

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

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

3

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34 **Abstract**

35 Tree species can influence rates of soil N transformations, but the question remains  
36 whether differences in N cycling rates are mirrored by the abundance of relevant functional  
37 genes. We studied whether the influence of tree species on soil N transformation processes and  
38 abundance of functional genes exist across two sites in British Columbia with different N  
39 availability. We used the <sup>15</sup>N pool-dilution method to estimate gross rates of ammonification and  
40 nitrification in forest floors of four conifers in a common garden experiment. The abundances of  
41 bacteria, fungi, nitrification (AOA *amoA*, AOB *amoA*) and denitrification (*nirS*, *nirK*) genes  
42 were determined by qPCR. Western red cedar (*Thuja plicata*) had the highest rates of gross  
43 ammonification and NH<sub>4</sub><sup>+</sup> consumption, followed by Sitka spruce (*Picea sitchensis*), hemlock  
44 (*Tsuga heterophylla*), and Douglas-fir (*Pseudotsuga menziesii*); all species showed net nitrate  
45 immobilization. Western red cedar forest floors had the greatest abundance of bacterial *16S*  
46 genes and ammonia-oxidizing archaea *amoA* genes. This suggests that tree species foster  
47 different abundances of ammonification and denitrification functional groups. Differences in N  
48 transformation rates between the sites were related to site N status, as reflected in C:N ratios of  
49 the forest floor and microbial biomass, and were more closely tied to rates of N consumption  
50 rather than gross mineralization. Rates of most N transformation processes were related to  
51 microbial C:N ratio, indicating that the N status of microbes rather than their biomass or activity  
52 level determined the rates of N cycling. Ammonification rates were associated with forest floor  
53 and microbial biomass C:N ratio as well as bacterial and fungal abundances. Nitrification rates  
54 and denitrification gene abundance were associated with microbial biomass C:N ratios and AOA  
55 *amoA* gene abundance. The forest floor's genetic potential for denitrification was positively  
56 correlated with its nitrification potential as indicated by ammonia-oxidizer abundance. We  
57 conclude that tree species influenced forest floor N cycling and soil microbial gene abundances,  
58 and that functional genetics can be useful for exploring mechanistic links between tree species  
59 and nitrogen cycling processes.

60

61 **Keywords:** Ammonia oxidizers, nitrifiers, <sup>15</sup>N pool dilution, tree species effects, nitrogen  
62 cycling, forest floors

63

64 **1. Introduction**

65 Nitrogen (N) availability is often the primary limitation on ecosystem productivity in  
66 conifer forests (Vitousek et al., 1997, 2002; LeBauer and Treseder, 2008), and factors that  
67 regulate N availability have far-reaching consequences for ecosystem properties such as  
68 microbial activity, biogeochemical cycling, carbon (C) sequestration, aboveground biomass  
69 production and greenhouse gas (GHG) fluxes. Soil N transformation processes (e.g.,  
70 mineralization of organic N to  $\text{NH}_4^+$  and nitrification of  $\text{NH}_4^+$  to  $\text{NO}_3^-$ ) are largely mediated by  
71 interactions between functional communities of soil microorganisms and their environment  
72 (Bengtsson et al., 2003; Balser and Firestone, 2005; Högberg et al., 2013). The balance between  
73 gross and net rates of mineralization and nitrification can indicate turnover and immobilization  
74 rates of  $\text{NH}_4^+$  and  $\text{NO}_3^-$ , respectively, which can be rapid in forest soil (Davidson et al., 1992;  
75 Stark and Hart, 1997). Rates of mineralization and nitrification have further implications for N  
76 loss through leaching of  $\text{NO}_3^-$  and denitrification to  $\text{N}_2\text{O}$  (Bengtsson et al., 2003; Szukics et al.,  
77 2010).

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

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

95 directly via leaf litter inputs and formation of forest floors (Hobbie et al., 2006; Vesterdal et al.,  
96 2012), and via root litter inputs and alteration of soil structure. Tree species also influence rates  
97 of litter decomposition, nutrient release, C turnover and soil respiration through differences in  
98 foliar N, Ca, Mg and lignin concentrations (Hobbie et al., 2006; Vesterdal et al., 2012,  
99 2013). Several studies have reported differences in rates of N-cycling processes in soils under  
100 different tree species (e.g., Ste-Marie and Paré, 1999; Malchair and Carnol, 2009; Christiansen et  
101 al., 2010), and distinct microbial communities have also been reported in soils and forest floors  
102 under different tree species (Leckie et al., 2005; Prescott and Grayston, 2014). However, despite  
103 decades of research on this question, categorization of species according to their influence of soil  
104 properties has proven elusive, as tree species effects have been inconsistent among studies, and  
105 even at different sites within a single study. For example, in a common garden experiment on  
106 Vancouver Island, the indirect influence of site factors (particularly slope position) on rates of  
107 net N mineralization and nitrification in the forest floors appeared to overwhelm the influence of  
108 tree species, with appreciable net rates detected only at the valley bottom sites where the forest  
109 floor C:N ratio was less than 35 (Prescott et al., 2000a). Likewise, Gurmesa et al. (2013) found  
110 an influence of broadleaved tree species on soil carbon only at relatively rich sites, which they  
111 attributed to the lack of earthworms at the infertile sites, regardless of tree species. These  
112 observations prompted Prescott and Vesterdal (2013) to propose that the expression of tree  
113 species influence on soils is context-dependent, and more likely to be detectable on rich or  
114 intermediate sites.

115         In the common garden experiment on Vancouver Island, previous studies of the forest  
116 floors discerned some distinct characteristics of the forest floors under four coniferous tree  
117 species, which have been summarized by Prescott and Vesterdal (2005). Western red cedar forest  
118 floors had the lowest rates of litter decomposition and net N mineralization, but the highest  
119 proportion of nitrate and the highest bacterial:fungal ratio of the four species. Western hemlock  
120 forest floors had low pH and low Ca concentrations, low bacteria:fungal ratios, and were  
121 dominated by  $\text{NH}_4^+$  rather than nitrate. Sitka spruce forest floors had intermediate to high  
122 concentrations of N, P, Ca, and K, low bacterial:fungal ratios, and moderate rates of net N  
123 mineralization. Finally, Douglas-fir forest floors had intermediate pH and Ca, high  
124 bacterial:fungal ratios, high N concentrations and rates of net N mineralization and nitrification.

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

132

## 133 **2. Materials and Methods**

### 134 *2.1 Study location*

135 We sampled two sites, as case studies rather than replicates, from the EP571 common  
136 garden experiment on Vancouver Island: San Juan (48°35'N, 124°12'W) and Fairy Lake  
137 (48°35'N, 124°19'W), both located near Port Renfrew. The two sites were comparable in terms  
138 of elevation (65-85 and 75-85 m, respectively), and were within the Sub-montane Very Wet  
139 Maritime Coastal Western Hemlock variant (Prescott et al., 2000a). San Juan is a valley-bottom  
140 site with understory largely composed of *Rubus spectabilis* Pursh (salmonberry) and *Polystichum*  
141 *munitum* (Kaulf.) Presl. (swordfern), while Fairy Lake is a mid-slope site with understory  
142 dominated by *Gaultheria shallon* Pursh (salal) and *Vaccinium parvifolium* Smith (red  
143 huckleberry). Significantly higher N mineralization and nitrification rates and concentrations of  
144 P and K were measured in forest floors at the San Juan site compared with the Fairy Lake site  
145 (Prescott et al. 2000a), which were related to the differences in slope position and understory  
146 vegetation. We selected these two study sites because of their contrast in terms of N cycling, to  
147 enable us to test whether species effects on gross N cycling and functional genes would be  
148 consistent across contrasting sites.

149 Replicate plots of each species (western red cedar, western hemlock, Douglas-fir, and  
150 Sitka spruce) were planted in 1961, as a part of Experimental Project No. 571. The previous  
151 forest cover of western hemlock, western red cedar, amabilis fir, and Sitka spruce had been clear-  
152 cut and slash burned. The full experimental design contained 24 0.07-ha plots at each site, with  
153 81 tree seedlings planted in three densities (2.7, 3.7, and 4.7 m); for the current study we sampled  
154 the two densest (2.7-m spacing) plots of each species. We used this pre-existing experimental  
155 design to determine if tree species differ in their dominant nutrient cycling characteristics

156 between N-rich and N-poor sites.

157

## 158 *2.2. Soil sampling and soil physical and chemical analyses*

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

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

177

## 178 *2.3 Gross rates of ammonification and nitrification*

179 The <sup>15</sup>N pool-dilution method (modified from Drury, 2008) was used to determine gross  
180 rates of ammonification and nitrification, with samples analyzed in triplicate. Six 15 g  
181 subsamples from each plot were passed through a 2-mm mesh sieve and transferred to 500 mL  
182 glass Mason jars and sealed with parafilm (n = 3 subsamples for both <sup>15</sup>NH<sub>4</sub><sup>+</sup> and <sup>15</sup>NO<sub>3</sub><sup>-</sup>). The  
183 parafilm seal was punctured to enable gas exchange and maintain aerobic conditions. Samples  
184 were incubated in the dark at room temperature for 24 hours prior to initial <sup>15</sup>N treatments. These  
185 treatments consisted of either: 4 mL of <sup>15</sup>NH<sub>4</sub>Cl solution (99 atom%; Cambridge Isotope  
186 Laboratories) or 4 mL of K<sup>15</sup>NO<sub>3</sub> (99 atom%; Cambridge Isotope Laboratories) added to the

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

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

206

#### 207 *2.4 Microbial biomass determination*

208 Microbial biomass nitrogen (MBN) and microbial biomass carbon (MBC) were  
209 determined using a modified chloroform-fumigation extraction (Brookes et al., 1985). Briefly,  
210 100 mL of 2 M KCl was used in lieu of 40 mL of 0.5 M  $\text{K}_2\text{SO}_4$  for extractions (Verchot et al.,  
211 1999). 20 mL extracts were analyzed for total organic C (TOC) and total N (TN) on a Shimadzu  
212 5000A TOC analyzer at the Analytical Services Laboratory of the University of Alberta. The  
213 additional remaining extractant was used for acid diffusion traps, identical to the above protocol  
214 for the  $^{15}\text{N}$  pool-dilution method. To determine if microbial communities were immobilizing N  
215 during the course of the incubation we assessed MBN and MBC in  $^{15}\text{N-NH}_4$  and  $^{15}\text{N-NO}_3$  added  
216 samples at the end of the pool-dilution experiment, as well as from unfertilized soil samples.

217



218           2.5 *DNA isolation and quantitative PCR* DNA was extracted from 0.1 g of field-moist  
219 forest floor using the MoBio Power Soil DNA isolation kit (MoBio Laboratories, Inc., Carlsbad,  
220 CA, USA). DNA quality and concentration was measured using a nanodrop spectrophotometer  
221 (Thermo Fisher Scientific Inc., Wilmington, DE, USA) and electrophoresis in agarose gels (1%  
222 w/v in TAE), then stored at -20°C prior to amplification.

223           Quantitative PCR (qPCR) was used to quantify gene copy numbers. Reactions were  
224 carried out with an Applied Biosystems® StepOnePlus™ real-time PCR system. Each 20 µl  
225 reaction contained 10 µl of SybrGreen (2x) PCR Master Mix (Life Technologies Corp., Carlsbad,  
226 CA, USA), 0.5 µl of each primer, 250 ng µl<sup>-1</sup> bovine serum albumin (BSA), and 1 µl of DNA  
227 template. Table 1 shows primer sequences for qPCR assays. PCR conditions for AOA *amoA*,  
228 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  
229 for 1 min, and 10 s at 80°C. Triplicate 10x standard curves ranged from 10<sup>2</sup> to 10<sup>7</sup> copy numbers  
230 of AOA and AOB *amoA* in linearized plasmids. PCR conditions for *nirK* and *nirS* were 10 min at  
231 95 °C and 40 cycles of 95 °C for 1 min, 60 °C for 1 min and 72 °C for 1 min, with fluorescence  
232 quantified at extension (Levy-Booth and Winder, 2010). The standard curves for *nirS* and *nirK*  
233 used a triplicate 10-fold serial dilutions of 10<sup>1</sup> to 10<sup>7</sup> gene copies from *Pseudomonas aeruginosa*  
234 (ATCC 47085) and *Pseudomonas chlororaphis* (ATCC 13985) genomic DNA, respectively.  
235 Bacterial 16S rRNA PCR conditions were 5 min at 95°C, followed by 40 cycles of 95°C for 30 s,  
236 30 s at 57°C, and 72°C for 1 min, and 10 s at 80°C. Florescence quantification occurred during  
237 annealing. Triplicate standard curves were run using a 10x dilution of 10<sup>2</sup> to 10<sup>7</sup> amplified 16S  
238 rRNA in linearized plasmids. PCR conditions for fungal ITS were 10 min at 95°C, followed by  
239 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  
240 at 80°C to reduce the formation of non-target and primer self-complementation structures.  
241 Triplicate standard curves for fungal ITS quantification were constructed using 10x dilutions  
242 between 10<sup>3</sup> to 10<sup>9</sup> ITS copies amplified from soil and *Aspergillus citrisporus* genomic DNA.  
243 Standard curve ranges are indicative of lower and upper limits of detection, respectively. All  
244 qPCR analyses were run in duplicate.

245

## 246 2.6 *Statistical analyses*

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

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

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

268

### 269 **3. Results**

#### 270 *3.1 Forest floor pH, C and N*

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

280

### 281 3.2 Microbial biomass C and N

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

291

### 292 3.3 Microbial gene abundance

293 Bacteria *16S* rRNA gene abundance was significantly greater in forest floors from San  
294 Juan than from Fairy Lake (Table 3,  $p = 0.01$ ), and differed significantly among tree species  
295 (Table 3,  $p = 0.05$ ) with no species-by-site interaction. Bacterial genes were more abundant in  
296 forest floors of western red cedar at both sites, with hemlock or spruce having the lowest  
297 abundance depending on the site (Figure 2). Fungal *ITS* abundance did not differ between sites ( $p$   
298  $= 0.55$ ) or tree species ( $p = 0.89$ ). AOA *amoA* had the most pronounced site effect of any of the  
299 microbial genes quantified in this study, with San Juan having several orders of magnitude more  
300 AOA *amoA* genes than Fairy Lake ( $p = 0.01$ ), but no species effects. AOB *amoA* genes were  
301 more abundant at San Juan than at Fairy Lake, but within the same order of magnitude ( $p=0.10$ ).  
302 The abundance of the Cu-nitrite reductase (*nirK*) gene did not differ by site ( $p = 0.19$ ), or tree  
303 species ( $p = 0.13$ ), although hemlock tended to have the lowest gene abundance. In contrast, Cd-  
304 nitrite reductase (*nirS*) genes were significantly more abundant in forest floor at San Juan than at  
305 Fairy Lake ( $p = 0.02$ ), but did not differ by tree species ( $p = 0.27$ ).

306

### 307 3.4 Gross and net N ammonification and nitrification rates

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

311 ammonification under western red cedar being significantly greater than under Douglas-fir ( $p =$   
312 0.03), hemlock ( $p = 0.006$ ) and Sitka spruce ( $p = 0.03$ ). Gross ammonium ( $\text{NH}_4^+$ ) consumption  
313 was significantly higher in forest floors from San Juan than Fairy Lake ( $p = 0.002$ ), and was  
314 significantly greater in forest floors under cedar than the other species (Figure 3b). Net  
315 ammonification rates were mostly negative, indicating net immobilization of  $\text{NH}_4^+$ , with  
316 occasional positive mineralization occurring only in Sitka spruce at San Juan (Figure 3c). San  
317 Juan forest floors had higher (i.e. less negative) rates of net ammonification than Fairy Lake  
318 forest floors ( $p = 0.01$ ), and net ammonification rates differed among tree species ( $p = 0.05$ ).

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

### 329 330 *3.5 Relationships between N ammonification and nitrification rates and microbial parameters*

331 In general, we had greater explanatory power for ammonification rates than nitrification  
332 rates (Table 4). Gross ammonification was best explained by the total number of bacterial *16S*  
333 rRNA and fungal *ITS* genes. Gross  $\text{NH}_4^+$  consumption was best explained by gross  
334 ammonification, pH, forest floor C:N ratio, and microbial biomass C:N ratio. Net  
335 ammonification was best explained by forest floor pH and C:N ratios and microbial biomass C:N  
336 ratios. Gross nitrification was best explained by microbial biomass C:N ratios and AOA *amoA*  
337 gene. Gross  $\text{NO}_3^-$  consumption was best explained by net ammonification, gross nitrification,  
338 microbial biomass C:N ratios, bacterial 16S and AOA and AOB *amoA* genes. Net nitrification  
339 rates were best explained by net ammonification, microbial biomass C:N ratios, bacterial 16S,  
340 and AOA and AOB *amoA* genes. The sum of AOA and AOB gene abundances showed a strong,  
341 positive relationship with the sum of the abundance of nitrite reductase genes (*nirK* and *nirS*)

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

#### 347 **4. Discussion**

348 The relationships between rates of production and consumption of N and microbial parameters  
349 uncovered in this study provide insights into the mechanisms underlying differences in N cycling  
350 and availability in forest floors. The prominence of forest floor C:N ratio in the relationships  
351 indicate that site N status exerts a dominant influence on N cycling. The proportion of  
352 mineralized N taken up by microbial biomass, rather than the gross mineralization rate, appeared  
353 to be the primary driver of N release, consistent with conclusions of Mooshammer et al. (2014).  
354 While gross ammonification was influenced by the abundance of bacteria and fungi (i.e. overall  
355 saprotrophic activity), consumption of the mineralized  $\text{NH}_4^+$  depended on microbial demand for  
356 N, as indicated by microbial C:N ratio. This is consistent with forest floors at these sites having  
357 different rates of N mineralization and nitrification despite similar rates of litter decomposition  
358 and C mineralization (Prescott et al., 2000). The strong relationships between rates of most N  
359 transformation processes and microbial C:N ratio indicate that the N status of microbes, i.e. the  
360 degree to which N is available excess to their needs, rather than their biomass or activity level,  
361 determined the amount of  $\text{NH}_4^+$  that remained available in the forest floor. High microbial N  
362 status (i.e. low microbial C:N ratio) led to a smaller proportion of the  $\text{NH}_4^+$  being consumed by  
363 microbes, and the resulting 'excess'  $\text{NH}_4^+$  stimulated nitrifying organisms (as indicated by the  
364 relationships between microbial biomass C:N ratios and *amoA* gene abundances). Nitrate  
365 production then creates conditions conducive to denitrifying organisms as indicated by the  
366 relationship between AOA and AOB gene abundances and abundance of nitrite reductase genes  
367 (*nirK* and *nirS*).

368  
369 The manner in which these interrelationships between site conditions, soil microorganisms and N  
370 cycling processes play out at the two study sites is illustrated in Figure 6. Despite similar rates of  
371 decomposition and C mineralization between sites, the high C:N ratio in organic matter and  
372 microbes at Fairy Lake causes all of the mineralized  $\text{NH}_4^+$  to be consumed by microbes and

373 plants. In contrast, at San Juan, the low C:N ratio of the organic matter causes more N to be  
374 mineralized per unit C mineralized, and only a portion of this is consumed by microbes and  
375 vegetation. The resulting accumulation of  $\text{NH}_4^+$  stimulates ammonia-oxidizers (as evident in the  
376 higher microbial gene abundance for AOA and AOB *amoA*), which liberate N in the form of  
377 nitrate. Some of this nitrate is consumed by microbes and plants, and some is used by  
378 denitrifiers, as evident in the detection of higher microbial gene abundance for *nirK*, and *nirS* in  
379 San Juan forest floors.

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

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

398 Tree species effects were smaller than site effects, and were more pronounced for  $\text{NH}_4^+$   
399 transformations than for  $\text{NO}_3^-$  transformations. Western red cedar had consistently high  
400 abundance of *16S* and AOA *amoA* at both sites, and higher rates of gross ammonification and  
401  $\text{NH}_4^+$  consumption, which made it the most ecologically extreme of the four tree species. This is  
402 consistent with previous studies highlighting the different N transformation pattern and microbial  
403 community structure in western red cedar forest floors (Turner and Franz, 1985; Prescott et al.,

404 2000a; Prescott and Grayston, 2005). Douglas-fir and Sitka spruce had similar abundances of all  
405 targeted genes, which did not significantly differ from each other. Some tree species effects  
406 appeared to depend on site N status. For example, Sitka spruce and western hemlock differed in  
407 rates of net ammonification, but only at the nutrient-poor site, Fairy Lake. The data from this  
408 study support both direct effects of tree species on soil N cycling and soil microbial  
409 communities, but also highlight the context-dependency of tree species effects (Prescott and  
410 Vesterdal 2013).

411         The positive correlation between gross ammonification rate and abundance of bacterial  
412 16S rRNA genes suggests an important role of bacteria in ammonification, which aligns with  
413 current thinking about N-cycling processes (Laverman et al., 2001, Kowalchuk and Stephen,  
414 2001, Wallenstein et al., 2006). The significantly positive correlations between the abundance of  
415 AOA *amoA* genes and rates of both gross and net nitrification indicate that nitrification in these  
416 forest floors is modulated primarily by archaeal ammonia-oxidation, with AOB playing a  
417 negligible role. Although archaea are considered to contribute little to soil microbial biomass  
418 (Gattinger et al., 2002; Bardgett and Griffiths, 1997), archaea in forest soils can have a functional  
419 role in N cycling akin to a keystone species (Prosser and Nicol, 2008; Verhamme et al., 2011).  
420 AOA are generally more abundant than AOB in acidic forest soils (Petersen et al., 2012) and  
421 AOA can have higher ammonia-oxidation rates relative to AOB under similar N availabilities  
422 (Wertz et al., 2012). The strong, positive relationship between the abundance of nitrite reductase  
423 genes (*nirK*, *nirS*) and the sum of AOA and AOB indicates that the genetic potential for  
424 denitrification in these forest floors was strongly influenced by ammonia oxidizer abundance.  
425 We measured gene abundance rather than directly assessing activity associated with specific  
426 genes; in other studies, gene abundances have shown a high degree of correlation with substrate  
427 concentrations and process rates (McGill et al., 2006; Wertz et al., 2009; Penton et al., 2013;  
428 Levy-Booth et al., 2014). Recent studies have characterized the comammox *Nitrosospira* species  
429 which contain enzymes that catalyze complete nitrification (van Kessel et al., 2015 and Daims et  
430 al., 2015), but these organisms were not considered in this study.

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

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

442

443 The net N transformation rates presented here were derived from the 24-hour gross-N  
444 incubations, not a standard 28-day incubation for rate of net N mineralization such as in the  
445 previous study of forest floor N dynamics in the common garden experiment (Prescott et al.,  
446 2000a). The short-term incubation study found tree species differences in N ammonification  
447 rates, but these were overshadowed by site effects. This is consistent with results of the longer-  
448 term incubations (Prescott et al., 2000a), although the values should not be directly compared,  
449 and more studies are needed to confirm the patterns. Nevertheless, this study demonstrates that a)  
450 differences between tree species and sites are discernible with these methods, and b) insights into  
451 the linkages between forest floor physico-chemical parameters, microbial gene abundance and  
452 biogeochemical cycling can be gained using these methods.

453

## 454 **5. Conclusions**

455 Quantification of key microbial marker genes involved in biogeochemical  
456 transformations were used to explore mechanistic links between site factors, tree species and N  
457 cycling processes. Rates of N transformation and microbial gene abundances were higher at the  
458 San Juan site, which had higher forest floor C:N ratios, higher microbial gene abundances related  
459 to nitrification and denitrification, and higher gross N transformation rates. Differences between  
460 the sites were related to site N status, as reflected in C:N ratios of the forest floor, and were more  
461 closely tied to rates of N consumption rather than gross mineralization. The relative contributions  
462 of AOA and AOB to gross and net nitrification in forests were mainly influenced by site N  
463 status. Tree species influenced gross and net ammonification and  $\text{NH}_4^+$  consumption. Western  
464 red cedar forest floors were the most distinct of the four tree species, with highest rates of  $\text{NH}_4^+$   
465 N transformation, and the most distinctive forest floor microbial communities in terms of *16S*



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

470

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476

#### 477 **Literature Cited**

- 478 Allison, S.D. 2005. Cheaters, diffusion and nutrients constrain decomposition by microbial  
479 enzymes in spatially structured environments. *Ecology Letters* 8, 627-635.
- 480 Andersen, M.J., and Legendre, P. 1999. An empirical comparison of permutation methods for  
481 tests of partial regression coefficients in a linear model. *Journal of Statistical*  
482 *Computation and Simulation* 62:271-303.
- 483 Baker G.C., et al. 2003. Review and re-analysis of domain-specific 16S primers. *Journal of*  
484 *Microbiological Methods*. 55: 541-555.
- 485 Balser, T.C., Firestone, M.K. 2005. Linking microbial community composition and soil  
486 processes in a California annual grassland and mixed-conifer forest. *Biogeochemistry* 73,  
487 395-415.
- 488 Bardgett, R.D. and Griffiths, B.S. 1997. Ecology and biology of soil protozoa, nematodes, and  
489 microarthropods. In: *Modern Soil Microbiology*. J.D. van Elsas, J.T. Trevors, E.M.H.  
490 Wellington (Eds.), Marcel Dekker, New York. pp. 129–163
- 491 Bates, S.T., et al. 2011. Examining the global distribution of dominant archaeal populations in  
492 soil. *The ISME Journal*. 5: 908–917.
- 493 Bengtsson, G., et al. 2003. Gross nitrogen mineralization-, immobilization-, and nitrification  
494 rates as a function of soil C/N ratio and microbial activity. *Soil Biology and Biochemistry*  
495 35, 143-154.
- 496 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 Gattinger, 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

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

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

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

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

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

540 Högberg, M.N., et al. 2013. Soil microbial community indices as predictors of soil solution  
541 chemistry and N leaching in *Picea abies* (L.) Karst. forests in S. Sweden. *Plant and Soil*  
542 372, 507-522.

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

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

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

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

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

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

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

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

559 and denitrification in forest ecosystems. *Soil Biology and Biochemistry* 75, 11-25.

560 Lovett, G.M., et al. 2002. Control of nitrogen loss from forested watersheds by soil  
561 carbon:nitrogen ratio and tree species composition. *Ecosystems* 5:0712-0718.

562 Malchair, S. and Carnol, M. 2009. Microbial biomass and C and N transformations in forest  
563 floors under European beech, sessile oak, Norway spruce, and Douglas-fir and four  
564 temperate forest sites. *Soil Biology and Biochemistry* 41: 831-839.

565 Malchair, S. and Carnol, M. 2013. AOB community structure and richness under European  
566 beech, sessile oak, Norway spruce and Douglas-fir at three temperate forest sites. *Plant  
567 and Soil Science* 366: 521–535.

568 Mengistu, S.G., et al. 2014. Searching for similarity in topographic controls on carbon, nitrogen  
569 and phosphorus export from forested headwater catchments. *Hydrological Processes*  
570 28:3201-3216.

571 Mooshammer, M., et al. 2014. Adjustment of microbial nitrogen use efficiency to carbon:  
572 nitrogen imbalances regulates soil nitrogen cycling. *Nature Communications* 5, doi:  
573 10.1038/ncomms4694.

574 Muyzer G., et al. 1995. Phylogenetic relationships of *Thiomicrospira* species and their  
575 identification in deep-sea hydrothermal vent samples by denaturing gradient gel  
576 electrophoresis of 16S rDNA fragments. *Archives of Microbiology* 164:165-172.

577 Petersen, D.G., et al. 2012. Abundance of microbial genes associated with nitrogen cycling as  
578 indices of biogeochemical process rates across a vegetation gradient in Alaska.  
579 *Environmental Microbiology* 14:993-1008.

580 Prescott, C. E. 2014. The scientific value of long-term field trials in forest soils and nutrition  
581 research: An opportunist's perspective. *Canadian Journal of Soil Science* 94: 255-262.

582 Prescott, C.E., et al. 2000a. Nutrient concentrations and nitrogen mineralization in forest floors  
583 of single species conifer plantations in coastal British Columbia. *Canadian Journal of  
584 Forest Research* 30:1341-1352.

585 Prescott, C.E., et al. 2000b. Nitrogen turnover in forest floors of coastal Douglas-fir at sites  
586 differing in soil nitrogen capital. *Ecology* 81:1878:1886.

587 Prescott, C.E., et al. 2000c. Humus in northern forests: friend or foe? *Forest Ecology and  
588 Management* 133:23-36.

589 Prescott C.E. and Grayston S.J. 2013. Tree species influence on microbial communities in litter

590 and soil: current knowledge and research needs. *Forest Ecology and Management* 309:  
591 19-27. *Special Issue from Eurosoil Session S10.3. 'Influences of Tree Species on Forest*  
592 *Soils', 2012, Bari, Italy.*

593 Prescott, C.E. and Preston, C.M. 1994. Nitrogen mineralization and decomposition in forest  
594 floors in adjacent plantations of western red cedar, western hemlock, and Douglas-fir.  
595 *Canadian Journal of Forest Research* 24: 2424-2431.

596 Prescott, C.E. and Vesterdal, L. 2013. Tree species effects on soils in temperate and boreal  
597 forests: emerging themes and research needs. *Forest Ecology and Management* 309: 1-3.

598 Prescott, C.E., and Vesterdal, L., 2005. Effects of British Columbia tree species on forest floor  
599 chemistry. Chapter 2. In: Binkley, D., Menyailo, O. (Eds.), *Tree Species Effects on Soils.*  
600 *Implications for Global Climate Change.* Springer, Dordrecht, The Netherlands, pp. 17–  
601 29.

602 Priha, O. and Smolander, A. 1997. Microbial biomass and activity in soil and litter under *Pinus*  
603 *sylvestris*, *Picea abies* and *Betula pendula* at originally similar field afforestation sites.  
604 *Biol Fertil Soils* 24: 45–51.

605 Prosser J.I. and Nicol G.W. 2008. Relative contributions of archaea and bacteria to aerobic  
606 ammonia oxidation in the environment. *Environmental Microbiology* 10: 2931–2941.

607 Rousk, J., et al. 2009. Contrasting Soil pH Effects on Fungal and Bacterial Growth Suggest  
608 Functional Redundancy in Carbon Mineralization. *Applied and Environmental*  
609 *Microbiology* 75: 1589-1596.

610 Seibert, J., et al. 2007. Topographical influences on soil properties in boreal forests. *Geoderma*  
611 141:139-148.

612 Ste-Marie, C., Paré, D. 1999. Soil, pH and N availability effects on net nitrification in the forest  
613 floors of a range of boreal forest stands. *Soil Biology and Biochemistry* 31, 1579-1589.

614 Turner, D.P., and Franz, E.H. 1985. The influence of western hemlock and western redcedar on  
615 microbial numbers, nitrogen mineralization, and nitrification. *Plant Soil* 88, 259-267.

616 van Kessel, M.A.H.J., et al. 2015. Complete nitrification by a single microorganism. *Nature*. 528,  
617 555-559.

618 Verchot, L.V., Holmes, Z., Mulon, L., et al. 2001. Gross vs. net rates of N mineralization and  
619 nitrification as indicators of functional differences between forest types. *Soil Biology and*

620 Biochemistry 33: 1889-1901.

621 Verhamme, D.T., Prosser, J.I., and Nicol, G.W. 2011. Ammonia concentration determines  
622 differential growth of ammonia-oxidising archaea and bacteria in soil microcosms. The  
623 ISME Journal 5:1067–1071.

624 Vesterdal, L., et al. 2012. Soil respiration and rates of soil carbon turnover differ among six  
625 common European tree species. Forest Ecology and Management 264:185-196.

626 Vesterdal, L., et al. 2013. Do tree species influence soil carbon stocks in temperate and boreal  
627 forests? Forest Ecology and Management 309:4-18.

628 Vilgalys, R., Hester, M. 1990. Rapid genetic identification and mapping of enzymatically  
629 amplified ribosomal DNA from several *Cryptococcus* species. Journal of Bacteriology.  
630 172: 4238-4246.

631 Wallenstein, M.D., Myrold, D.D., Firestone, M., and Voytek, M. 2006. A synthesis of  
632 denitrification. Ecological Applications. 16: 2143-2152.

633 Wardle, D.A. 1998. Controls of temporal variability of the soil microbial biomass: a global-scale  
634 synthesis. Soil Biology and Biochemistry 30:1627–1637.

635 Wertz, S., Leigh, A.K.K., Grayston, S.J., 2012. Effects of long-term fertilization of forest soils  
636 on potential nitrification and on the abundance and community structure of ammonia  
637 oxidizers and nitrite oxidizers. FEMS Microbiology Ecology 79, 142-154

638 White, T.J., et al. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for  
639 phylogenetics. In: Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J. (Eds.), PCR  
640 Protocols — a Guide to Methods and Applications. Academic Press, San Diego, CA, pp.  
641 315–322.

642 Wickham, H. 2009. ggplot2: elegant graphics for data analysis. Springer New York, USA. Book.  
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645

646 **Figure legends**

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

650

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

656

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

660 .

661

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

667

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

675

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

678

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 <sup>a</sup> ( <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 <sup>b</sup> ( <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 <sup>a</sup>Ammonia Oxidizing Archaea, <sup>b</sup>Ammonia 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 <sup>-1</sup> )	Total N (mg g <sup>-1</sup> )	C:N ratio
San	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
Juan	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	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
Lake	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

684 **Table 3.** F-statistics following permutation ANOVA testing of tree species (western red cedar,  
 685 Douglas-fir, western hemlock, Sitka spruce, df=3), site (Fairy Lake, San Juan, df=1), and  
 686 interaction (T x S, df=3) effects on forest floor chemistry (pH, total C, total N, C:N ratio),  
 687 microbial biomass (C, N and C:N ratios), N transformations (gross and net nitrogen  
 688 ammonification and nitrification, and NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> consumption) and microbial gene  
 689 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

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

691 **Table 4.** Multiple linear regressions of gross and net N ammonification and nitrification rates,  
 692 and consumption rates with adjusted R<sup>2</sup> values, with best models selected using the lowest AIC  
 693 values.  
 694

<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			

695 Ammonification rates: mg N kg<sup>-1</sup> soil (dw) d<sup>-1</sup>; nitrification rates: mg N g<sup>-1</sup> soil (dw) d<sup>-1</sup>; microbial C and N, mg N g<sup>-1</sup> soil (dw);  
 696 Bacterial *16S*, Fungal *ITS*, AOA and AOB *amoA*: genes g<sup>-1</sup> soil (dw).  
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