Linking microbial communities, functional genes and nitrogen-cycling processes in forest floors under four tree species

Ribbons, Relena; Levy-Booth, David J.; Masse, Jacynthe; Grayston, Sue J.; McDonald, Morag; Vesterdal, Lars; Prescott, Cindy E.

Soil Biology and Biochemistry

DOI:

[10.1016/j.soilbio.2016.07.024](https://doi.org/10.1016/j.soilbio.2016.07.024)

Published: 01/12/2016

Peer reviewed version

[Cyswllt i'r cyhoeddiad / Link to publication](#)

Dyfyniad o'r fersiwn a gyhoeddwyd / Citation for published version (APA):

Ribbons, R., Levy-Booth, D. J., Masse, J., Grayston, S. J., McDonald, M., Vesterdal, L., & Prescott, C. E. (2016). Linking microbial communities, functional genes and nitrogen-cycling processes in forest floors under four tree species. *Soil Biology and Biochemistry*, 103(December), 181-191. <https://doi.org/10.1016/j.soilbio.2016.07.024>

Hawliau Cyffredinol / General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal ?

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Linking microbial communities, functional genes and nitrogen-cycling processes in forest floors under four tree species

Authors: Relena R. Ribbons^{123*}, David J. Levy-Booth⁴, Jacynthe Masse¹, Sue J. Grayston¹, Morag A. McDonald², Lars Vesterdal³, and Cindy E. Prescott¹

Affiliations:

¹Department of Forest and Conservation Sciences, Faculty of Forestry, University of British Columbia-Vancouver, Forest Sciences Centre 3006, 2424 Main Mall, Vancouver, British Columbia, V6T 1Z4 Canada; ²School of Environment, Natural Resources, and Geography, Bangor University, Thoday Building, Bangor, LL57 2UW, Wales, United Kingdom; ³Department of Geosciences and Natural Resource Management, University of Copenhagen, Rolighedsvej 23, DK-1958 Frederiksberg C, Denmark; ⁴Department of Microbiology and Immunology, University of British Columbia, Vancouver, British Columbia V6T 1Z4, Canada

Author for correspondence:

Relena R. Ribbons

Tel: +45 01248 382450

Email: rribbons@gmail.com

Abstract

Tree species can influence rates of soil N transformations, but the question remains whether differences in N cycling rates are mirrored by the abundance of relevant functional genes. We studied whether the influence of tree species on soil N transformation processes and abundance of functional genes exist across two sites in British Columbia with different N availability. We used the ^{15}N pool-dilution method to estimate gross rates of ammonification and nitrification in forest floors of four conifers in a common garden experiment. The abundances of bacteria, fungi, nitrification (AOA *amoA*, AOB *amoA*) and denitrification (*nirS*, *nirK*) genes were determined by qPCR. Western red cedar (*Thuja plicata*) had the highest rates of gross ammonification and NH_4^+ consumption, followed by Sitka spruce (*Picea sitchensis*), hemlock (*Tsuga heterophylla*), and Douglas-fir (*Pseudotsuga menziesii*); all species showed net nitrate immobilization. Western red cedar forest floors had the greatest abundance of bacterial *16S* genes and ammonia-oxidizing archaea *amoA* genes. This suggests that tree species foster different abundances of ammonification and denitrification functional groups. Differences in N transformation rates between the sites were related to site N status, as reflected in C:N ratios of the forest floor and microbial biomass, and were more closely tied to rates of N consumption rather than gross mineralization. Rates of most N transformation processes were related to microbial C:N ratio, indicating that the N status of microbes rather than their biomass or activity level determined the rates of N cycling. Ammonification rates were associated with forest floor and microbial biomass C:N ratio as well as bacterial and fungal abundances. Nitrification rates and denitrification gene abundance were associated with microbial biomass C:N ratios and AOA *amoA* gene abundance. The forest floor's genetic potential for denitrification was positively correlated with its nitrification potential as indicated by ammonia-oxidizer abundance. We conclude that tree species influenced forest floor N cycling and soil microbial gene abundances, and that functional genetics can be useful for exploring mechanistic links between tree species and nitrogen cycling processes.

Keywords: Ammonia oxidizers, nitrifiers, ^{15}N pool dilution, tree species effects, nitrogen cycling, forest floors

1. Introduction

Nitrogen (N) availability is often the primary limitation on ecosystem productivity in conifer forests (Vitousek et al., 1997, 2002; LeBauer and Treseder, 2008), and factors that regulate N availability have far-reaching consequences for ecosystem properties such as microbial activity, biogeochemical cycling, carbon (C) sequestration, aboveground biomass production and greenhouse gas (GHG) fluxes. Soil N transformation processes (e.g., mineralization of organic N to NH_4^+ and nitrification of NH_4^+ to NO_3^-) are largely mediated by interactions between functional communities of soil microorganisms and their environment (Bengtsson et al., 2003; Balser and Firestone, 2005; Högberg et al., 2013). The balance between gross and net rates of mineralization and nitrification can indicate turnover and immobilization rates of NH_4^+ and NO_3^- , respectively, which can be rapid in forest soil (Davidson et al., 1992; Stark and Hart, 1997). Rates of mineralization and nitrification have further implications for N loss through leaching of NO_3^- and denitrification to N_2O (Bengtsson et al., 2003; Szukics et al., 2010).

Quantification of marker genes for biogeochemical functions can be used to characterize N cycling processes and communities (Levy-Booth et al., 2014). Ammonia-oxidizing archaea (AOA) and bacteria (AOB) carry out the first, and potentially rate-limiting, step of nitrification. AOA and AOB abundance can be quantified using the ammonia-monooxygenase (*amoA*) gene (Rotthauwe et al., 1997; Francis et al., 2005; Leininger et al., 2006; Szukics et al., 2010). While AOB have long been thought to dominate ammonia-oxidation, the quantitative importance of AOA in soil has recently been recognized (Leininger et al., 2006; Petersen et al., 2012), and AOA are generally more abundant than AOB in acidic forest soil (Petersen et al., 2012). The relative contributions of AOA and AOB to gross and net nitrification in forests with different tree species remain to be seen. Denitrification is a biological pathway through which N returns to the atmosphere from soil or water by the reduction of nitrate to nitrous oxide (Henry et al., 2004) via the nitrite reductase enzyme genes *nirS* and *nirK*. Genes that encode enzymes involved in biogeochemical cycling can be used to compare measured N mineralization and nitrification rates with bacterial, fungal, nitrifier and denitrifier population sizes (inferred from gene abundances) and in so doing, link N-cycling pathways to functional microbial groups.

Tree species have been shown to influence chemical and biological properties of soil, particularly forest floor layers, and rates of processes therein. Tree species influence soils

directly via leaf litter inputs and formation of forest floors (Hobbie et al., 2006; Vesterdal et al., 2012), and via root litter inputs and alteration of soil structure. Tree species also influence rates of litter decomposition, nutrient release, C turnover and soil respiration through differences in foliar N, Ca, Mg and lignin concentrations (Hobbie et al., 2006; Vesterdal et al., 2012, 2013). Several studies have reported differences in rates of N-cycling processes in soils under different tree species (e.g., Ste-Marie and Paré, 1999; Malchair and Carnol, 2009; Christiansen et al., 2010), and distinct microbial communities have also been reported in soils and forest floors under different tree species (Leckie et al., 2005; Prescott and Grayston, 2014). However, despite decades of research on this question, categorization of species according to their influence of soil properties has proven elusive, as tree species effects have been inconsistent among studies, and even at different sites within a single study. For example, in a common garden experiment on Vancouver Island, the indirect influence of site factors (particularly slope position) on rates of net N mineralization and nitrification in the forest floors appeared to overwhelm the influence of tree species, with appreciable net rates detected only at the valley bottom sites where the forest floor C:N ratio was less than 35 (Prescott et al., 2000a). Likewise, Gurmesa et al. (2013) found an influence of broadleaved tree species on soil carbon only at relatively rich sites, which they attributed to the lack of earthworms at the infertile sites, regardless of tree species. These observations prompted Prescott and Vesterdal (2013) to propose that the expression of tree species influence on soils is context-dependent, and more likely to be detectable on rich or intermediate sites.

In the common garden experiment on Vancouver Island, previous studies of the forest floors discerned some distinct characteristics of the forest floors under four coniferous tree species, which have been summarized by Prescott and Vesterdal (2005). Western red cedar forest floors had the lowest rates of litter decomposition and net N mineralization, but the highest proportion of nitrate and the highest bacterial:fungal ratio of the four species. Western hemlock forest floors had low pH and low Ca concentrations, low bacteria:fungal ratios, and were dominated by NH_4^+ rather than nitrate. Sitka spruce forest floors had intermediate to high concentrations of N, P, Ca, and K, low bacterial:fungal ratios, and moderate rates of net N mineralization. Finally, Douglas-fir forest floors had intermediate pH and Ca, high bacterial:fungal ratios, high N concentrations and rates of net N mineralization and nitrification.

In this study, we revisit the common garden experiment on Vancouver Island and compare the forest floors that have developed under four tree species at one nutrient-rich, valley-bottom site and one nutrient-poor, mid-slope site. We examine differences in forest floors among tree species and the extent to which these differ according to the site. We measure rates of N mineralization and nitrification using ^{15}N pool-dilution and apply quantitative PCR of microbial genes involved in N-cycling processes to explore interactions among microbial communities (nitrifiers and denitrifiers) and rates of N-cycling processes.

2. Materials and Methods

2.1 Study location

We sampled two sites, as case studies rather than replicates, from the EP571 common garden experiment on Vancouver Island: San Juan ($48^{\circ}35'\text{N}$, $124^{\circ}12'\text{W}$) and Fairy Lake ($48^{\circ}35'\text{N}$, $124^{\circ}19'\text{W}$), both located near Port Renfrew. The two sites were comparable in terms of elevation (65-85 and 75-85 m, respectively), and were within the Sub-montane Very Wet Maritime Coastal Western Hemlock variant (Prescott et al., 2000a). San Juan is a valley-bottom site with understory largely composed of *Rubus spectabilis* Pursh (salmonberry) and *Polystichum munitum* (Kaulf.) Presl. (swordfern), while Fairy Lake is a mid-slope site with understory dominated by *Gaultheria shallon* Pursh (salal) and *Vaccinium parvifolium* Smith (red huckleberry). Significantly higher N mineralization and nitrification rates and concentrations of P and K were measured in forest floors at the San Juan site compared with the Fairy Lake site (Prescott et al. 2000a), which were related to the differences in slope position and understory vegetation. We selected these two study sites because of their contrast in terms of N cycling, to enable us to test whether species effects on gross N cycling and functional genes would be consistent across contrasting sites.

Replicate plots of each species (western red cedar, western hemlock, Douglas-fir, and Sitka spruce) were planted in 1961, as a part of Experimental Project No. 571. The previous forest cover of western hemlock, western red cedar, amabilis fir, and Sitka spruce had been clear-cut and slash burned. The full experimental design contained 24 0.07-ha plots at each site, with 81 tree seedlings planted in three densities (2.7, 3.7, and 4.7 m); for the current study we sampled the two densest (2.7-m spacing) plots of each species. We used this pre-existing experimental design to determine if tree species differ in their dominant nutrient cycling characteristics

between N-rich and N-poor sites.

2.2. Soil sampling and soil physical and chemical analyses

We focused on the F-layer of the forest floor. This is the layer in which the greatest differences in soil microbial communities of these tree species have previously been found (Grayston and Prescott, 2005), and where soil fungi and fauna are expected to be most abundant and active (Kurbatova et al., 2009). It also ensured that we were comparing the influence of tree species on microbial communities at the same stage of decay, as recommended by Prescott and Grayston (2013).

Composite samples were collected from nine randomly selected 450 cm² samples of the F-layer in each plot in June of 2014. Brockett et al. (2012) showed that composite samples provide similar results to individually analyzed samples for plot-level values, albeit at a loss of information regarding spatial heterogeneity. Each composite forest floor sample was passed through a 2-mm mesh sieve and one 15-g subsample was immediately removed for the ¹⁵N pool-dilution analysis. The remaining bulk sample was stored at -20° C for DNA extraction. For pH analysis, a 5-g field-moist subsample of forest floor was added to a small jar with 20 mL distilled water, shaken for 30 minutes then measured with a pH meter. A subsample of field-moist forest floor was also removed for C and N analysis. These samples were oven dried to 70°C for 48 h, ground using a mortar and pestle, and 5 mg weighed into tin capsules and analyzed for C and N by high-temperature flash combustion using a Vario EL Cube elemental analyzer (Elementar Americas Inc., Mount Laurel, NJ).

2.3 Gross rates of ammonification and nitrification

The ¹⁵N pool-dilution method (modified from Drury, 2008) was used to determine gross rates of ammonification and nitrification, with samples analyzed in triplicate. Six 15 g subsamples from each plot were passed through a 2-mm mesh sieve and transferred to 500 mL glass Mason jars and sealed with parafilm (n = 3 subsamples for both ¹⁵NH₄⁺ and ¹⁵NO₃⁻). The parafilm seal was punctured to enable gas exchange and maintain aerobic conditions. Samples were incubated in the dark at room temperature for 24 hours prior to initial ¹⁵N treatments. These treatments consisted of either: 4 mL of ¹⁵NH₄Cl solution (99 atom%; Cambridge Isotope Laboratories) or 4 mL of K¹⁵NO₃ (99 atom%; Cambridge Isotope Laboratories) added to the

forest floor samples in each respective jar, which was an equivalent application rate of 12 $\mu\text{g N g}^{-1}$ forest floor. Labeled N was injected into the samples in 1 mL intervals four times over one minute, and gently homogenized to ensure isotopic labeled N was applied uniformly throughout the forest floor sample, and the parafilm seal was replaced.

i) Immediately after ^{15}N addition and ii) 24 hours after ^{15}N addition, a 5-g forest floor subsample was removed from each Mason jar, this subsample was added to 100 mL of 2.0 M KCl, shaken for 1 hour, and filtered through glass fiber filter paper (Fisher Inc.). 10 mL of the KCl extract were analyzed for $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ using a flow injection analyzer (Lachat; Quickchem 8000) at the Environmental Engineering Department at the University of British Columbia. The remainders of the extracts were used for microdiffusion of $^{15}\text{NH}_4\text{-N}$ and $^{15}\text{NO}_3\text{-N}$ with the use of acid traps, and the sequential addition of MgO and Devarda's alloy according to the protocol developed by the International Atomic Energy Agency (IAEA, 2001). Acid traps were dried, packaged in tin cups, and sent to the University of Saskatchewan Isotope Laboratory for stable isotope ratio analysis using a Costech ECS4010 elemental analyzer coupled to a Delta V mass spectrometer with a Conflo IV interface. Net rates of ammonification were calculated as the difference in NH_4^+ between the incubated samples and the initial soil extractions at the start of the incubation. Net nitrification was calculated as the difference in NO_3^- between the initial and incubated samples. The gross rates of ammonification, nitrification and microbial consumption were calculated following Hart et al. (1994).

2.4 Microbial biomass determination

Microbial biomass nitrogen (MBN) and microbial biomass carbon (MBC) were determined using a modified chloroform-fumigation extraction (Brookes et al., 1985). Briefly, 100 mL of 2 M KCl was used in lieu of 40 mL of 0.5 M K_2SO_4 for extractions (Verchot et al., 1999). 20 mL extracts were analyzed for total organic C (TOC) and total N (TN) on a Shimadzu 5000A TOC analyzer at the Analytical Services Laboratory of the University of Alberta. The additional remaining extractant was used for acid diffusion traps, identical to the above protocol for the ^{15}N pool-dilution method. To determine if microbial communities were immobilizing N during the course of the incubation we assessed MBN and MBC in $^{15}\text{N-NH}_4$ and $^{15}\text{N-NO}_3$ added samples at the end of the pool-dilution experiment, as well as from unfertilized soil samples.

2.5 DNA isolation and quantitative PCR DNA was extracted from 0.1 g of field-moist forest floor using the MoBio Power Soil DNA isolation kit (MoBio Laboratories, Inc., Carlsbad, CA, USA). DNA quality and concentration was measured using a nanodrop spectrophotometer (Thermo Fisher Scientific Inc., Wilmington, DE, USA) and electrophoresis in agarose gels (1% w/v in TAE), then stored at -20°C prior to amplification.

Quantitative PCR (qPCR) was used to quantify gene copy numbers. Reactions were carried out with an Applied Biosystems® StepOnePlus™ real-time PCR system. Each 20 µl reaction contained 10 µl of SybrGreen (2x) PCR Master Mix (Life Technologies Corp., Carlsbad, CA, USA), 0.5 µl of each primer, 250 ng µl⁻¹ bovine serum albumin (BSA), and 1 µl of DNA template. Table 1 shows primer sequences for qPCR assays. PCR conditions for AOA *amoA*, AOB *amoA* were 10 min at 95°C, followed by 40 cycles of 95°C for 30 s, 30 s at 57°C, and 72°C for 1 min, and 10 s at 80°C. Triplicate 10x standard curves ranged from 10² to 10⁷ copy numbers of AOA and AOB *amoA* in linearized plasmids. PCR conditions for *nirK* and *nirS* were 10 min at 95 °C and 40 cycles of 95 °C for 1 min, 60 °C for 1 min and 72 °C for 1 min, with fluorescence quantified at extension (Levy-Booth and Winder, 2010). The standard curves for *nirS* and *nirK* used a triplicate 10-fold serial dilutions of 10¹ to 10⁷ gene copies from *Pseudomonas aeruginosa* (ATCC 47085) and *Pseudomonas chlororaphis* (ATCC 13985) genomic DNA, respectively. Bacterial 16S rRNA PCR conditions were 5 min at 95°C, followed by 40 cycles of 95°C for 30 s, 30 s at 57°C, and 72°C for 1 min, and 10 s at 80°C. Florescence quantification occurred during annealing. Triplicate standard curves were run using a 10x dilution of 10² to 10⁷ amplified 16S rRNA in linearized plasmids. PCR conditions for fungal ITS were 10 min at 95°C, followed by 40 cycles of 95°C for 1 min, 30 s at 53°C, 50 s at 72°C and 10 s at 80°C. Fluorescence was read at 80°C to reduce the formation of non-target and primer self-complementation structures. Triplicate standard curves for fungal ITS quantification were constructed using 10x dilutions between 10³ to 10⁹ ITS copies amplified from soil and *Aspergillus citrisporus* genomic DNA. Standard curve ranges are indicative of lower and upper limits of detection, respectively. All qPCR analyses were run in duplicate.

2.6 Statistical analyses

At each site one composite forest floor sample from the two replicate plots for each tree species was sampled (n=2). The influences of tree species and sites on forest floor chemistry,

microbial biomass C and N, process rates and gene abundances were evaluated using two-way permutational analysis of variance (with 999 permutations). Permutational analysis of variance was used to address the low sample sizes in this study (Andersen and Legendre, 1999) with tree species and site as fixed effects. Microbial gene abundances were $\log_{10}$ transformed prior to two-way permutational analysis of variance (with 999 permutations). We used multiple linear regressions to determine which environmental variables were the best predictors of N transformations on standardized data (*decostand* function in the *vegan* package in R (Oksanen et al., 2013)). Rates of N transformations, including gross and net ammonification and nitrification, were related to all potentially regulating parameters using multiple linear regressions, following the removal of collinear variables. Model selection was based on stepwise variable selection with Akaike's Information Criterion (AIC), whereby the lowest value indicates the model with the highest explanatory power.

Redundancy analysis (RDA), a form of constrained ordination that determines how much of the variation from one set of variables explains the variation in another set of variables, was performed to test relationships between site and forest floor parameters and gene abundances (*vegan* package in R). Forward selection of explanatory variables for RDA was carried out using the *packfor* function using the method recommended by Blanchet et al. (2008). The significance of the RDA model and its individual terms were calculated using Monte-Carlo tests with 10000 permutations. All analyses were conducted with R v. 2.15.3 (R Core Team, 2013).

3. Results

3.1 Forest floor pH, C and N

There were no significant differences in pH, although Sitka spruce and western red cedar had the highest average pH values (Table 2). Concentrations of total C and N also did not significantly differ between forest floors under the four tree species or at the two sites (Table 3). However, forest floor C:N ratio was significantly lower ($p = 0.02$) at the San Juan (nutrient-rich) site (36.5 ± 1.1) than at Fairy Lake (nutrient-poor) (42.2 ± 1.5). C:N ratio also differed among tree species, with hemlock forest floors having significantly higher C:N ratio (43.3 ± 2.3) than Sitka spruce (36.4 ± 2.1 ; $p = 0.018$) and Douglas-fir (36.6 ± 1.2 ; $p = 0.015$). There were no significant species-by-site interaction effects, indicating consistent tree species effects on C:N ratios across sites.

3.2 Microbial biomass C and N

Microbial biomass C (MBC) differed among tree species across both sites and was significantly lower in forest floors at San Juan than Fairy Lake (Figure 1, Table 3). Significant species-by-site interaction indicated that influences of tree species on MBC differed between the two sites ($p = 0.03$). At Fairy Lake, Sitka spruce had lower MBC than other species whereas cedar had lower MBC than other tree species at San Juan (Figure 1). Microbial biomass N (MBN) did not differ significantly between sites ($p=0.89$) or among tree species across sites (Table 3, $p = 0.39$), but at San Juan, Douglas-fir had higher MBN than other species (Figure 1). Microbial C:N ratios did not differ significantly between sites or tree species, although there was a tendency for lower microbial C:N at San Juan (Figure 1, $p = 0.10$).

3.3 Microbial gene abundance

Bacteria 16S rRNA gene abundance was significantly greater in forest floors from San Juan than from Fairy Lake (Table 3, $p = 0.01$), and differed significantly among tree species (Table 3, $p = 0.05$) with no species-by-site interaction. Bacterial genes were more abundant in forest floors of western red cedar at both sites, with hemlock or spruce having the lowest abundance depending on the site (Figure 2). Fungal ITS abundance did not differ between sites ($p = 0.55$) or tree species ($p = 0.89$). AOA *amoA* had the most pronounced site effect of any of the microbial genes quantified in this study, with San Juan having several orders of magnitude more AOA *amoA* genes than Fairy Lake ($p = 0.01$), but no species effects. AOB *amoA* genes were more abundant at San Juan than at Fairy Lake, but within the same order of magnitude ($p=0.10$). The abundance of the Cu-nitrite reductase (*nirK*) gene did not differ by site ($p = 0.19$), or tree species ($p = 0.13$), although hemlock tended to have the lowest gene abundance. In contrast, Cd-nitrite reductase (*nirS*) genes were significantly more abundant in forest floor at San Juan than at Fairy Lake ($p = 0.02$), but did not differ by tree species ($p = 0.27$).

3.4 Gross and net N ammonification and nitrification rates

Gross ammonification rates were significantly higher in forest floors at San Juan than at Fairy Lake (Figure 3a), with mean rates of 32.9 and 19.4 mg N g⁻¹ soil (dw) day⁻¹, respectively. A highly significant trees species effect on gross ammonification was observed with gross

ammonification under western red cedar being significantly greater than under Douglas-fir ($p = 0.03$), hemlock ($p = 0.006$) and Sitka spruce ($p = 0.03$). Gross ammonium (NH_4^+) consumption was significantly higher in forest floors from San Juan than Fairy Lake ($p = 0.002$), and was significantly greater in forest floors under cedar than the other species (Figure 3b). Net ammonification rates were mostly negative, indicating net immobilization of NH_4^+ , with occasional positive mineralization occurring only in Sitka spruce at San Juan (Figure 3c). San Juan forest floors had higher (i.e. less negative) rates of net ammonification than Fairy Lake forest floors ($p = 0.01$), and net ammonification rates differed among tree species ($p = 0.05$).

Rates of gross nitrification, nitrate consumption and net nitrification did not differ between species, but did differ between sites with slightly more N transformed at Fairy Lake than San Juan ($p < 0.01$). At Fairy Lake, western red cedar had the highest rates of gross nitrification, which significantly differed from hemlock and Sitka spruce, but not from Douglas-fir. Douglas-fir and Sitka spruce had the highest and similar rates of nitrate consumption, followed by western red cedar, spruce, and hemlock at Fairy Lake. At San Juan, Sitka spruce had the highest rates of gross nitrification. Douglas-fir, western red cedar, and hemlock all had negligible rates of gross nitrification and nitrate consumption. Net nitrification rates were negative, indicating net nitrate immobilization in all plots except Sitka spruce at San Juan (Figure 3f).

3.5 Relationships between N ammonification and nitrification rates and microbial parameters

In general, we had greater explanatory power for ammonification rates than nitrification rates (Table 4). Gross ammonification was best explained by the total number of bacterial *16S* rRNA and fungal *ITS* genes. Gross NH_4^+ consumption was best explained by gross ammonification, pH, forest floor C:N ratio, and microbial biomass C:N ratio. Net ammonification was best explained by forest floor pH and C:N ratios and microbial biomass C:N ratios. Gross nitrification was best explained by microbial biomass C:N ratios and AOA *amoA* gene. Gross NO_3^- consumption was best explained by net ammonification, gross nitrification, microbial biomass C:N ratios, bacterial *16S* and AOA and AOB *amoA* genes. Net nitrification rates were best explained by net ammonification, microbial biomass C:N ratios, bacterial *16S*, and AOA and AOB *amoA* genes. The sum of AOA and AOB gene abundances showed a strong, positive relationship with the sum of the abundance of nitrite reductase genes (*nirK* and *nirS*)

(Figure 4). Redundancy analysis showed clear separation of the two sites largely due to differences in forest floor C:N ratio and pH, but no clear grouping of microbial abundance patterns according to tree species (Figure 5). Fairy Lake was associated with high C:N ratio, and San Juan with higher microbial gene abundance for AOA and AOB *amoA*, *nirK*, *nirS*, and *16S*.

4. Discussion

The relationships between rates of production and consumption of N and microbial parameters uncovered in this study provide insights into the mechanisms underlying differences in N cycling and availability in forest floors. The prominence of forest floor C:N ratio in the relationships indicate that site N status exerts a dominant influence on N cycling. The proportion of mineralized N taken up by microbial biomass, rather than the gross mineralization rate, appeared to be the primary driver of N release, consistent with conclusions of Mooshammer et al. (2014). While gross ammonification was influenced by the abundance of bacteria and fungi (i.e. overall saprotrophic activity), consumption of the mineralized NH_4^+ depended on microbial demand for N, as indicated by microbial C:N ratio. This is consistent with forest floors at these sites having different rates of N mineralization and nitrification despite similar rates of litter decomposition and C mineralization (Prescott et al., 2000). The strong relationships between rates of most N transformation processes and microbial C:N ratio indicate that the N status of microbes, i.e. the degree to which N is available excess to their needs, rather than their biomass or activity level, determined the amount of NH_4^+ that remained available in the forest floor. High microbial N status (i.e. low microbial C:N ratio) led to a smaller proportion of the NH_4^+ being consumed by microbes, and the resulting 'excess' NH_4^+ stimulated nitrifying organisms (as indicated by the relationships between microbial biomass C:N ratios and *amoA* gene abundances). Nitrate production then creates conditions conducive to denitrifying organisms as indicated by the relationship between AOA and AOB gene abundances and abundance of nitrite reductase genes (*nirK* and *nirS*).

The manner in which these interrelationships between site conditions, soil microorganisms and N cycling processes play out at the two study sites is illustrated in Figure 6. Despite similar rates of decomposition and C mineralization between sites, the high C:N ratio in organic matter and microbes at Fairy Lake causes all of the mineralized NH_4^+ to be consumed by microbes and

plants. In contrast, at San Juan, the low C:N ratio of the organic matter causes more N to be mineralized per unit C mineralized, and only a portion of this is consumed by microbes and vegetation. The resulting accumulation of NH_4^+ stimulates ammonia-oxidizers (as evident in the higher microbial gene abundance for AOA and AOB *amoA*), which liberate N in the form of nitrate. Some of this nitrate is consumed by microbes and plants, and some is used by denitrifiers, as evident in the detection of higher microbial gene abundance for *nirK*, and *nirS* in San Juan forest floors.

For many of the variables in this study, the influence of site N status on N cycling processes was greater than the influence of tree species. Observed gross ammonification rates support earlier findings of greater net N mineralization rates and higher bacterial:fungal ratio in forest floors at nutrient-rich, valley-bottom sites (including San Juan) than at nutrient-poor, mid-slope sites (including Fairy Lake) (Prescott et al., 2000a; Grayston and Prescott, 2005). Microbial gene abundances were also strongly affected by site, with forest floors from the San Juan site have a higher potential for nitrification and denitrification than those from Fairy Lake.

Forest floor C:N ratios were the primary feature of site influence on microbial biomass, gene abundance and N transformations. This is consistent with earlier findings that rates of N mineralization in forest floors along a site fertility gradient in Douglas-fir stands were most closely related to forest floor C:N ratios, with net mineralization being appreciable only at sites where the forest floor C:N ratio was less than 35. Similarly, Högberg et al. (2007) found forest floor C:N ratios to be as important as pH and base cations in predicting microbial community composition in forest floors in a boreal Fennoscandian forest ecosystem, while Chen and Högberg (2007) found negative correlations between fungal:bacteria ratios, forest floor C:N ratios and gross mineralization rates in forest floors. Bates et al. (2011) found soil C:N ratio to be the best predictor for archaeal relative abundances, with higher C:N ratios leading to higher archaeal relative abundances.

Tree species effects were smaller than site effects, and were more pronounced for NH_4^+ transformations than for NO_3^- transformations. Western red cedar had consistently high abundance of *16S* and AOA *amoA* at both sites, and higher rates of gross ammonification and NH_4^+ consumption, which made it the most ecologically extreme of the four tree species. This is consistent with previous studies highlighting the different N transformation pattern and microbial community structure in western red cedar forest floors (Turner and Franz, 1985; Prescott et al.,

2000a; Prescott and Grayston, 2005). Douglas-fir and Sitka spruce had similar abundances of all targeted genes, which did not significantly differ from each other. Some tree species effects appeared to depend on site N status. For example, Sitka spruce and western hemlock differed in rates of net ammonification, but only at the nutrient-poor site, Fairy Lake. The data from this study support both direct effects of tree species on soil N cycling and soil microbial communities, but also highlight the context-dependency of tree species effects (Prescott and Vesterdal 2013).

The positive correlation between gross ammonification rate and abundance of bacterial 16S rRNA genes suggests an important role of bacteria in ammonification, which aligns with current thinking about N-cycling processes (Laverman et al., 2001, Kowalchuk and Stephen, 2001, Wallenstein et al., 2006). The significantly positive correlations between the abundance of AOA *amoA* genes and rates of both gross and net nitrification indicate that nitrification in these forest floors is modulated primarily by archaeal ammonia-oxidation, with AOB playing a negligible role. Although archaea are considered to contribute little to soil microbial biomass (Gattinger et al., 2002; Bardgett and Griffiths, 1997), archaea in forest soils can have a functional role in N cycling akin to a keystone species (Prosser and Nicol, 2008; Verhamme et al., 2011). AOA are generally more abundant than AOB in acidic forest soils (Petersen et al., 2012) and AOA can have higher ammonia-oxidation rates relative to AOB under similar N availabilities (Wertz et al., 2012). The strong, positive relationship between the abundance of nitrite reductase genes (*nirK*, *nirS*) and the sum of AOA and AOB indicates that the genetic potential for denitrification in these forest floors was strongly influenced by ammonia oxidizer abundance. We measured gene abundance rather than directly assessing activity associated with specific genes; in other studies, gene abundances have shown a high degree of correlation with substrate concentrations and process rates (McGill et al., 2006; Wertz et al., 2009; Penton et al., 2013; Levy-Booth et al., 2014). Recent studies have characterized the comammox *Nitrosospora* species which contain enzymes that catalyze complete nitrification (van Kessel et al., 2015 and Daims et al., 2015), but these organisms were not considered in this study.

Bacterial 16S and *nirK* varied amongst tree species, which suggests that tree species foster different abundances of denitrifying bacteria, in addition to the elevated AOA *amoA* in western red cedar plots. AOA *amoA* can oxidize ammonia via an alternate pathway that requires less oxygen than the bacterial (AOB *amoA*) channel, which enables ammonia oxidation in anoxic

soils (Schleper and Nicol, 2010; Levy-Booth et al., 2014). Similarly, organisms that contain the gene *nirS* often do not contain *nirK*, which suggests that tree species-specific soil microbial communities are associated with specific denitrifying bacteria (Levy-Booth et al., 2014). Consistent with previous studies of forest floor microbial communities associated with these tree species (Grayston and Prescott, 2005; Turner and Franz, 1985), we found cedar forest floors to be more bacteria-dominated (*16S*) while forest floors of all four tree species were similar in fungal abundance (fungal *ITS*).

The net N transformation rates presented here were derived from the 24-hour gross-N incubations, not a standard 28-day incubation for rate of net N mineralization such as in the previous study of forest floor N dynamics in the common garden experiment (Prescott et al., 2000a). The short-term incubation study found tree species differences in N ammonification rates, but these were overshadowed by site effects. This is consistent with results of the longer-term incubations (Prescott et al., 2000a), although the values should not be directly compared, and more studies are needed to confirm the patterns. Nevertheless, this study demonstrates that a) differences between tree species and sites are discernible with these methods, and b) insights into the linkages between forest floor physico-chemical parameters, microbial gene abundance and biogeochemical cycling can be gained using these methods.

5. Conclusions

Quantification of key microbial marker genes involved in biogeochemical transformations were used to explore mechanistic links between site factors, tree species and N cycling processes. Rates of N transformation and microbial gene abundances were higher at the San Juan site, which had higher forest floor C:N ratios, higher microbial gene abundances related to nitrification and denitrification, and higher gross N transformation rates. Differences between the sites were related to site N status, as reflected in C:N ratios of the forest floor, and were more closely tied to rates of N consumption rather than gross mineralization. The relative contributions of AOA and AOB to gross and net nitrification in forests were mainly influenced by site N status. Tree species influenced gross and net ammonification and NH_4^+ consumption. Western red cedar forest floors were the most distinct of the four tree species, with highest rates of NH_4^+ N transformation, and the most distinctive forest floor microbial communities in terms of *16S*

and *nirK* gene abundances. The coupling of techniques for assessing ecosystem process rates with molecular techniques, such as functional gene abundances, can provide a greater mechanistic understanding of links between tree species and N transformation processes, as demonstrated in this study.

Acknowledgements

The authors would like to thank Israel Del Toro for field assistance, Mary Berbee for providing *Aspergillus* DNA, and Andy Smith and John Healey for comments on study design. The research was funded by NSERC grants to CEP and SJG, and a FONASO fellowship to RRR. The authors declare no conflict of interest.

Literature Cited

- Allison, S.D. 2005. Cheaters, diffusion and nutrients constrain decomposition by microbial enzymes in spatially structured environments. *Ecology Letters* 8, 627-635.
- Andersen, M.J., and Legendre, P. 1999. An empirical comparison of permutation methods for tests of partial regression coefficients in a linear model. *Journal of Statistical Computation and Simulation* 62:271-303.
- Baker G.C., et al. 2003. Review and re-analysis of domain-specific 16S primers. *Journal of Microbiological Methods*. 55: 541-555.
- Balser, T.C., Firestone, M.K. 2005. Linking microbial community composition and soil processes in a California annual grassland and mixed-conifer forest. *Biogeochemistry* 73, 395-415.
- Bardgett, R.D. and Griffiths, B.S. 1997. Ecology and biology of soil protozoa, nematodes, and microarthropods. In: *Modern Soil Microbiology*. J.D. van Elsas, J.T. Trevors, E.M.H. Wellington (Eds.), Marcel Dekker, New York. pp. 129–163
- Bates, S.T., et al. 2011. Examining the global distribution of dominant archaeal populations in soil. *The ISME Journal*. 5: 908–917.
- Bengtsson, G., et al. 2003. Gross nitrogen mineralization-, immobilization-, and nitrification rates as a function of soil C/N ratio and microbial activity. *Soil Biology and Biochemistry* 35, 143-154.
- Binkley, D. and Giardina, C.1998. Why do tree species affect soils? The Warp and Woof of tree-

497 soil interactions. Biogeochemistry 42: 89-106.

498 Blanchet, F.G., et al. 2008. Forward selection of explanatory variables. Ecology. 89: 2623-2632.

499 Braker, G., et al. 1998. Development of PCR primer systems for amplification of nitrite reductase

500 genes (*nirK* and *nirS*) to detect denitrifying bacteria in environmental samples. Applied

501 Environmental Microbiology. 64: 3769-3775.

502 Booth, M.S., et al. 2005. Controls on nitrogen cycling in terrestrial ecosystems: a synthetic

503 analysis of literature data. Ecological Monographs 75: 139-157.

504 Brockett B.F.T., et al. 2012. Soil moisture is the major factor influencing microbial community

505 structure and enzyme activities across seven biogeoclimatic zones in western Canada.

506 Soil Biology and Biochemistry 44: 9-20.

507 Brooks, P.D., et al. 1989. Diffusion method to prepare soil extracts for automated nitrogen-15

508 analysis. Soil Science Society of America Journal 53: 1707–1711.

509 Brookes, P. C., et al. 1985. Chloroform fumigation and the release of soil nitrogen: a rapid direct

510 extraction method to measure microbial biomass nitrogen in soil. Soil Biology and

511 Biochemistry 17:837-842.

512 Chu, H., Grogan, P. 2010. Soil microbial biomass, nutrient availability and nitrogen

513 mineralization potential among vegetation-types in a low arctic tundra landscape. Plant

514 and Soil. 329, 411-420.

515 Dairns, H. et al. 2015. Complete nitrification by *Nitrospira* bacteria. Nature. 528, 504-509.

516 Drury, C.F., et al. 2008. Chapter 38: Nitrification techniques for soils. In Soil Sampling and

517 Methods of Analysis, Second Edition. Edited by M.R. Carter and E.G. Gregorich. DOI:

518 110.1201/9781420005271.ch38

519 Fierer, N., et al. 2005. Assessment of soil microbial community structure by use of taxon-

520 specific quantitative PCR assays. Applied and Environmental Microbiology 71, 4117-

521 4120.

522 Gardes, M., and Bruns, T.D. 1993. ITS primers with enhanced specificity for basidiomycetes—

523 application to the identification of mycorrhizae and rusts. Molecular Ecology. 2: 113–

524 118.

525 Gatteringer, A., et al. 2002. Microbial community structure varies in different soil zones of a

526 potato field. Journal of Plant Nutrition and Soil Science 165: 421–428.

527 Grayston, S.J. and C.E. Prescott. 2005. Microbial communities in forest floors under four tree

species in coastal British Columbia. *Soil Biology and Biochemistry* 37: 1157-1167.

Hart, S.C., et al. 1994. Nitrogen mineralization, immobilization, and nitrification. In: Weaver, R.W., Angle, S., Bottomley, P., Bezdicek, D., Smith, S., Tabatabi, A., and Wollum, A. (Eds.). *Methods of Soil Analysis: Part 2. Microbiological and Biochemical Properties*. Soil Science Society of America, Madison, WI, pp. 985—1018.

Hobbie, S.E., et al. 2006. Tree species effects on decomposition and forest floor dynamics in a common garden. *Ecology* 87, 2288-2297.

Högberg, M.N., et al. 2007. Is microbial community composition in boreal forest soils determined by pH, C-to-N ratio, the trees, or all three? *Oecologia* 150:590-601.

Högberg, M.N., et al. 2007. Gross nitrogen mineralisation and fungi-to-bacteria ratios are negatively correlated in boreal forests. *Biology and Fertility of Soils* 44:363-366.
DOI: 10.1007/s00374-007-0215-9

Högberg, M.N., et al. 2013. Soil microbial community indices as predictors of soil solution chemistry and N leaching in *Picea abies* (L.) Karst. forests in S. Sweden. *Plant and Soil* 372, 507-522.

Kowalchuk, G.A., and Stephen, J.R. 2001. Ammonia-oxidizing bacteria: A model for molecular microbial ecology. *Annual Reviews in Microbiology*. 55: 485-529.

Lauber, C.L., et al. 2008. The influence of soil properties on the structure of bacterial and fungal communities across land-use types. *Soil Biology and Biochemistry* 40: 2407-2415.

Laverman, A.M., et al. 2001. Spatiotemporal stability of an ammonia-oxidizing community in a nitrogen-saturated forest soil. *Microbial ecology* 42:35-45.

Leckie, S.E., et al. 2004. Characterization of humus microbial communities in adjacent forest types that differ in nitrogen availability. *Microbial Ecology* 48: 29–40.

Leininger, S., et al. 2006. Archaea predominate among ammonia-oxidizing prokaryotes in soils. *Nature* 442, 806-809.

LeJon, D.P.H., et al. 2005. Microbial Community Structure and Density Under Different Tree Species in an Acid Forest Soil (Morvan, France). *Microbial Ecology* 50: 614-625.

Levy-Booth, D.J., Winder, R.S. 2010. Quantification of nitrogen reductase and nitrite reductase genes in soil of thinned and clear-cut Douglas-fir stands by using real-time PCR. *Applied and Environmental Microbiology* 76, 7116-7125.

Levy-Booth., et al. 2014. Microbial functional genes involved in nitrogen fixation, nitrification

- and denitrification in forest ecosystems. *Soil Biology and Biochemistry* 75, 11-25.
- Lovett, G.M., et al. 2002. Control of nitrogen loss from forested watersheds by soil carbon:nitrogen ratio and tree species composition. *Ecosystems* 5:0712-0718.
- Malchair, S. and Carnol, M. 2009. Microbial biomass and C and N transformations in forest floors under European beech, sessile oak, Norway spruce, and Douglas-fir and four temperate forest sites. *Soil Biology and Biochemistry* 41: 831-839.
- Malchair, S. and Carnol, M. 2013. AOB community structure and richness under European beech, sessile oak, Norway spruce and Douglas-fir at three temperate forest sites. *Plant and Soil Science* 366: 521–535.
- Mengistu, S.G., et al. 2014. Searching for similarity in topographic controls on carbon, nitrogen and phosphorus export from forested headwater catchments. *Hydrological Processes* 28:3201-3216.
- Mooshammer, M., et al. 2014. Adjustment of microbial nitrogen use efficiency to carbon:nitrogen imbalances regulates soil nitrogen cycling. *Nature Communications* 5, doi: 10.1038/ncomms4694.
- Muyzer G., et al. 1995. Phylogenetic relationships of *Thiomicrospira* species and their identification in deep-sea hydrothermal vent samples by denaturing gradient gel electrophoresis of 16S rDNA fragments. *Archives of Microbiology* 164:165-172.
- Petersen, D.G., et al. 2012. Abundance of microbial genes associated with nitrogen cycling as indices of biogeochemical process rates across a vegetation gradient in Alaska. *Environmental Microbiology* 14:993-1008.
- Prescott, C. E. 2014. The scientific value of long-term field trials in forest soils and nutrition research: An opportunist's perspective. *Canadian Journal of Soil Science* 94: 255-262.
- Prescott, C.E., et al. 2000a. Nutrient concentrations and nitrogen mineralization in forest floors of single species conifer plantations in coastal British Columbia. *Canadian Journal of Forest Research* 30:1341-1352.
- Prescott, C.E., et al. 2000b. Nitrogen turnover in forest floors of coastal Douglas-fir at sites differing in soil nitrogen capital. *Ecology* 81:1878:1886.
- Prescott, C.E., et al. 2000c. Humus in northern forests: friend or foe? *Forest Ecology and Management* 133:23-36.
- Prescott C.E. and Grayston S.J. 2013. Tree species influence on microbial communities in litter

- and soil: current knowledge and research needs. *Forest Ecology and Management* 309: 19-27. *Special Issue from Eurosoil Session S10.3. 'Influences of Tree Species on Forest Soils', 2012, Bari, Italy.*
- Prescott, C.E. and Preston, C.M. 1994. Nitrogen mineralization and decomposition in forest floors in adjacent plantations of western red cedar, western hemlock, and Douglas-fir. *Canadian Journal of Forest Research* 24: 2424-2431.
- Prescott, C.E. and Vesterdal, L. 2013. Tree species effects on soils in temperate and boreal forests: emerging themes and research needs. *Forest Ecology and Management* 309: 1-3.
- Prescott, C.E., and Vesterdal, L., 2005. Effects of British Columbia tree species on forest floor chemistry. Chapter 2. In: Binkley, D., Menyailo, O. (Eds.), *Tree Species Effects on Soils. Implications for Global Climate Change*. Springer, Dordrecht, The Netherlands, pp. 17–29.
- Priha, O. and Smolander, A. 1997. Microbial biomass and activity in soil and litter under *Pinus sylvestris*, *Picea abies* and *Betula pendula* at originally similar field afforestation sites. *Biol Fertil Soils* 24: 45–51.
- Prosser J.I. and Nicol G.W. 2008. Relative contributions of archaea and bacteria to aerobic ammonia oxidation in the environment. *Environmental Microbiology* 10: 2931–2941.
- Rousk, J., et al. 2009. Contrasting Soil pH Effects on Fungal and Bacterial Growth Suggest Functional Redundancy in Carbon Mineralization. *Applied and Environmental Microbiology* 75: 1589-1596.
- Seibert, J., et al. 2007. Topographical influences on soil properties in boreal forests. *Geoderma* 141:139-148.
- Ste-Marie, C., Paré, D. 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* 31, 1579-1589.
- Turner, D.P., and Franz, E.H. 1985. The influence of western hemlock and western redcedar on microbial numbers, nitrogen mineralization, and nitrification. *Plant Soil* 88, 259-267.
- van Kessel, M.A.H.J., et al. 2015. Complete nitrification by a single microorganism. *Nature*. 528, 555-559.
- Verchot, L.V., Holmes, Z., Mulon, L., et al. 2001. Gross vs. net rates of N mineralization and nitrification as indicators of functional differences between forest types. *Soil Biology and*

- Biochemistry 33: 1889-1901.
- Verhamme, D.T., Prosser, J.I., and Nicol, G.W. 2011. Ammonia concentration determines differential growth of ammonia-oxidising archaea and bacteria in soil microcosms. The ISME Journal 5:1067–1071.
- Vesterdal, L., et al. 2012. Soil respiration and rates of soil carbon turnover differ among six common European tree species. Forest Ecology and Management 264:185-196.
- Vesterdal, L., et al. 2013. Do tree species influence soil carbon stocks in temperate and boreal forests? Forest Ecology and Management 309:4-18.
- Vilgalys, R., Hester, M. 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. Journal of Bacteriology. 172: 4238-4246.
- Wallenstein, M.D., Myrold, D.D., Firestone, M., and Voytek, M. 2006. A synthesis of denitrification. Ecological Applications. 16: 2143-2152.
- Wardle, D.A. 1998. Controls of temporal variability of the soil microbial biomass: a global-scale synthesis. Soil Biology and Biochemistry 30:1627–1637.
- Wertz, S., Leigh, A.K.K., Grayston, S.J., 2012. Effects of long-term fertilization of forest soils on potential nitrification and on the abundance and community structure of ammonia oxidizers and nitrite oxidizers. FEMS Microbiology Ecology 79, 142-154
- White, T.J., et al. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J. (Eds.), PCR Protocols — a Guide to Methods and Applications. Academic Press, San Diego, CA, pp. 315–322.
- Wickham, H. 2009. ggplot2: elegant graphics for data analysis. Springer New York, USA. Book.

Figure legends

Figure 1. Microbial biomass C, N and C:N ratio in forest floors of four tree species at the two sites (blue bars=Fairy Lake; green bars=San Juan; DF=Douglas-fir; SS=Sitka spruce; WH=western hemlock; WRC=western red cedar).

Figure 2. Mean ($\pm$ SE) gene abundance (log of gene copies/g soil) of total ammonia oxidizers (AOA *amoA* and AOB *amoA*) and total denitrifiers (*nirK* and *nirS*, bacteria (*16S*), and fungi (*ITS*) in the forest floors of four tree species at the two sites. Blue bars=Fairy Lake; green bars=San Juan; DF=Douglas-fir; SS=Sitka spruce; WH=western hemlock; WRC=western red cedar.

Figure 3. Rates of nitrogen transformations in the forest floors of four tree species at the two sites; (mean $\pm$ SE). Blue bars=Fairy Lake; green bars=San Juan; DF=Douglas-fir; SS=Sitka spruce; WH=western hemlock; WRC=western red cedar.

Figure 4. Regression of the sum of denitrification (*nirK* and *nirS*) and ammonia-oxidation (AOA and AOB *amoA*) gene abundance by site ($p > 0.05$, *; $p > 0.01$, **, $p > 0.001$, ***). Sites are differentiated in the coordinate plot by colour (blue=Fairy Lake; green=San Juan) and tree species are differentiated by symbols (circle=hemlock; triangle=Douglas-fir; diamond=spruce; square=cedar).

Figure 5. Canonical redundancy analysis (RDA) and variation partitioning to determine the factors contributing to the abundance of functional gene abundance at Fairy Lake and San Juan sites. RDA was used to ordinate gene abundance measurements for total bacteria (*16S*), AOA (*amoA*), AOB (*amoA*) and nitrite reducers (*nirK* and *nirS*) against forest floor physico-chemical factors (pH, total C, total N and C:N ratio). Sites are differentiated in the coordinate plot by colour (orange, Fairy Lake; green, San Juan) and tree species are differentiated by symbols (circle=hemlock; triangle=Douglas-fir; diamond=spruce; square=cedar).

Figure 6. Conceptual model of N cycling processes at the two study sites with differing N status – Fairy Lake and San Juan.

679 **Table 1.** Group-specific primers for qPCR gene quantification assays.

<i>Primer Target</i>	<i>Primer Name</i>	<i>Primer Sequence (5'-3')</i>	<i>Reference</i>
All Bacteria (16S rRNA)	519F	CAG CMG CCG CGG TAA NWC	Baker et al. (2003)
	907R	CCG TCA ATT CMT TTR AGTT	Muyzer et al. (1995)
All Fungi (ITS)	ITS-1F	TCC GTA GGT GAA CCT GCG G	Gardes and Bruns (1993)
	5.8s	CGC TGC GTT CTT CAT CG	Vilgalys and Hester (1990)
AOA ^a (<i>amoA</i>)	<i>amoA</i> -23F	ATG GTC TGG CTW AGA CG	Francis et al. (2005)
	<i>amoA</i> - 616R	GCC ATC CAT CTG TAT GTC CA	
AOB ^b (<i>amoA</i>)	<i>amoA</i> -1F	GGG GTT TCT ACT GGT GGT	Rotthauwe et al. (1997)
	<i>amoA</i> -2R	CCC CTC KGS AAA GCC TTC TTC	
Cd-nitrite reductase (<i>nirS</i>)	<i>nirS</i> -1F	CCT AYT GGC CGG CRC ART	Braker et al. (1998)
	<i>nirS</i> -3R	GCC GCC GTC RTG VAG GAA	
Cu-nitrite reductase (<i>nirK</i>)	<i>nirK</i> -1F	GGG CAT GAA CGG CGC GCT CAT GGT G	Braker et al. (1998)
	<i>nirK</i> -1R	CGG GTT GGC GAA CTT GCC GGT GGT C	

680 ^aAmmonia Oxidizing Archaea, ^bAmmonia Oxidizing Bacteria

681 **Table 2.** Forest floor chemistry: pH, total soil C (mg/g), N (mg/g), and C:N ratios of the four tree
682 species at the two sites. Mean $\pm$ SE.

Site	Tree species	pH	Total C (mg g ⁻¹)	Total N (mg g ⁻¹)	C:N ratio
San Juan	WRC	4.01 $\pm$ 0.18	436 $\pm$ 31	110 $\pm$ 0	38.5 $\pm$ 3.1
	DF	3.95 $\pm$ 0.02	466 $\pm$ 9	135 $\pm$ 1	34.6 $\pm$ 0.9
	WH	3.98 $\pm$ 0.21	449 $\pm$ 17	114 $\pm$ 1	39.4 $\pm$ 1.0
	SS	4.63 $\pm$ 0.45	369 $\pm$ 85	112 $\pm$ 26	33.6 $\pm$ 0.9
Fairy Lake	WRC	4.31 $\pm$ 0.30	485 $\pm$ 8	11.0 $\pm$ 0.1	44.0 $\pm$ 0.6
	DF	4.05 $\pm$ 0.01	430 $\pm$ 52	11.1 $\pm$ 1.1	38.5 $\pm$ 0.9
	WH	3.75 $\pm$ 0.03	498 $\pm$ 4	10.5 $\pm$ 0.2	47.2 $\pm$ 0.7
	SS	4.29 $\pm$ 0.05	460 $\pm$ 18	11.8 $\pm$ 0.5	39.2 $\pm$ 3.1

683 WRC=western red cedar; DF=Douglas-fir; WH=western hemlock; SS=Sitka spruce

Table 3. F-statistics following permutation ANOVA testing of tree species (western red cedar, Douglas-fir, western hemlock, Sitka spruce, df=3), site (Fairy Lake, San Juan, df=1), and interaction (T x S, df=3) effects on forest floor chemistry (pH, total C, total N, C:N ratio), microbial biomass (C, N and C:N ratios), N transformations (gross and net nitrogen ammonification and nitrification, and NH₄⁺ and NO₃⁻ consumption) and microbial gene abundances.

<i>Forest floor chemistry</i>	Tree (T)		Site (S)		Tree x Site (T x S)	
	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>
pH	2.93	0.10	0.08	0.79	0.90	0.48
C	0.89	0.49	2.03	0.20	0.97	0.43
N	0.58	0.64	0.87	0.40	0.71	0.59
C:N	8.49	0.01	23.77	<0.01	0.45	0.71
<i>Microbial biomass</i>						
MBC	6.77	0.02	33.16	<0.01	5.25	0.03
MBN	2.26	0.18	0.02	0.90	1.11	0.39
MBC:N	1.62	0.28	3.90	0.10	1.67	0.29
<i>N transformation rates</i>						
Gross ammonification	18.43	<0.01	64.57	<0.01	1.08	0.40
Ammonium consumption	6.75	0.01	7.31	0.03	0.50	0.73
Net ammonification	4.43	0.05	18.27	0.01	0.73	0.58
Gross nitrification	0.74	0.58	20.81	<0.01	1.95	0.18
Nitrate consumption	1.19	0.38	132.72	<0.01	1.68	0.25
Net nitrification	1.41	0.34	67.09	<0.01	5.84	0.02
<i>Microbial gene abundances</i>						
Bacteria <i>16S</i>	3.88	0.05	10.20	0.01	1.24	0.32
Fungal <i>ITS</i>	0.20	0.90	0.38	0.55	0.99	0.45
AOA <i>amoA</i>	0.76	0.51	18.62	0.01	0.02	1.00
AOB <i>amoA</i>	1.06	0.46	4.02	0.07	1.42	0.33
<i>nirK</i>	2.73	0.13	1.94	0.20	0.97	0.45
<i>nirS</i>	1.47	0.27	8.54	0.02	1.73	0.22

C=carbon; N=nitrogen; MB=microbial biomass

Table 4. Multiple linear regressions of gross and net N ammonification and nitrification rates, and consumption rates with adjusted R² values, with best models selected using the lowest AIC values.

<i>Gross ammonification</i> = bacterial <i>16S</i> gene copies - fungal <i>ITS</i> gene copies			
Variable	Coefficient	t value	p-value
bacteria <i>16S</i> gene copies	1.057	6.253	2.96E-05
fungal <i>ITS</i> gene copies	-0.537	-3.179	0.007
F-statistics: 19.68 on 2 and 13 degrees of freedom; Adj. R2: 0.71; p-value: 0.0001168			
<i>Gross NH4+ consumption</i> = gross ammonification + C:N ratio + MBC:N ratio			
Variable	Coefficient	t value	p-value
Gross ammonification	1.055	13.084	1.84E-08
Forest Floor C:N ratio	0.243	2.68	0.020
Microbial Biomass C:N ratio	0.262	3.178	0.008
F-statistics: 61.5 on 3 and 12 degrees of freedom; Adj. R2: 0.92; p-value: <0.001			
<i>Net ammonification</i> = - C:N ratio - MBC:N ratio			
Variable	Coefficient	t value	p-value
C:N ratio	-0.526	0.1538	0.005
Microbial biomass C:N ratio	-0.486	0.1538	0.008
F-statistics: 21.73 on 2 and 13 degrees of freedom; Adj. R2: 0.73; p-value: <0.001			
<i>Gross nitrification</i> = MB C:N ratio - <i>amoA</i> AOA gene copies			
Variable	Coefficient	t value	p-value
Microbial biomass C:N ratio	0.3401	1.77	0.100
<i>amoA</i> AOA gene copies	-0.5998	-3.179	0.008
F-statistics: 7.265 on 2 and 13 degrees of freedom; Adj. R2: 0.46; p-value: 0.008			
<i>Gross NO3- consumption</i> = gross nitrification			
Variable	Coefficient	t value	p-value
gross nitrification	0.7552	0.1752	<0.001
F-statistics: 18.58 on 1 and 14 degrees of freedom; Adj. R2: 0.54; p-value: <0.001			
<i>Net nitrification</i> = <i>amoA</i> AOB gene copies			
Variable	Coefficient	t value	p-value
<i>amoA</i> AOB gene copies	0.4775	2.034	0.061
F-statistics: 4.135 on 1 and 14 degrees of freedom; Adj. R2: 0.1729; p-value: 0.061			

Ammonification rates: mg N kg⁻¹ soil (dw) d⁻¹; nitrification rates: mg N g⁻¹ soil (dw) d⁻¹; microbial C and N, mg N g⁻¹ soil (dw); Bacterial *16S*, Fungal *ITS*, AOA and AOB *amoA*: genes g⁻¹ soil (dw).