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REGULAR ARTICLE

Soil properties associated with net nitrification following watershed conversion from Appalachian hardwoods to Norway spruce

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Abstract Nitrate (NO_3-N) in soil solution and streamwater can be an important vector of nitrogen (N) loss from forested watersheds, and nitrification is associated with negative consequences of soil acidification and eutrophication of aquatic ecosystems. The purpose of this study was to identify vegetation-mediated soil properties that may control potential net nitrification dynamics and to determine if net nitrification is a function of abiotic retention or biotic inhibition. We performed a soil inoculation and incubation study and analyzed a suite of soil chemical and biological properties in soils from a 40-year-old Appalachian hardwood forest and an adjacent 37-year-old Norway spruce forest converted from Appalachian hardwoods. Our results indicate that net NO_3-N production was nine times higher in hardwood soil (mean = 183.51 mg N/kg/28 days) than in the spruce soil (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 in the central Appalachian hardwood ecoregion significantly altered N cycling, likely depleted soil N stores, increased soil acidity, and altered soil organic matter dynamics, thus leading to low net nitrification rates.

Keywords Nitrification · Norway spruce · Appalachian hardwoods · Fernow Experimental Forest · Forest conversion · Soil organic matter

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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 ecosystems if uptake rates are less than nitrification rates (Robertson 1982). Nitrate (NO_3^-) is highly mobile in soil solution and is easily leached, inducing eutrophication in downstream aquatic systems (Vitousek et al.

1982). Oxidation of NH_4^+ also produces acidity (H^+ protons), thus decreases soil pH, increases mobility of phytotoxic Al^{3+}, and displaces important base cations such as Ca^{2+} and Mg^{2+} from the soil complex (Fenn et al. 1998; Christ et al. 2002).

Many ecological studies have had 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 N (Drury and Beauchamp 1991).

Many ecosystem factors have been identified that can influence net nitrification rates in forest soils, as reviewed by Ste-Marie and Pare (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 allelochemical 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 generally exhibiting relatively low net nitrification (e.g. Carlyle et al. 1990; Ste-Marie and Pare 1999). However, other studies have shown net nitrification to occur even in soils with pH <3.0 (Robertson 1982; De Boer 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 eight 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 et al. 1998). This was attributed to effects of a higher mycorrhizal fungi abundance in the soils with high C:N ratio, which upon sieving, released relatively high amounts of inorganic N (Gundersen et al. 1998). Organic matter quality (i.e., as measured by lignin concentration) has also been correlated 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 N abiotically into stable soil organic matter, as observed beneath oak (*Quercus spp.*) and beech (*Fagus grandifolia*) (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 (FEF), West Virginia, US. 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). These watersheds are of similar size and geomorphology and have nearly identical management histories, soil, and climate, differing primarily by vegetation cover (Kelly 2010). 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 et al. 2003; Kelly 2010). In contrast to the N export patterns associated with these spruce and hardwood stands within the FEF, several spruce forests in Europe exhibit stream NO_3^- export that greatly exceeds the NO_3^- export of associated hardwood forests (generally *P. abies* versus European beech, *Fagus sylvatica*; see review by Gundersen et al. 2006).

The goal of our research was to identify key soil properties that influence potential net nitrification dynamics in these watersheds. Specifically, 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 inhibition of nitrifying microbes by compounds produced in spruce vegetation 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 as suitable microbial substrate and determine 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 (WS6 and WS7) near Parsons, West Virginia, USA. Soils in both watersheds are mapped mainly 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. For a complete description of these nearly adjacent watersheds and management histories, see Kelly (2010). Both watersheds were clearcut logged in sections, beginning in 1964 and concluding in 1967, and maintained vegetation-free using herbicides until 1969.

Watershed 6 (22 ha) was planted with Norway spruce 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 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,* 0.99% of total basal area), yellow poplar (*Liriodendron tulipifera,*1.8% of total basal area), red maple (*Acer rubrum,* 0.40% of total basal area), and sourwood (*Oxydendron arboreum,* 0.35% of total basal area). 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 in WS7 is 17 m^2/ha (Kelly 2010).

Soil incubation design

Sampling transects were established perpendicular to the topographic contour lines, from the stream towards the upper reaches of each 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) following removal of the O-horizon 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. Within 7 days of collection, soils were sieved through 2 mm mesh and allowed to air dry to approximately 50% water content by weight as measured by percent weight loss at 105°C after 24 h

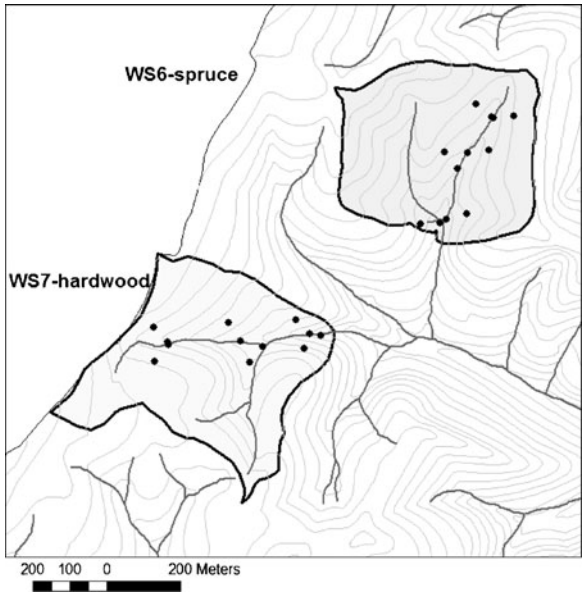

Fig. 1 Proximity of study watersheds WS6 (Norway spruce) and WS7 (native hardwoods) within the Fernow Experimental Forest, WV and locations of transects and soil collection sites within each watershed (indicated by *black circles*)

(Table 1). 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 (Ste-Marie and Pare 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

Table 1 Measured values for all soil chemical and biological properties and Spearman's correlation coefficients as associated with soil net nitrification after 28 days incubation from five soil mixtures (H, 2, 3, 4, and S)

Soil property	Soil mixture					Spearman's ρ	Prob. $>\|\rho\|$
	H	2	3	4	S		
Total soil C (g/kg)	85.56 (4.59)	84.16 (3.90)	78.59 (1.96)	77.99 (2.69)	79.36 (3.84)	0.34	0.0725
*Total soil N (g/kg)	6.30 (0.42)^{a}	5.91 (0.37)^{ab}	5.15 (0.18)^{bc}	4.69 (0.11)^{c}	4.32 (0.12)^{c}	0.94	<0.0001
*C:N ratio	13.66 (0.35)^{c}	14.33 (0.44)^{bc}	15.33 (0.58)^{bc}	16.59 (1.01)^{ab}	18.43 (1.03)^{a}	−0.89	<0.0001
*Hydrophobic C (% of total DOC)	68.71 (4.49)^{a}	68.85 (2.02)^{ab}	73.31 (2.86)^{ab}	76.20 (3.31)^{b}	84.05 (3.20)^{b}	−0.62	0.0003
*Hydrophobic N (% of total DN)	23.02 (1.35)	22.74 (1.36)	17.60 (3.62)	23.10 (1.64)	15.87 (2.57)	0.47	0.0098
pH	3.81 (0.04)	3.82 (0.04)	3.83 (0.05)	3.85 (0.05)	3.83 (0.04)	0.13	0.496
Exchangeable acidity (cmolq/kg)	5.65 (0.45)	4.97 (0.31)	5.11 (0.37)	5.17 (0.39)	5.47 (0.17)	−0.15	0.4275
Exchangeable Al^{3+} (mg/kg)	448.97 (53.21)	435.59 (65.92)	378.81 (24.67)	425.76 (55.78)	409.74 (52.16)	−0.10	0.6237
*Sulfate (mg/kg)	10.22 (3.47)^{ab}	8.21(0.31)^{b}	10.21 (0.34)^{ab}	11.73 (1.46)^{ab}	29.80 (10.21)^{a}	−0.73	<0.0001
*Oxalate (mg/kg)	0.79 (0.03)^{ab}	0.80 (0.06)^{a}	0.62 (0.03)^{bc}	0.49 (0.04)^{c}	0.57 (0.05)^{c}	0.70	<0.0001
Citrate (mg/kg)	1.14 (0.83)	8.24 (7.18)	0 (0)	9.01 (5.49)	0 (0)	0.29	0.1297
Formate (mg/kg)	2.08 (0.13)	2.00 (0.07)	2.56 (0.30)	2.30 (0.34)	2.08 (0.09)	−0.10	0.5916
Lactate	1.55 (0.08)	2.21 (0.15)	2.05 (0.21)	1.43 (0.10)	1.57 (0.10)	0.23	0.232
*Exchangeable Ca^{2+} (mg/kg)	714.33 (128.92)	666.02 (148.49)	618.65 (113.84)	568.89 (111.36)	405.47 (62.41)	0.60	0.0006
*Exchangeable K^{+} (mg/kg)	214.37 (19.72)	222.93 (37.94)	168.26 (17.90)	169.35 (18.78)	142.18 (3.97)	0.79	<0.0001
Exchangeable Mg^{2+} (mg/kg)	74.12 (11.71)	82.94 (20.89)	89.64 (23.03)	106.17 (28.81)	97.87 (24.97)	0.13	0.4867
Exchangeable Mn^{2+} (mg/kg)	107.23 (22.96)	134.48 (22.28)	128.12 (10.20)	119.07 (14.94)	43.80 (8.96)	0.29	0.1288
*Exchangeable $Fe^{2+/3+}$ (mg/kg)	0.22 (0.05)^{b}	0.25 (0.05)^{b}	0.28 (0.05)^{b}	0.47 (0.08)^{ab}	0.64 (0.09)^{a}	−0.74	<0.0001
*Ca:Al	1.89 (0.53)	1.90 (0.56)	1.76 (0.41)	1.55 (0.43)	1.17 (0.29)	0.47	0.0094
*Sum of base cations (mg/kg)	1110.0 (139.16)	1106.4 (186.24)	1004.7 (144.36)	963.5 (148.67)	689.3 (96.54)	0.63	0.0003
Extractable P (mg/kg)	4.49 (0.65)	4.37 (0.38)	4.10 (0.31)	4.02 (0.41)	3.57 (0.78)	0.24	0.2052
Soil moisture (%)	53.84 (1.61)	53.49 (1.44)	52.16 (1.12)	50.89 (1.13)	51.55 (0.82)	0.35	0.0875
*Microbial biomass C (mg/kg)	229.65 (19.06)^{a}	173.17 (19.46)^{ab}	156.40 (14.61)^{b}	138.55 (3.57)^{b}	169.38 (14.51)^{ab}	0.49	0.0117
Microbial biomass N (mg/kg)	94.17 (15.70)	78.82 (11.19)	77.91 (12.22)	69.18 (6.11)	83.99 (10.32)	0.27	0.185
Microbial biomass C:N ratio	2.55 (0.22)	2.28 (0.16)	2.22 (0.30)	2.24 (0.24)	2.14 (0.35)	0.15	0.4500
Autotroph nitrifiers log (cells/g)	8.59 (0.22)	9.48 (0.37)	9.93 (0.73)	10.09 (0.80)	10.07 (1.10)	−0.06	0.7489
Heterotroph nitrifiers log (cells/g)	10.95 (0.42)	10.06 (0.60)	11.37 (0.49)	11.41 (0.36)	9.73 (0.61)	0.05	0.8103

*Properties that are significantly correlated to net nitrification according to Spearman's correlation (α<0.05); these terms were utilized to create the Stepwise regression model described. For each soil property, means followed by different letters are significantly different according to Tukey's HSD (at α=0.05). Soil mixture *H* 100% hardwood soils, *S* 100% spruce soils, *2–4* represent mixes of H and S

replicates; n=5 soil mixtures, n=2 landscape positions; total N=30).

Soil extractions using 2 M 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). Remaining soil mixtures were then incubated for 28 days in the dark at 24°C (Paavolainen et al. 1998).

After 28 days 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. 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 analysis and all further analyses were completed within 45 days of conclusion of the incubation period. Soil pH was measured from a 2:1 extraction of 0.01 M $CaCl_2$ (Hendershot et al. 1984) and $CaCl_2$ was used rather than water because the exchangeable properties of the soil were measured using salt solutions (Warby et al. 2007). Total N and C were analyzed on a CN elemental analyzer (Elementar VarioMax CNS, Hanau, Germany) (Pella and Colombo 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 deionized water (pH-adjusted to 3.8 with HCl).This analysis was completed approximately 3 weeks after the conclusion of the incubation period. 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 h 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 deionized 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™ 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 began 34 days following incubation and 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 min 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 biotic controls affecting NO_3-N production in the soil mixes (Schmidt et al. 2004), beginning 3 days following the incubation period. 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 (2009) MPN calculator; Build 23), based on the equation by Hurley and Roscoe (1983).

Microbial biomass C and N were determined 3 weeks following the incubation period 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 h. Equivalent soil samples were also placed in vacuum-sealed chambers for 24 h 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:

$$\mathrm{MBC} = \frac{\text{extractable C}_{\text{fumigated}} - \text{extractable C}_{\text{unfumigated}}}{0.35} \quad (1)$$

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:

$$\mathrm{MBN} = \frac{\text{extractable N}_{\text{fumigated}} - \text{extractable N}_{\text{unfumigated}}}{0.35} \quad (2)$$

Data analysis

Predicted values for net nitrification and mineralization were calculated as a weighted value based on the 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 Pare 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 α=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 in stepwise regression to select those properties most 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 because all significant variables 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-N production in the soil mixtures were interpreted as follows. If the inhibition of NO_3-N accumulation noted in the spruce soils is caused by persistent allelochemical 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-N following incubation of the soil mixtures, as demonstrated in a similar inoculation and incubation study of forest floor material (Ste-Marie and Pare 1999). If NO_3-N 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-N in the mixtures than hardwood soil alone. If NO_3-N production is related to the degree of suitability of substrate in these soils (i.e. C availability for microbial processing), no differences will be seen in observed versus predicted outcomes.

Results

Nitrogen fluxes

Observed values of net NO_3-N production in the five soil mixes did not differ significantly from predicted values after 28 days incubation ($p>0.05$; Fig. 2). Thus, the "No interaction" theoretical outcome was observed in the net production of NO_3-N in these soil mixtures in this experiment.

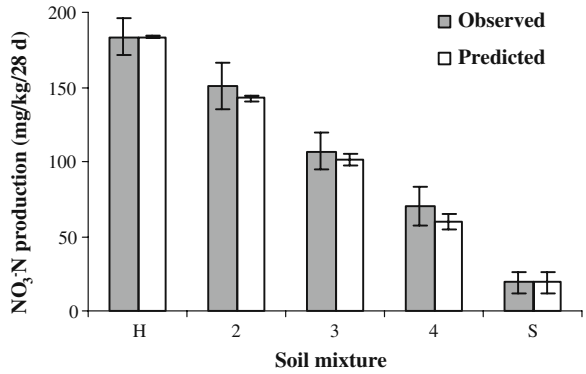

Fig. 2 Observed and predicted values of net nitrification after 28 days incubation in soil mixes. Error bars represent standard error of the mean. Soil mixture *H* 100% hardwood soils, *S* 100% spruce soils, *2–4* represent mixes of H and S

Net NO_3-N production was highest in 100% hardwood soils (mean = 183.51 mg N/kg/28 days), and declined linearly to lowest production in 100% spruce soils (mean = 18.97 mg N/kg/28 days) (Fig. 3a). 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. 3b). 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. 3c). However, total N mineralization was approximately 40% lower in the spruce soil than the hardwood soil (Fig. 3c; 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 relationships between each measured soil chemical and biotic property and net NO_3-N production are listed in Table 1, with associated *p*-values. Twelve 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 all soil properties measured are also shown in Table 1, with 12 soil properties that were significantly corre-

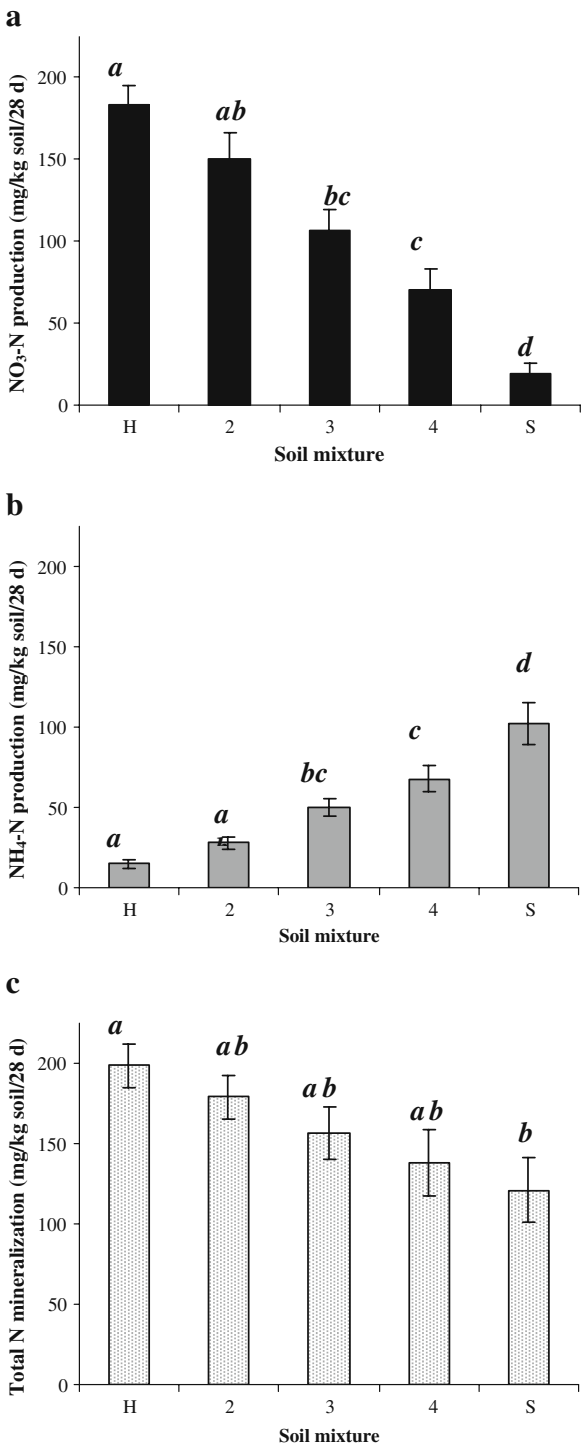

Fig. 3 **a** Net nitrification, **b** net ammonification and **c** total net N mineralization in soil mixtures after 28 days incubation. For each N compound, soil mixtures with different letters represent means significantly different ($\alpha<0.05$), according to Tukey's HSD. Error bars represent standard error of the mean. Soil mixture *H* 100% hardwood soils, *S* 100% spruce soils, *2–4* represent mixes of H and S

lated to net NO_3-N production highlighted. 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 ($p=0.0039$) (Table 1). 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; $p=0.0250$) (Table 1). 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; Table 1). 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 ($p=0.0698$; Table 1). The sum of bases was nearly twice as high in the hardwood soils (mean 1,110.04 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) (Table 1). 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. 4; soil mixtures 2–4 and S), with NO_3-N only slightly and insignificantly higher 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. 4).

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), a predictive model was developed using stepwise regression. Of the twelve terms introduced, four terms remained in the model (R^2 model value of 0.9458; $P<0.0001$) (Fig. 5). 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 the incubated soil mixtures:

$$\begin{aligned}\text{Net NO}_3\text{ – N production(mg/kg/28 d)} &= -91.08 - 6.63(\textit{soil C : N}) \\ &\quad - 26.41(\textit{log sulfate}\ \text{mg/kg}) \\ &\quad + 186.31(\textit{log total soil N}\ \text{g/kg}) \\ &\quad + 89.66(\textit{oxalate}\ \text{mg/kg}) \qquad (3)\end{aligned}$$

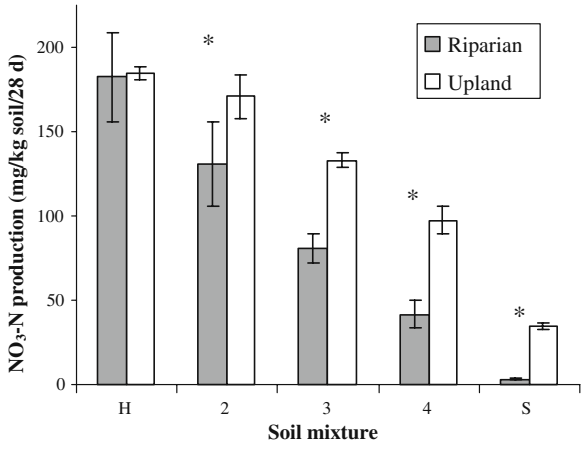

Fig. 4 Effect of landscape position on net nitrification within each soil mixture. Error bars represent standard error of the mean. Soil mixture *H* 100% hardwood soils, *S* 100% spruce soils, *2–4* represent mixes of H and S. *Asterisks* represent significantly different means by landscape position for each soil mixture according to Tukey's HSD ($\alpha<0.05$)

Differences between landscape positions also occurred in soil properties that remained in the stepwise regression model (Eq. 3). 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).

Using the variables that were significantly correlated to NH_4-N production from all soil mixtures, a predictive model was created using stepwise regression. Of the twelve terms introduced, three terms remained in the model (R^2 model value of 0.8204; $p<0.0001$). These terms included 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 the incubated soil mixtures:

$$\begin{aligned}\text{Net NH}_4\text{ – N production(mg/kg/28 d)} &= 196.59 - 207.83(\log \textit{total soil N}\ \text{g/kg}) \\ &\quad + 1.81(\textit{total soil C}\ \text{g/kg}) \\ &\quad + 0.59\left(\textit{exchangeable}\ Mg^{2+}\ \text{mg/kg}\right) \qquad (4)\end{aligned}$$

Using the variables that were significantly correlated to total net N mineralization from all soil mixtures, a predictive model was created using stepwise regression. Of the twelve terms introduced, four terms remained in the model (R^2 model value of 0.92; $p<0.0001$). These terms included 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 $R^2=0.1112$;

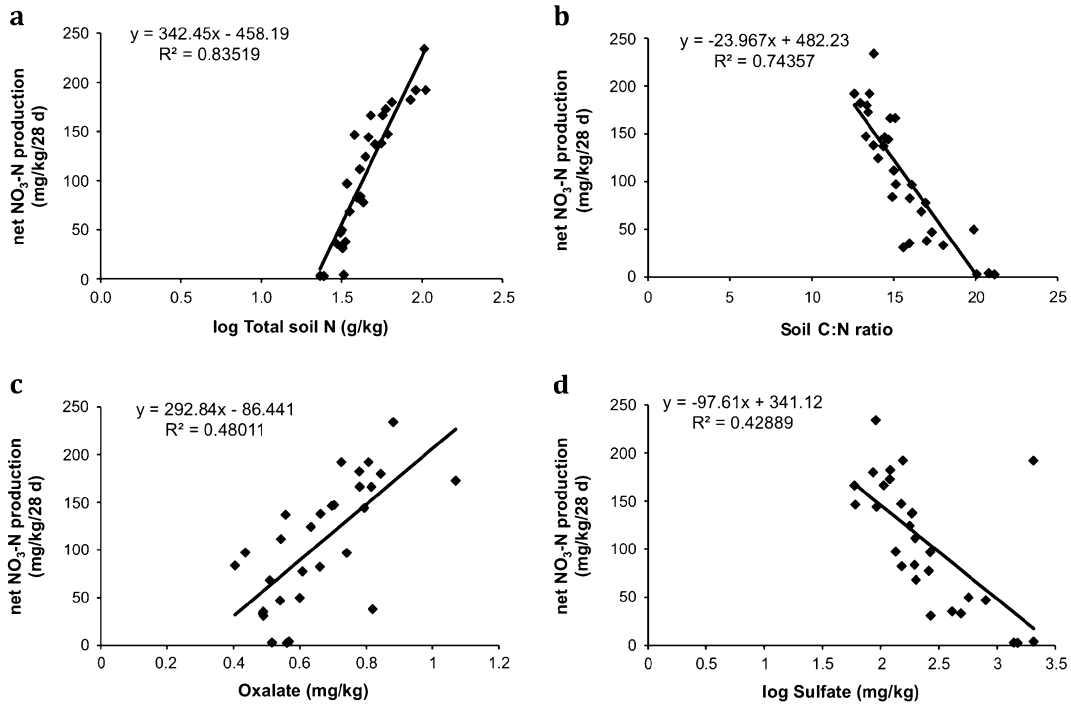

Fig. 5 Net nitrification after 28 days incubation from all soil mixtures, as related to those soil parameters included in the final predictive model **a** total soil N, **b** soil C:N ratio, **c** soil oxalate concentration, and **d** soil sulfate concentration

p=0.0717). The resultant model can be used to predict total net N mineralization within the incubated soil mixtures:

$$\begin{aligned}&\text{Net N mineralization}(\text{mg/kg/28 d})\\&\quad = 67.06 + 92.45(\textit{total soil N}\ \text{g/kg})\\&\qquad - 10.87(\textit{soil C : N ratio})\\&\qquad + 0.51\left(\textit{exchangeable}\,Mg^{2+}\ \text{mg/kg}\right)\\&\qquad + 0.15\left(\textit{exchangeable}\,Al^{3+}\ \text{mg/kg}\right)\end{aligned} \tag{5}$$

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. 3a). There were no significant differences between observed and predicted values of net NO_3-N production in any soil mixture (Fig. 2). This suggests that no biological interaction occurred between the two soil types upon mixing, and the observed pattern of NO_3-N production was assumed to be present as a result of the unsuitability of the spruce soil to provide favorable substrate for use by introduced soil microbes. No evidence of persistent allelochemical 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 Pare 1999).

It is possible that the design of this experiment, which entails mixing soils developed beneath different vegetation, may not provide physical opportunities for all soil microsites to interact chemically or biologically. However, sieving soils through 2 mm mesh likely broke apart large soil aggregates and provided sufficient newly exposed soil surfaces that would allow for a large degree of biological interaction upon mixing of the soil types, if it were to occur. In addition, a previous soil incubation study demonstrated that sufficient interaction occurred in mixtures of sieved mineral soil and organic matter fractions to alter N mineralization in a variety of soil types (Whalen et al. 2000).

Lower production of NO_3-N exhibited by spruce soil could result from 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, 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, 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 hypothesis is supported by Gilliam et al. (2001), who concluded that very low nitrification at some sites in a reference watershed at the FEF (WS4) was probably a result of high levels of available Al^{3+} and low Ca^{2+} concentrations in the soil, and not the lack of NH_4^+ availability. The authors attributed this to the presence 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 by increasing soil acidity and by incorporating N compounds into organic complexes that are then unavailable for biotic processing (Straker 1996; Read and Perez-Moreno 2003). In addition to the influence of species, across several watersheds in their study, Gilliam et al. (2001) reported net nitrification was most strongly correlated to soil moisture and total soil N content.

Influence of soil chemical properties

Total N was significantly lower in the spruce soils, positively correlated to NO_3-N production, and also positively correlated to total net N mineralization. A 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). Assumed mass losses of soil C and N upon conversion to the conifer plantation following the hardwood harvest that occurred from 1967 to 1969 may explain some of the differences in measures of soil N, though soil C and N data prior to conversion at these sites are not available. 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% decrease in soil C was documented when native broadleaf forests were converted to conifer plantations, whereas no changes in soil C and N were observed in plots that recovered to native broadleaf forest following harvest. This loss of soil C and N associated with conversion to conifers may be attributed to (1) disturbance, (2) changes in amount and composition of plant material returned to the soil via litter and root processes (Lugo and Brown 1993), and (3) 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 (Chapela et al. 2001). Using stable C isotopic tools and radiocarbon dating of fungal tissue, the authors demonstrated that ectomycorrhizal fungi can utilize stabilized soil C stores as an energy source (Chapela et al. 2001).

Soil C:N ratio was significantly greater in the spruce soil relative to the hardwood soil, and this term was negatively correlated to NO_3-N production in these soil mixtures. 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, and this term was negatively correlated to NO_3-N production. The hydrophobic fraction consists of slowly degrading compounds of humic substances, humic and fulvic acids, tannins, and phenols. 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). It has previously been demonstrated that higher hydrophobicity of organic material leads to lower microbial mineralization and respiration rates in incubated soil columns (Spaccini 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). The authors concluded that lignin:N ratios were the best predictor of wood decay rates in their study. Norway spruce litter also contains significantly higher concentrations of lignin and cellulose and lower concentrations of water-soluble compounds (rhamnan and xylan) than white birch litter (*B. pubescens*), where cellulose content was 28.8% in spruce and 21.3% in birch litter and water-soluble compounds comprised 13.2% in spruce and 23.1% in birch litter (Johansson 1995). 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 (Eq. 3).

Surprisingly, only one organic acid compound, oxalate, was significantly correlated with NO_3-N production in these soil mixtures, and oxalate concentrations were significantly greater in hardwood soil. Acetate has been previously shown to be an inhibitor of nitrification (De Boer 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 tissue oxalate concentrations were shown to occur in association with higher soil NH_4^+ content. High soil NH_4^+ can induce greater NH_4^+ uptake 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 1998; Ma 2000; Pineros 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).

Sulfate concentrations were significantly higher in spruce soil and SO_4^{2-} was negatively correlated to NO_3-N production in these soil mixtures. Sulfur (in the form of sulfide) has been shown to inhibit nitrification (Joye and Hollibaugh 1995) by effectively competing for oxygen in oxygen-limited environments. However, soils in this incubation were well aerated and nitrification inhibition by sulfur probably did not occur. Nitrification can increase 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, 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).

Influence of soil biotic properties

Soil microbial biomass C (MBC) was the only measured biotic property that was significantly correlated with NO_3-N production in these soil mixtures. Soil MBC tended to be greater in the hardwood soil 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 on soil organic matter composition (Zak et al. 1990) because

soil microorganisms generally are C limited (Anderson and 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 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 and the MPN values for these populations were not correlated to NO_3-N production. The methods used in this study to culture and quantify nitrifying bacteria may not allow for enumeration of the entire population of such bacteria in the soils, as not all bacteria can be easily cultured. However, this technique yields an index of relative population size of bacteria capable of nitrification (Papen and von Berg 1998). 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 conclusions that C compounds within the spruce soil are unsuitable for nitrifying microbes, and persistent allelochemical 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. This is somewhat unexpected because seasonal in situ net nitrification measurements exhibited no difference between 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). However, other studies have demonstrated riparian soils to be strong transformers of or sinks for NO_3-N in the field (Cooper 1990; Hill 1996). 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. It may be possible that slightly higher soil moisture in the upland soil incubations stimulated higher net nitrification, because mean soil moisture in upland and riparian soil incubations was 54.2% and 50.6%, respectively.

Laboratory incubations like the one presented in this study 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). The differences in net nitrification patterns between our incubated soils and field measurements in the same watersheds may be a function of the absence of plant uptake and the presence of constant soil moisture within the incubated soils.

Conclusions

This study documented relatively high net nitrification in soils collected beneath native hardwoods compared to soils occurring in a similar site converted to Norway spruce, and we conclude that soil substrate properties resulting from spruce vegetation led to decreased net nitrification in these soils. Differences in net nitrification could not be attributed to either processes of abiotic retention of N or allelochemical inhibition of biotic activity. 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. It cannot be determined from the data reported here if these properties 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 soil organic matter dynamics, thus leading to low net nitrification. These results are useful for management activities, including forest tree 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 total soil C and N storage and biogeochemistry following vegetation conversion. Additional studies should include efforts to isolate soil properties that strongly alter nitrification processes.

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