NH_4-N to yield NO_3-N that was apparently inhibited in spruce soil. This accumulation of NH_4-N in soils was also shown by Sahrawat (1980), where NH_4-N accumulated to an average of 70 µg/g soil, and NO_3-N production was zero following an incubation of acidic sulfate soils for 2 weeks.

Thus, availability of NH_4-N is probably not the limiting factor causing the low net NO_3-N production exhibited by the spruce soil in this study.

Soil properties related to soil chemistry and substrate availability appear to hinder the activity of the nitrifier population and lead to the low rates of net NO_3-N production observed in the spruce soil. This is supported by Gilliam et al (2001), who concluded that in a reference watershed at the FEF (WS4), very low nitrification at some sites was probably a result of high levels of available Al^{3+} and low Ca^{2+} concentrations in the soil, and not lack of NH_4^+ availability. This soil condition was thought to be a result of ericaceous mycorrhizae associated with hillside blueberry (*Vaccinium pallidum*), the presence of which was highly correlated to very low soil solution NO_3-N in some areas within this watershed. Ericoid mycorrhizae secrete organic acids that inhibit nitrifying microbes and incorporate N compounds into organic complexes that are then unavailable for biotic processing (Straker 1996; Read and Perez-Moreno 2003).

Influence of soil chemical properties

Total N was significantly lower in the spruce soils (p=0.0039; Fig. 6), and this soil property was strongly positively correlated to NO_3-N production (p<0.0001; Table 1), and also positively correlated to total net N mineralization (p<0.0001). This pattern of lower N content in the spruce soil has been previously documented within these watersheds, where the total ecosystem N budget in the spruce watershed accounted for approximately 35% less N than the hardwood watershed (Kelly 2010; Dissertation Chapter 2). This was explained by assumed mass losses of soil C and N upon conversion to the conifer plantation following the hardwood harvest that occurred from 1967-1969. Significant ecosystem losses of C and N following conversion to conifer from hardwood vegetation have also been shown by Kasel and Bennett (2007), who documented a 30% decrease in soil C content with conversion of native broadleaf forest to pine plantation after 37 years in Australia. Guo and Gifford (2002) also observed this pattern in a meta-analysis of land-use change. A 12-15% loss of soil C was documented when native broadleaf forests were converted to conifer plantations, whereas no changes in soil C and N were observed upon recovery to native broadleaf forest without conversion to conifer

plantations. This loss of soil C and N associated with conversion to conifers may be attributed to both disturbance and changes in amount and composition of plant material returned to the soil via litter and root processes (Lugo and Brown 1993), and possibly to the presence of a large abundance of ectomycorrhizal fungi introduced into the watershed upon conversion of hardwood vegetation to conifer (Chapela et al 2001). Introduced ectomycorrhizal fungi have previously been shown to induce a 30% soil C depletion within 20 years of establishment of an exotic Radiata pine (*Pinus radiata*) plantation in Ecuador in a study that utilized stable C isotopic tools and radiocarbon dating of fungal tissue to show that ectomycorrhizal fungi can utilize stable soil C stores as an energy source (Chapela et al 2001).

Soil C:N ratio was significantly higher in the spruce soil relative to the hardwood soil (p=0.0039; Fig. 6), and this term was negatively correlated to NO_3-N production in these soil mixtures (p<0.0001; Table 1). Soil C:N ratio has been identified as an important regulator of net nitrification in many studies (e.g. van Veen et al 1984; Aber 1992; Bradbury et al 1993; Janssen 1996; Ross et al 2004; Christenson et al 2009), and C:N ratio is often a function of vegetation cover and the degradability of litter inputs (Christ et al 2002). Data from the current study support soil C:N ratio as an important factor influencing net NO_3-N production in these soil mixes.

As expected, the hydrophobic C fraction comprised a significantly greater proportion of the total DOC in spruce soil relative to hardwood soil (p=0.0250; Fig. 6), and this term was negatively correlated to NO_3-N production (Table 1). The hydrophobic fraction in this method consists of slowly degrading compounds of humic substances, humic and fulvic acids, tannins, and phenols and the hydrophilic materials not retained on the resin are the more easily degradable compounds of carbohydrates, carboxylic acids, aromatic amines, and amino sugars, amino acids, and free peptides and proteins (Yu et al 2002). Spruce vegetation produces litter that is less degradable than that produced by most hardwoods (Melillo et al 1983). For example, black spruce (*Picea mariana*) and Douglas fir (*Pseudotsuga menziesii*) in Canada contained higher amounts of lignin in wood than associated alder (*Alnus rugosa),* birch (*Betula papyrifera*), and aspen (*Populus*

tremuloides) (24.6 %, 25.9%, 13.1%, 8.2%, and 12.0% lignin in wood, respectively) and lignin:N ratios were highest in spruce wood relative to the hardwood species (e.g.. 647 in spruce and 57 in birch) (Melillo et al 1983). Lignin:N ratios were the best determinant of wood decay rates in their study. Thus, spruce vegetation produces organic compounds that are composed of materials that are more recalcitrant for microbial use as substrate, resulting in a higher proportion of hydrophobic materials in the dissolved C fraction. However, the hydrophobic C term did not remain in the best stepwise regression model developed to predict NO_3^-N production in these soil mixtures (Equation 3).

Surprisingly, only one organic acid compound, oxalate, was significantly correlated to NO_3-N production in these soil mixtures ($p<0.0001$; Table 1), and oxalate concentrations were significantly greater in hardwood soil (mean oxalate = 0.79 and 0.57 mg/kg in hardwood and spruce soils, respectively; Fig. 6). Acetate has been previously shown to be an inhibitor of nitrification (DeBouer and Laanbroek 1989), though we could not discern acetate concentrations from glycolate concentrations in our analysis of organic acids, leading to inconclusive data. Inhibition of nitrification has been shown to yield decreased concentrations of oxalate in other studies (Ombodi et al 1999) and low oxalate concentrations were shown to occur in association with higher soil NH_4^+ content. High soil NH_4^+ can induce NH_4^+ nutrition in plants, leading to a decreased uptake of base cations, which lowers organic acid production within the plant (Ombodi et al 1999). Oxalate and other organic acids have also been shown to chelate toxic Al^{3+} compounds in soils (Jones et al 1993; Ma 2000; Piñeros et al. 2002; Kochian et al. 2004; Klugh and Cumming 2007), and Al^{3+} has been identified as a potential inhibitor of nitrification (e.g. Brar and Giddens 1968). Thus, concentration of oxalate in the soil is positively correlated to net NO_3-N production in our soil mixtures (Table 1).

Sulfate concentration was significantly higher in spruce soil ($p=0.0250$; Fig. 6) and SO_4^{2-} was negatively correlated to NO_3-N production in these soil mixtures ($p<0.0001$; Table 1). Sulfur (in the form of sulfide) has been shown to inhibit nitrification (Joye and Hollibaugh 1995) by effectively competing for oxygen in anaerobic environments. However, soils in this incubation were well aerated and nitrification inhibition by sulfur

was probably not occurring. Nitrification increases the retention of SO_4^{2-} in forest soils by increasing the protonation of Fe and Al oxides on soil surfaces (Johnson and Cole 1980; Nodvin et al 1986; Nodvin et al 1988). Additionally, sulfate adsorption declines as pH decreases below 4.0, and this can be attributed to the dissolution of Al oxides (Chao et al 1964, in Nodvin et al 1986). Spruce soil in this study had a pH value of 3.75, and hardwood soil had a pH of 3.95 (Kelly 2010, Dissertation Chapter 2. Thus, soils with relatively high rates of net nitrification and higher pH are associated with low extractable SO_4^{2-}, which agrees with the results of the current study.

Influence of soil biotic properties

Soil microbial biomass C (MBC) was the only measured biotic property to be significantly correlated with NO_3-N production in these soil mixtures (p=0.0117; Table 1). Soil MBC tended to be greater in the hardwood soil (p=0.0864; mean MBC = 229.65 and 169.38 mg/kg in hardwood and spruce soils, respectively; Fig. 6) and was associated with increased net NO_3-N production. Generally, microbial biomass can be a good indicator of N cycling processes at landscape or regional scales in northern hardwood forests (Bohlen et al. 2001). Microbial biomass depends upon soil organic matter composition (Zak et al. 1990) because soil microorganisms generally are C limited (Anderson & Domsch 1985; Wardle 1992). The microbial biomass C responses to hardwood and conifer vegetation type observed in this study have also been documented at the Harvard Forest in Massachusetts, where hardwood soils in the control plots contained 534 μg MBC/g soil and conifer soils contained only 431 μg MBC/g soil (Frey et al 2004). Microbial biomass C was also shown to be positively correlated to net N mineralization (R^2= 0.638) in an old-field chronosequence study in Minnesota, though the relationship between MBC and net nitrification was not as strong (Zak et al 1990). This agrees with our results, demonstrating that MBC is indicative of general soil N cycling, but may not always be a strong indicator of net NO_3-N production across vegetation types.

No differences were detected in autotrophic or heterotrophic nitrifier populations (MPN) in these soil mixtures (p>0.05) and the MPN values for these populations were not

correlated to NO_3-N production ($p>0.05$; Table 1). In the heterotrophic assay, a labile C substrate is supplied to the microbes in the form of meat peptone, and microbes from spruce soils produced NO_3-N to the same extent as microbes from hardwood soils. This result supports the conclusion that C compounds within the spruce soil are unsuitable for nitrifying microbes, and persistent alleleochemical inhibition of microbes is not evident in these soils.

Influence of landscape

Soils collected from the upland landscape position produced more NO_3-N after 28 days incubation than soils collected from the riparian position in all soil mixtures except the 100% hardwood soil ($p<0.0001$; Fig 7). This is somewhat unexpected because seasonal *in situ* net nitrification measurements exhibited no difference by landscape position within these watersheds, and soil solution patterns showed that riparian concentrations of NO_3-N were often higher than concentrations observed at upland locations (Kelly 2010 Dissertation Chapter 3). However, other studies have demonstrated riparian soils to be strong transformers of or sinks for NO_3-N in the field (Cooper 1990; Hill et al 2000). Cooper (1990) demonstrated that 56-100% of NO_3^-N transformation occurred in the riparian soil of a New Zealand headwater stream, which was anoxic and high in denitrifying enzymes and available C. Laboratory incubations isolate soil processes from vegetation uptake, temperature, and hydrologic influences, allowing the effects of such factors as C availability and enzyme activity on N cycling to be better expressed. For example, through the use of laboratory soil incubations, investigators have demonstrated that temperature affects the chemical processes of SOM adsorption and desorption onto mineral surfaces, and soil moisture levels driven by drainage, precipitation, and evapotranspiration regulate the diffusion efficiency, and thus availability, of organic substrates and extracellular enzymes for microbial processes (Davidson and Janssens 2006).

CONCLUSIONS

This study documented relatively high net nitrification in soils collected beneath native hardwoods compared to soils influenced by spruce, and it is concluded that soil substrate

properties resulting from spruce vegetation led to decreased net nitrification in these soils. We isolated several key soil properties that influence substrate characteristics that were correlated with NO_3-N production from the soil mixtures in this study, including total soil N, soil C:N ratio, and concentrations of oxalate and sulfate. In order to determine causation, in addition to correlation, additional laboratory studies need to be conducted to isolate the specific effects of substrate and of each influential soil property identified by this study. For example, it is difficult to discern the specific effect of sulfate in soil solution from the effect of soil pH and other acidity parameters that are often cited as indicators of nitrification potential in soils (De Boer and Kowalchuk 2001). It cannot be determined from the data reported here if these parameters are by-products of N cycling in these soils, or are truly factors regulating the production of NO_3-N.

Results of this study show that establishment of a spruce monoculture at the FEF significantly altered N cycling, likely depleted soil N stores, increased soil acidity, and altered organic matter dynamics, thus leading to low net nitrification. These results are useful for management activities, including vegetative species selection in areas managed to minimize N export to aquatic systems, such as in riparian-zone restoration efforts. However, caution should be taken with respect to effects on soil C and N storage upon vegetation conversion. Additional studies should include efforts to isolate soil properties that determine actual causation of strongly altered nitrification processes.

WORKS CITED

Aber JD (1992) Nitrogen cycling and nitrogen saturation in temperate forest ecosystems. Trends in Ecology and Evolution 7: 220–223.

Anderson JPE and KH Domsch (1978) Mineralization of bacteria and fungi in chloroform-fumigated soils. Soil Biology and Biochemistry. 10(3): 207-213.

Anderson TH and KH Domsch (1985) Determination of ecophysiological maintenance carbon requirements of soil microorganisms in a dormant state. Biology and Fertility of Soils. 1: 81-89.

Bengtsson G, Bengtson P, and KF Mansson (2003) Gross nitrogen mineralization-, immobilization-, and nitrification rates as a function of soil C/N ratio and microbial activity. Soil Biology and Biochemistry. 35(1): 143-154.

Blum U, Worsham AD, King LD, and TM Gerig (1994) Use of water and EDTA extractions to estimate available (free and reversibly bound) phenolic acids in Cecil soils. Journal of Chemical Ecology. 20:341-359.

Bohlen PJ, Groffman PM, Driscoll CT, Fahey TJ, and TG Siccama (2001) Plant-soil-microbial interactions in a northern hardwood forest. Ecology. 82(4): 965-978.

Bradbury NJ, Whitmore AP, Hart, PBS, and DS Jenkinson (1993) Modelling the fate of nitrogen in crop and soil in the years following application of 15N-labelled fertilizer to winter wheat. Journal of Agricultural Science 121: 363–379.

Brar SS and J Giddens (1968) Inhibition of nitrification in Bladen Grassland soil. Soil Science Society of America Journal. 32: 821-823.

Carlyle JC, Lowther JR, Smethurst PJ, and EKS Nambiar (1990) Influence of chemical properties on nitrogen mineralization and nitrification in podzolized sands. Implications for forest management. Australian Journal of Soil Research. 28: 981-1000.

Carter MR (1993) Soil Sampling and Methods of Analysis. Canadian Society of Soil Science. Lewis Publishers.

Chao TT, Harward ME and SC Fang (1964) Iron or aluminum coatings in relation to sulfate adsorption characteristics of soils. Soil Science Society of America Proceedings. 28: 632-635.

Chapela IH, Osher LJ, Horton TR, and MR Henn (2001) Ectomycorrhizal fungi introduced with exotic pine plantations induce soil carbon depletion. Soil Biology and Biochemistry. 33: 1733-1740.

Christ MJ, Peterjohn WT, Cumming JR, and Adams MB (2002) Nitrification potentials and landscape, soil and vegetation characteristics in two Central Appalachian watersheds differing in NO_3^- export. Forest Ecology and Management. 159:145-158.

Christopher SF, Mitchell MJ, McHale MR, Boyer EW, Burns DA, and C Kendall (2008) Factors controlling nitrogen release from two forested catchments with contrasting hydrochemical responses. Hydrological Processes. 22: 46-62.

Cooper BA (1990) Nitrate depletion in the riparian zone and stream channel of a small headwater catchment. Hydrobiologia. 202(1-2).

Curiale, M. MPN Calculator; Build 23. <http://members.ync.net/mcuriale/mpn/>.

De Boer W and HJ Laanbroek (1989) Ureolytic nitrification at low pH by *Nitrospira* spec. Archives of Microbiology. 152: 178-181.

Davidson EA and IA Janssens (2006) Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. Nature. 440: 165-174.

De Boer W and GA Kowalchuk (2001) Nitrification in acid soils: micro-organisms and mechanisms. Soil Biology and Biochemistry. 33(7-8): 853-866.

Drury CF and EG Beauchamp (1991) Ammonium fixation, release, nitrification, and immobilization in high- and low-fixing soils. Soil Science Society of America Journal. 55(1): 125-129.

Fenn (1998) Nitrogen excess in North American ecosystems: Predisposing factors, ecosystem responses, and management strategies. Ecological Applications. 8(3):706-733.

Fitzhugh RD, Lovett GM, and RT Venterea (2003) Biotic and abiotic immobilization of ammonium, nitrite, and nitrate in soils developed under different tree species in the Catskill Mountains, New York, USA. Global Change Biology. 9: 1591-1601.

Frey SD, Knorr M, Parrent JL, and RT Simpson (2004) Chronic nitrogen enrichment affects the structure and function of the soil microbial community in temperate hardwood and pine forests. Forest Ecology and Management. 196(1): 159-171.

Goodale CL and JD Aber (2001) The long-term effects of land-use history on nitrogen cycling in northern hardwood forests. Ecological Applications. 11(1): 253-267.

Gundersen P, Callesen I, and W de Vries (1998) Nitrate leaching in forest ecosystems is related to forest floor C/N ratios. Environmental Pollution. 102(1,1): 402-407.

Guo LB and RM Gifford (2002) Soil carbon stocks and land use change: a meta analysis. Global Change Biology. 8(4): 345-360.

Hendershot WH, Lalande H, and M Duquette (1984) Soil Reaction and Exchangeable Acidity: In Soil Sampling and Methods of Analysis. Carter, Ed. Canadian Society of Soil Science. 142-145.

Hill AR (1996) Nitrate removal in stream riparian zones. Journal of Environmental Quality. 25:743–55.

Huang Y, Zou J, Zheng X, Wang Y, and X Xu (2004) Nitrous oxide emissions as influenced by amendment of plant residues with different C:N ratios. Soil Biology and Biochemistry. 36(6): 973-981.

Hurley MA and ME Roscoe (1983) Automated statistical analysis of microbial enumeration by dilution series. Journal of Applied Bacteriology. 55: 159–164.

Janssen BH (1996) Nitrogen mineralization in relation to C/N ratio and decomposability of organic materials. Plant and Soil 181: 39–45.

Johnson DW and DW Cole (1980) Anion mobility in soils: relevance to nutrient transport from forest ecosystems. Environment International. 3(1): 79-90.

Joye SB and JT Hollibaugh (1995) Influence of sulfide inhibition of nitrification of nitrogen regeneration in sediments. Science. 270(5236): 623-625.

Kasel S and LT Bennett (2007) Land-use history, forest conversion, and soil organic carbon in pine plantations and native forests of south eastern Australia. Geoderma. 137(3-4): 401-413.

Klugh KR and JR Cumming (2007) Variations in organic acid exudation and aluminum resistance among arbuscular mycorrhizal species colonizing *Liriodendron tulipifera.* Tree Physiology. 27:1103-1112.

Kochian LV, Hoekenga OA, and MA Pineros (2004) How do crop plants tolerate acid soils? Mechanisms of aluminum tolerance and phosphorus efficiency. Annual Review of Plant Biology. 55: 459-493.

Jones DL (1993) Organic acids in the rhizosphere – a critical review. Plant and Soil. 205(1): 25-44.

Klugh-Stewart K and JR Cumming (2009) Organic acid exudation by mycorhhizal *Andropogon virinicus* L. (broomsedge) roots in response to aluminum. Soil Biology and Biochemistry. 41(2): 367-373.

Lovett GM, Weathers, KC, and MA Arthur (2002) Control of nitrogen losses from forested watersheds by soil carbon:nitrogen ratio and tree species composition. Ecosystems. 5(7):712-718.

Lugo AE and S Brown (1993) Management of tropical soils as sinks or sources of atmospheric carbon. Plant and Soil. 149(1): 27-41.

Ma JF (2000) Role of organic acids in detoxification of aluminum in higher plants. Plant and Cell Physiology. 41(4): 383-390.

Melillo JM, Naimen RJ, Aber JD, and KN Eschleman (1983) The influence of substrate quality and stream size on wood decomposition dynamics. Oecologia. 58(3): 281-285.

Nodvin SC, Driscoll CT, and GE Likens (1986) The effect of pH on sulfate adsorption by a forest soil. Soil Science. 142(2): 69-75.

Nodvin SC, Driscoll CT, and GE Likens (1988) Soil processes and sulfate loss at the Hubbard Brook Experimental Forest. Biogeochemistry. 5(2): 185-199.

Ombodi A, Miyoshi S, and M Saigusa (1999) Effects of band applications of polyolefin-coated fertilizers on the nitrate and oxalate content in spinach. Tohoku Journal of Agricultural Research. 49(3-4): 101-109.

Paavolainen L, Kitunen V, and A Smolander (1998) Inhibition of nitrification in forest soil by monoterpenes. Plant and Soil. 205:147-154.

Papen H and R von Berg (1998) A Most Probable Number method (MPN) for the estimation of cell numbers of heterotrophic nitrifying bacteria in soil. Plant and Soil. 199(1): 123-130.

Pella E and B Colombo (1973) Study of carbon, hydrogen and nitrogen determination by combustion-gas chromatography. Mikrochim. Acta (Wein) 697-719.

Peterjohn WP, Foster CJ, Christ MJ, and MB Adams (1999) Patterns of nitrogen availability within a forested watershed exhibiting symptoms of nitrogen saturation. Forest Ecology and Management. 119:247-257.

Pineros MA, Magalhaes JV, Carvalho Alves VM, and LV Kochian (2002) The physiology and biophysics of an aluminum tolerance mechanism based on root citrate exudation in maize. Plant Physiology. 129: 1194-1206.

Read DJ and J Perez-Moreno (2003) Mycorrhizas and nutrient cycling in ecosystems—a journey towards relevance. New Phytologist 157:475–492.

Robertson GP and PM Vitousek (1981) Nitrification potentials and secondary succession. Ecology. 62(2): 376-386.

Robertson GP (1982) Nitrification in forested ecosystems. Philosophical Transactions of the Royal Society of London. 296(1082): 445-457.

Ross DS, Lawrence GB, and F Fredriksen (2004) Mineralization and nitrification patterns at eight northeastern USA forested research sites. Forest Ecology and Management. 188(1-3): 317-335.

Sahrawat KL (1980) Nitrogen mineralization in acid sulfate soils. Plant and Soil. 57(1): 143-146.

Schmidt EL and LW Belser (1994) Autotrophic nitrifying bacteria. Methods of Soil Analysis, Part 2. Microbiological and Biochemical Properties. Soil Science Society of America, Madison.

Schmidt SK, Lipson DA, Ley RE, Fisk MC, and AE West (2004) Impacts of chronic nitrogen additions vary seasonally and by microbial functional group in tundra soils. Biogeochemistry. 69(1):1-17.

Soil Survey Staff, Natural Resources Conservation Service, United States Department of Agriculture. Web Soil Survey. Available online at http://websoilsurvey.nrcs.usda.gov/ accessed [2/10/2010].

Ste-Marie C and D Pare (1999) Soil, pH and N availability effects on net nitrification in the forest floors of a range of boreal forest stands. Soil Biology and Biochemistry. 1579-1589.

Straker, C.J. 1996. Ericoid mycorrhiza: Ecological and host specificity. Mycorrhiza 6:215–225.

Strobel BW (2001) Influence of vegetation on low-molecular-weight carboxylic acids in soil solution-a review. Geoderma. 99(3-4):169-198.

Thomas GW (1982) Exchangeable cations. In: Page AL et al, Eds. Methods of Soil Analysis. Agronomy No. 9, 2nd ed. American Society of Agronomy, Madison, WI.

Thurman EM and RL Malcolm (1981) Preparative isolation of aquatic humic substances. Environmental Science Technology. 15(4): 463-466.

Tietema A, Emmett BA, Gundersen P, Kjonaas OJ, and CJ Koopmans (1998) The fate of 15N-labelled nitrogen deposition in coniferous forest ecosystems. Forest Ecology and Management. 101(1-3): 19-27.

Tran TS and RR Simard (1993) Mehlich III-extractable elements. In: Carter MR, ed. Soil Sampling and Methods of Analysis. Canadian Society of Soil Science. Lewis Publishers. van Veen JA, Ladd JN, and MJ Frissel (1984) Modelling C and N turnover through the microbial biomass in soil. Plant and Soil 76: 257–274.

Vitousek PM and PA Matson (1985) Disturbance, nitrogen availability, and nitrogen losses in an intensively managed loblolly pine plantation. Ecology. 66(4): 1360-1376.

Wardle DA (1992) A comparative assessment of factors which influence microbial biomass carbon and nitrogen levels in soil. Biological Reviews. 67(3): 321-358.

Woomer PL (1994) Most probable number counts. In: Methods of Soil Analysis, Part 2. Microbiological and Biochemical Properties. Pp. 59-79. Soil Science Society of America, Madison.

Yu Z, Zhang Q, Kraus TEC, Dahlgren RA, Anastasio C, and RJ Zasoski (2002) Contribution of amino compounds to dissolved organic nitrogen in forest soils. Biogeochemistry. 61:173-198.

Zak DR, Groffman PM, Pregitzer KS, Christensen S, and JM Tiedje (1990) The vernal dam: Plant-microbe competition for nitrogen in northern hardwood forests. Ecology. 71(2):651-656.
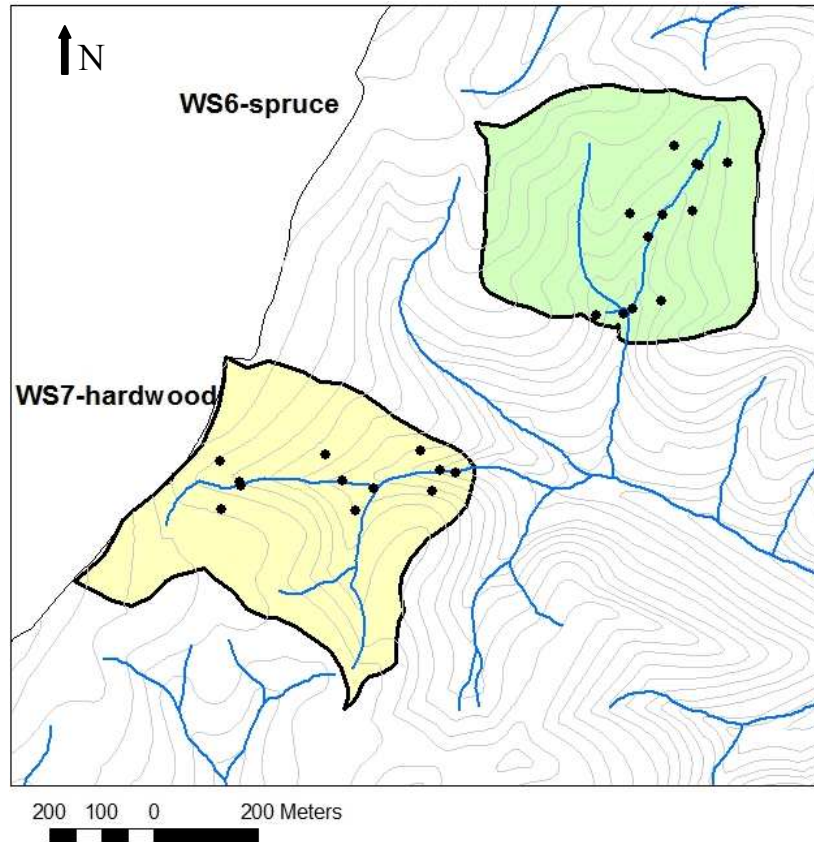
Figure 4.1. Proximity of study watersheds WS6 (Norway spruce) and WS7 (native hardwoods) within the Fernow Experimental Forest, WV and locations of transects and soil collection sites within each watershed (indicated by black circles).
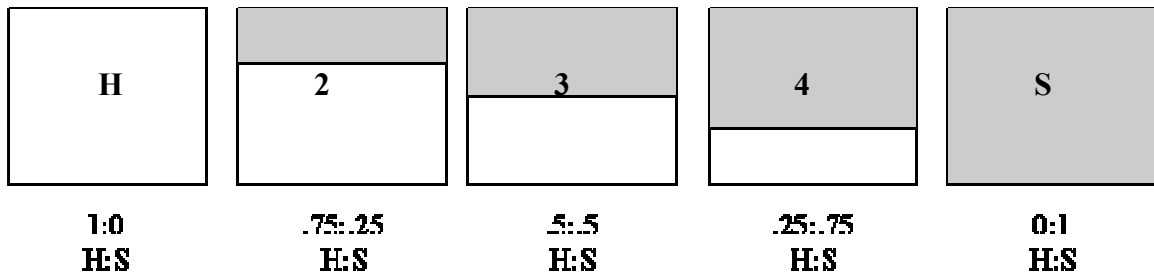
Figure 4.2. Gradient of five soil mixes used to study controls of N mineralization and nitrification in two watersheds with contrasting vegetation in the Fernow Experimental Forest, West Virginia. Ratios represent proportion of mixture comprised of hardwood soil (H) and spruce soil (S).
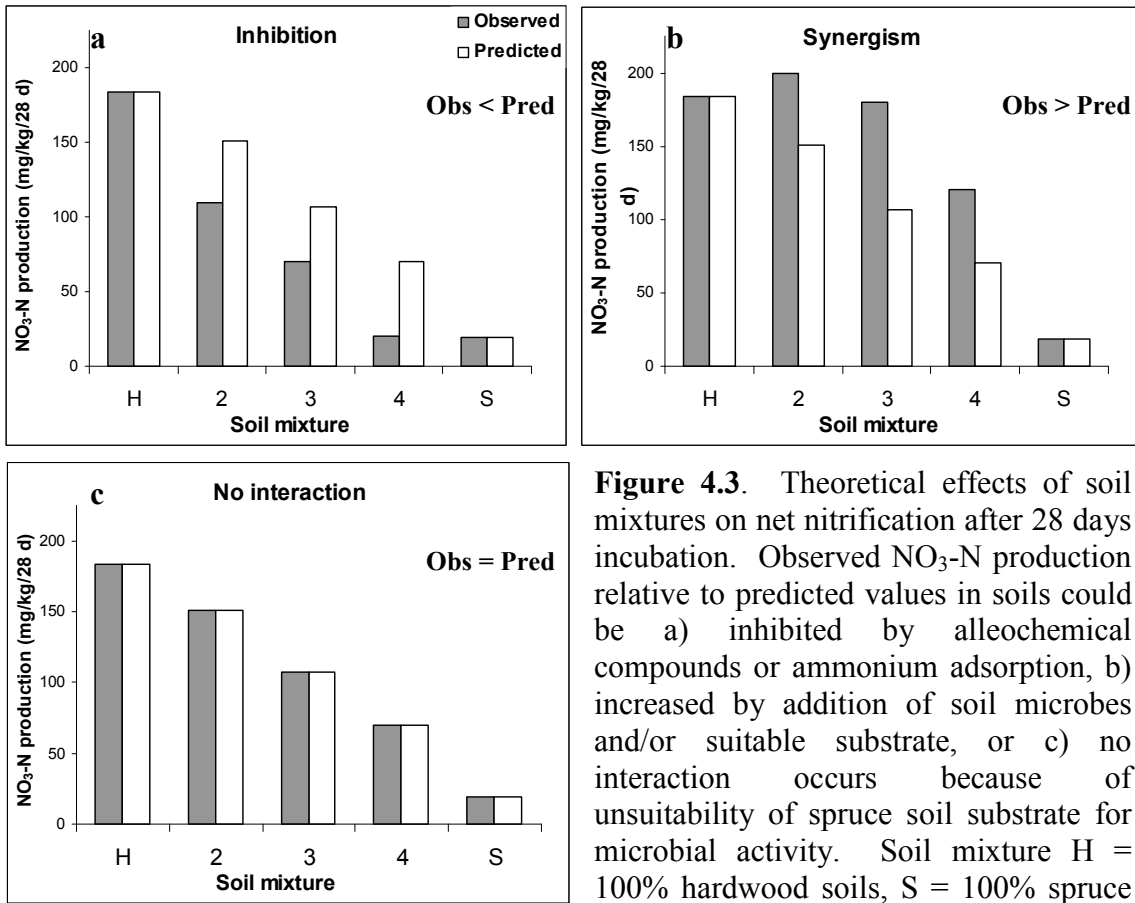
Figure 4.3. Theoretical effects of soil mixtures on net nitrification after 28 days incubation. Observed NO_3-N production relative to predicted values in soils could be a) inhibited by alleochemical compounds or ammonium adsorption, b) increased by addition of soil microbes and/or suitable substrate, or c) no interaction occurs because of unsuitability of spruce soil substrate for microbial activity. Soil mixture H = 100% hardwood soils, S = 100% spruce soils, 2-4 represent mixes of H and S.
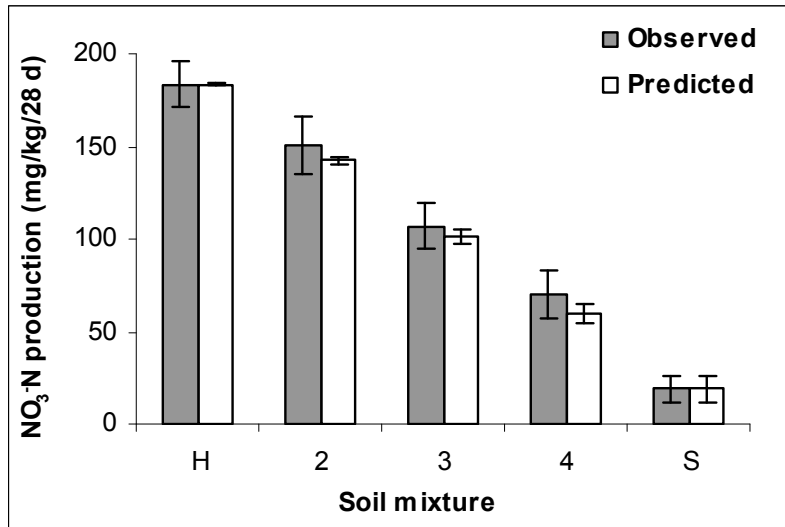
Figure 4.4. Observed and predicted values of net nitrification after 28 days incubation in soil mixes. Error bars represent standard error of the mean. Soil mixture H = 100% hardwood soils, S = 100% spruce soils, 2-4 represent mixes of H and S
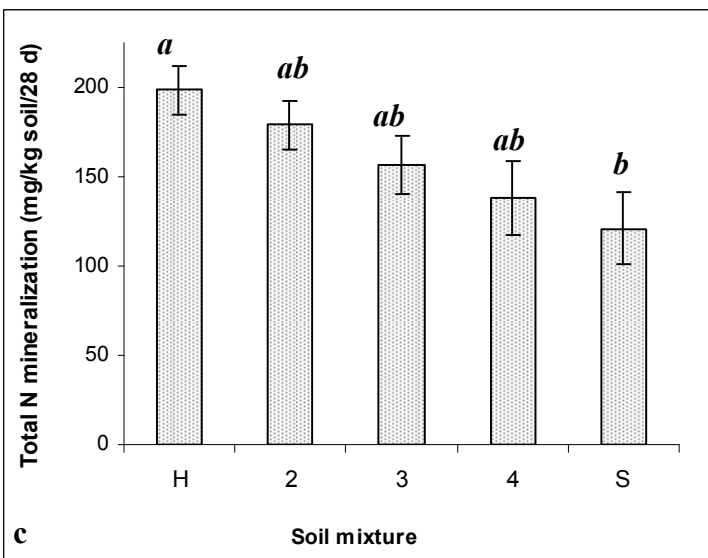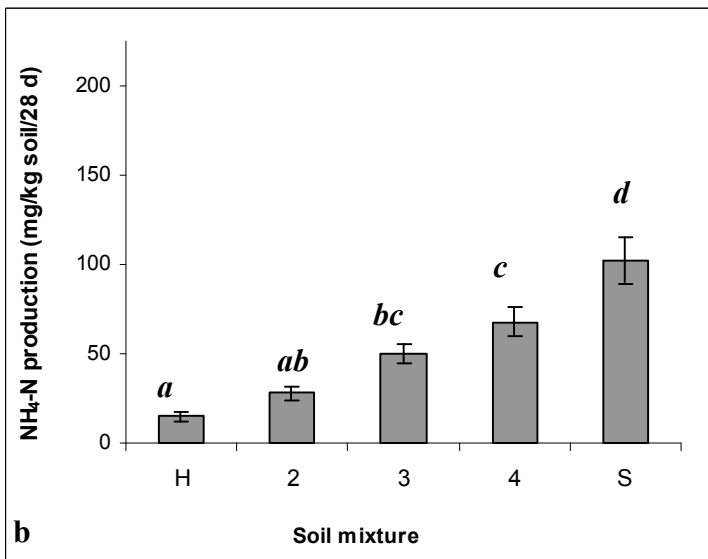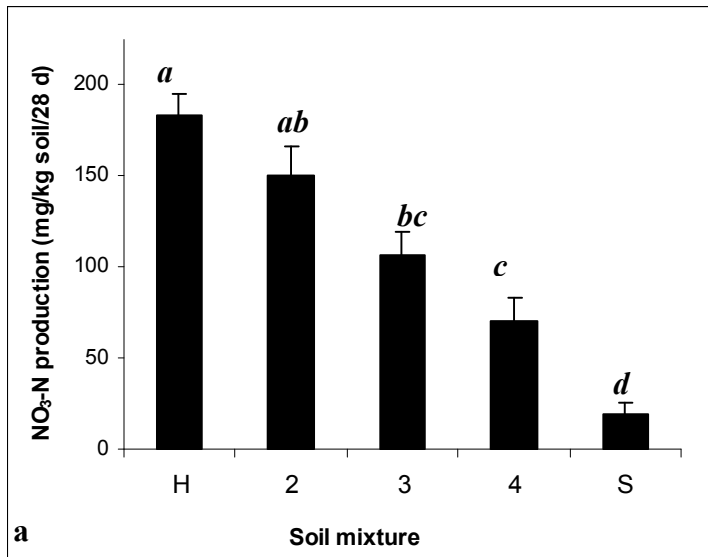
Figure 4.5. (a) Net nitrification, (b) net ammonification and (c) total net N mineralization in soil mixtures after 28 days incubation. For each N compound, soil mixtures with different letters represent means significantly different ($\alpha<0.05$), according to Tukey's HSD. Error bars represent standard error of the mean. Soil mixture H = 100% hardwood soils, S = 100% spruce soils, 2-4 represent mixes of H and S.
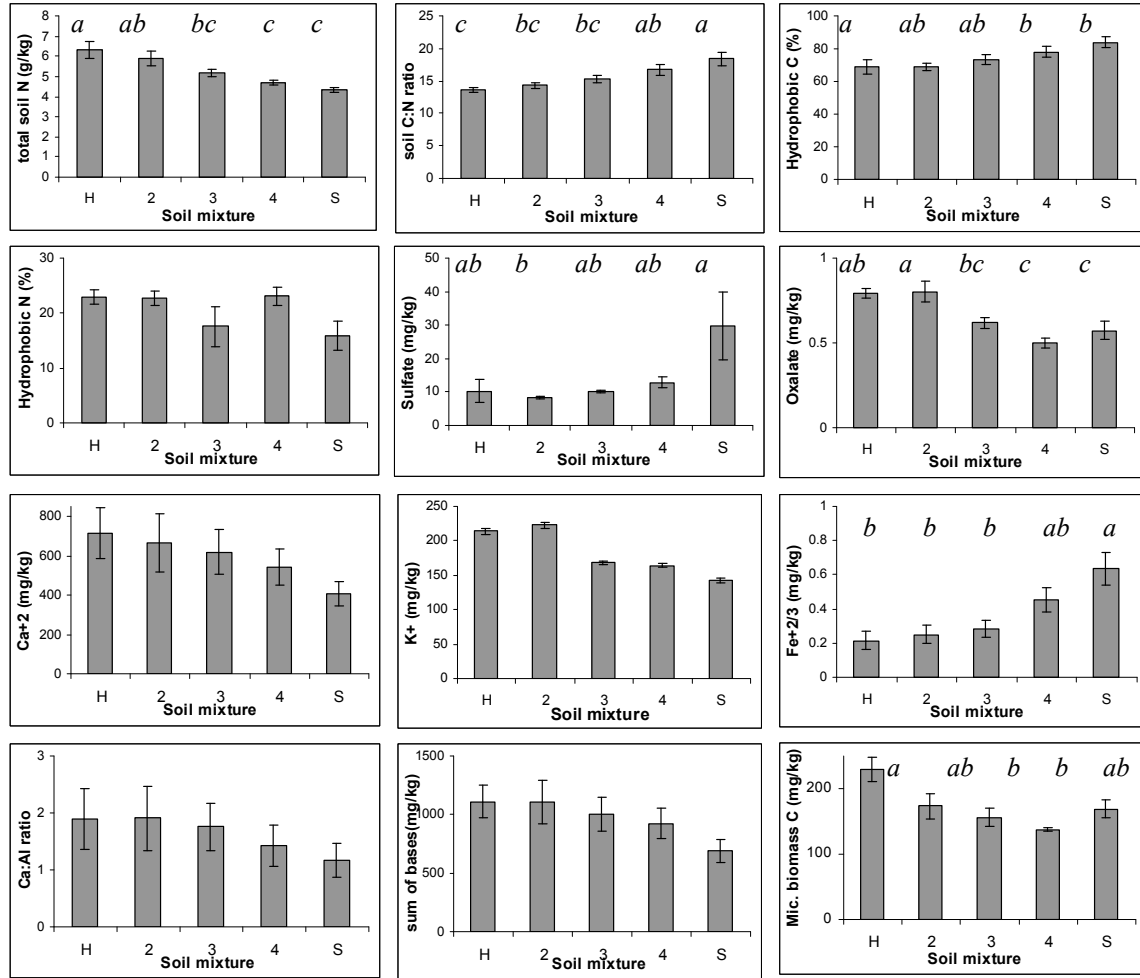
Figure 4.6. Gradients of soil chemical and biological properties crated by soil mixtures after 28 days incubation. For each soil property, means with different letters are significantly different at α=0.05 according to Tukey's HSD. Soil mixture H = 100% hardwood soils, S = 100% spruce soils, 2-4 represent mixes of H and S.

119

Table 4.1. Spearman's correlation coefficients for soil chemical and biological properties associated with soil net nitrification after 28 days incubation from 5 soil mixtures.

Category	Property	unit	Spearman's ρ	Probability $> \|\rho\|$
Organic matter	Total soil C	g/kg	0.34	0.0725
	Total soil N*	g/kg	0.94	<.0001
	C:N ratio*	-----	-0.89	<.0001
	Hydrophobic C*	% of total dissolved DOC	-0.62	0.0003
	Hydrophobic N*	% of total dissolved TN	0.47	0.0098
Acidity	pH	-----	0.13	0.496
	Exchangeable acidity	cmolq/kg	-0.15	0.4275
	Exchangeable Al^{3+}	mg/kg	-0.10	0.6237
Organic acids	Sulfate*	mg/kg	-0.73	<.0001
	Oxalate	mg/kg	0.70	<.0001
	Citrate	mg/kg	0.29	0.1297
	Formate	mg/kg	-0.10	0.5916
	Lactate	mg/kg	0.23	0.232
Exchangeable cations	Exchangeable Ca^{2+}*	mg/kg	0.60	0.0006
	Exchangeable K^{+}*	mg/kg	0.79	<.0001
	Exchangeable Mg^{2+}	mg/kg	0.13	0.4867
	Exchangeable Mn^{2+}	mg/kg	0.29	0.1288
	Exchangeable $Fe^{2+/3+}$*	mg/kg	-0.74	<.0001
	Ca:Al*	-----	0.47	0.0094
	Sum of base cations*	mg/kg	0.63	0.0003
Phosphorus	Extractable P	mg/kg	0.24	0.2052
Microbial	Microbial biomass C*	mg/kg	0.49	0.0117
	Microbial biomass N	mg/kg	0.27	0.185
	Microbial biomass C:N ratio	-----	0.15	0.4500
	Autotrophic nitrifiers	log(# cells/ g soil)	-0.06	0.7489
	Heterotrophic nitrifiers	log(# cells/ g soil)	0.05	0.8103

* properties that are significantly correlated to net nitrification according to Spearman's correlation and these terms were utilized to create the Stepwise regression model described ($\alpha < 0.05$).
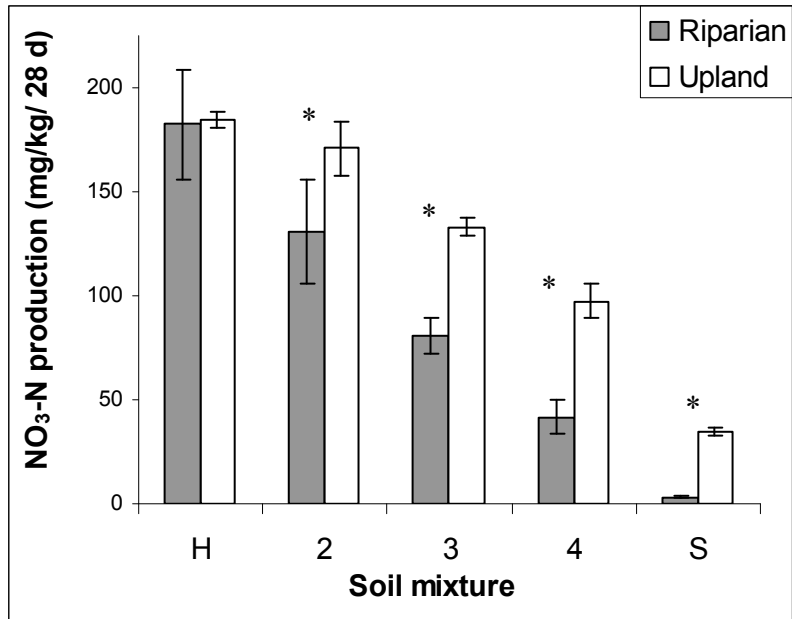
Figure 4.7. Effect of landscape position on net nitrification within each soil mixture. Error bars represent standard error of the mean. Soil mixture H = 100% hardwood soils, S = 100% spruce soils, 2-4 represent mixes of H and S. Asterisks represent significantly different means by landscape position for each soil mixture according to Tukey's HSD ($\alpha<0.05$).
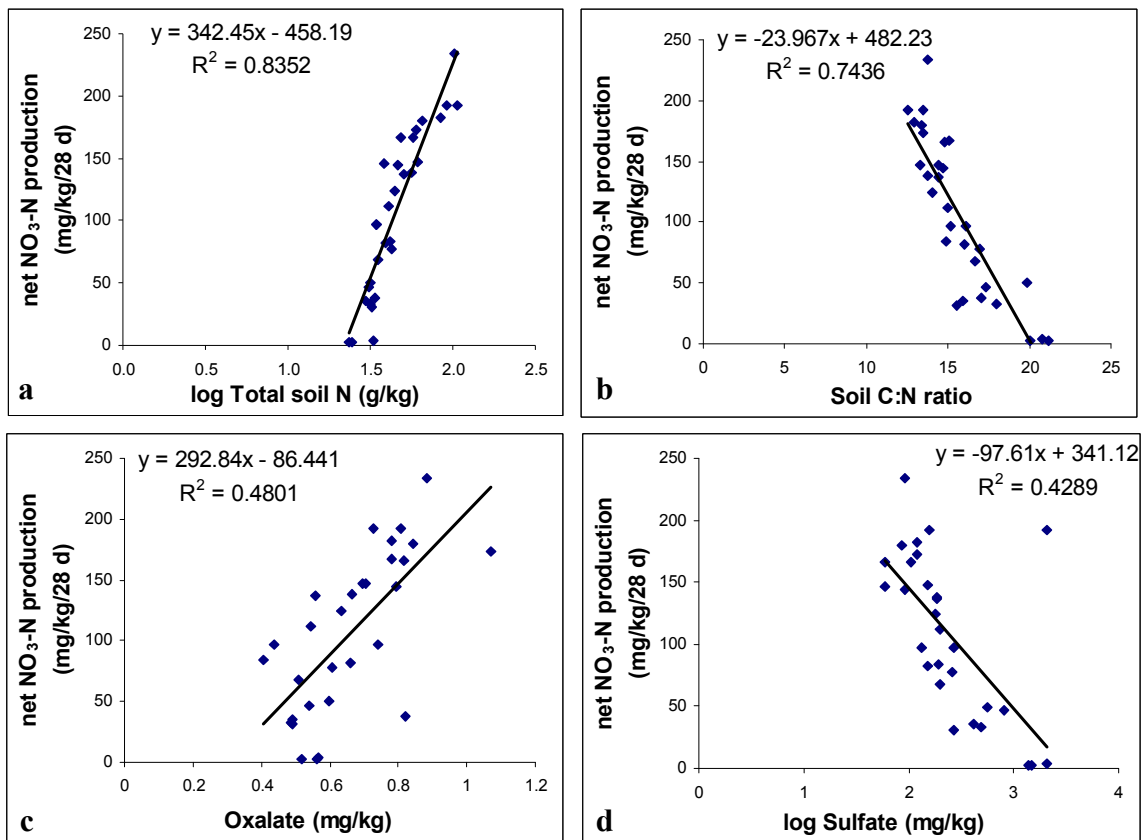
Figure 4.8. Net nitrification after 28 days incubation from all soil mixtures, as related to a) total soil N, b) soil C:N ratio, c) soil oxalate concentration, and d) soil sulfate concentration.

Chapter 5. Summary and Conclusions

Important differences in biogeochemical cycling of carbon (C) and nitrogen (N) have been documented in watersheds with contrasting forest vegetation at the Fernow Experimental Forest (FEF) in West Virginia. Soils influenced by a monoculture of Norway spruce (*Picea abies)* established 37 yrs ago exhibited lower soil pH and base cation content and higher Al^{3+} mobility, along with lower net N mineralization and N export to the stream draining the watershed in comparison with soils in an adjacent watershed supporting a 39-yr-old, naturally regenerated, native Appalachian hardwood forest.

Results from a comparative analysis of ecosystem C and N budgets between the two watersheds presented in Chapter 2 suggest that a significant loss of C and N from ecosystem pools likely occurred following conversion from native Appalachian hardwoods to the monoculture of Norway spruce. Total C and N pools were lower in the spruce watershed in nearly every compartment measured, as was total net N mineralization and dissolved N in streamwater. It was hypothesized that low net nitrification rates documented in the spruce watershed may occur as a result of abiotic immobilization and adsorption of N compounds onto the surface of mineral soils. This hypothesis was not supported in this study because total N content in the spruce soil was significantly lower than the hardwood soil. Thus, N does not appear to be accumulating in the soil profile in the spruce watershed. Total C and N pools were 28% and 35% less, respectively, in the spruce watershed as compared to the hardwood watershed. The B-horizon soil compartment exhibited the largest difference in both C and N stores between the two watersheds (32% less C and 38% less N in soils in the spruce watershed), followed by the A-horizon soil compartment, where there was nearly 20% less C and 34% less N in the spruce watershed than in the hardwood watershed. These results were contrary to the hypothesis that soil and forest floor C and N stores would be higher in the spruce watershed, thereby accounting for 40 years of relatively high atmospheric N input and very low stream export of NO_3-N from the spruce watershed in contrast to high

atmospheric N input and high stream export of NO_3-N from the hardwood watershed during the same period.

These observations suggest that soils within the spruce watershed may have been subjected to a phase of soil organic matter degradation as the spruce vegetation became established, inducing a loss of soluble C and N that was not detected in the long-term stream data that were limited to measures of NO_3-N loss, and did not measure dissolved organic C (DOC) or dissolved organic N (DON)) export in streamwater. These results also suggest that converting native hardwood forests to planted spruce forests may not provide the ecosystem service of C sequestration that is commonly associated with planted forests because potential C losses from the soil profile in response to conversion to a conifer forest may offset any increases in C storage in biomass.

This study also highlights the importance of including measures of DON in assessments of N cycling at the watershed scale. Monthly measures of total dissolved N in stream export (measured from July 2007-Sept 2009) indicate that annual DON export comprised nearly 50% of the total N export from the native hardwood watershed (mean = 10.03 kg DON/ha/yr and mean = 10.70 kg NO_3-N/ha/yr) and approximately 90% of the total N export in streamwater from the spruce watershed (mean = 0.48 kg DON/ha/yr and mean = 0.53 kg TN/ha/yr).

In Chapter 3, patterns of selected *in situ* dissolved C and N dynamics along transects from stream and riparian zone to the upland soils of the two contrasting watersheds were investigated to 1) determine specific soil properties that were potentially altered by the influence of the spruce vegetation relative to the native Appalachian hardwood soils and 2) analyze how these soil properties may be related to soil solution and in-stream fluxes of dissolved C and N spatially and temporally within the two watersheds.

Key soil and site properties with the strongest associations to soil solution NO_3-N concentrations across both watersheds included mineral soil exchangeable K^+, plot basal area, and total soil N content. The hardwood watershed contained areas of disturbance

caused by gap formation in the canopy, which are not present in the dense monoculture of spruce. Within these areas of low basal area in the hardwood watershed, the soil solution contained relatively high concentrations of mobile NO_3-N. This was likely caused by higher net nitrification rates resulting from possible soil disturbance from fallen trees, soil moisture increases with less vegetative uptake, and temperature increases with more sunlight reaching the forest floor. Thus, in addition to vegetation-mediated differences in soil properties, basal area stocking is an important factor controlling NO_3-N in soil solution. This result may be important for water quality associated with silvicultural treatments of single-tree selection or thinning near streams, especially in areas influenced by high levels of atmospheric N deposition and subjected to N saturation.

A coupled relationship between DOC and DON in soil solution and streamwater was generally lacking in the hardwood watershed, and this was in contrast to the tight coupling of DOC and DON in soil solution and streamwater in the spruce watershed. Additionally, soil solution NO_3-N concentrations were highest within the riparian soils of the hardwood watershed, indicating a diminished capacity of the riparian area to transform N or function as a sink for mobile forms of N emanating from upslope locations. In contrast, spruce vegetation imposed a strong limitation to net soil nitrification and soil solution NO_3-N was very low across the entire watershed. These patterns of an uncoupled relationship between DOC and DON and low sink strength of the riparian soils within the hardwood watershed provide further evidence that this watershed has reached a state of N saturation, whereas the spruce watershed ecosystem is probably limited by N as indicated by the low export of dissolved N in streamwater.

In addition to examining *in situ* relationships between soil solution NO_3-N and soil properties influenced by contrasting vegetation, soils from the two watersheds were utilized to perform a soil inoculation and incubation study in the laboratory as reported in Chapter 4. Laboratory measures of net nitrification exhibited the same pattern of relatively high net NO_3-N production occurring in the hardwood soil compared to the spruce soil that was documented in Chapter 2. Soil properties related to soil chemistry and substrate suitability within the spruce soil appear to hinder the activity of the nitrifier

population and lead to low rates of net NO_3-N production observed in the controlled laboratory conditions. Key soil properties most strongly correlated to net nitrification across a gradient of five soil mixtures ranging from 100% hardwood soil to 100% spruce soil in this laboratory study included total soil N content, soil C:N ratio, and concentrations of oxalate and sulfate.

Results presented in this dissertation highlight the scale of influence that forest management activities may have upon watershed C and N cycling, especially in forests subjected to high N deposition and in planted conifer forests. Carbon and N properties and processes in the soil profile should be taken into consideration in forests managed for ecosystem services including C sequestration and improvement or maintenance of water quality through alleviation of N inputs into aquatic ecosystems.

A management option that may provide C and N sequestration in both the soil profile and in tree biomass may be to establish forests of mixed vegetation type, and this may be especially beneficial in riparian buffer zones. In hardwood forests exhibiting symptoms of N saturation, addition of conifer species may impart a stronger ecosystem sink for high levels of N deposition and thereby improve water quality.