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Bacterial and fungal contributions to soil nitrogen cycling under Douglas fir and red alder at two sites in Oregon

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Abstract

A study was conducted at two experimental tree plantations in the Pacific Northwest to assess the roles of bacteria and fungi in nitrogen (N) cycling. Soils from red alder (*Alnus rubra*) and Douglas-fir (*Pseudotsuga menziesii*) plots in low- (H.J. Andrews) and high- (Cascade Head) productivity stands were sampled in 2005 and 2006. Fungal:bacterial ratios were determined using phospholipid fatty acid (PLFA) profiles and quantitative (Q)-PCR. Ratios from these two molecular methods were highly correlated and showed that microbial biomass varied significantly between the two experimental sites and to a lesser extent between tree types with fungal:bacterial biomass ratios lower in more N-rich plots. ^{15}N isotope dilution experiments, with ammonium (NH_4^+) and nitrate (NO_3^-), were paired with antibiotics that blocked bacterial (bronopol) and fungal (cycloheximide) protein synthesis. This modified isotope dilution technique was used to determine the relative contribution of bacteria and fungi to net N mineralization and gross rates of ammonification and nitrification. When bacterial protein synthesis was blocked NH_4^+ consumption and nitrification rates decreased in all treatments except for NH_4^+ consumption in the Douglas-fir plots at H.J. Andrews, suggesting that prokaryotic nitrifiers are a major sink for mineral NH_4^+ in forest soils with higher N availability. Cycloheximide consistently increased NH_4^+ consumption, however the trend was not statistically significant. Both antibiotics additions also significantly increased gross ammonification, which may have been due to continued activity of extra- and intracellular enzymes involved in producing NH_4^+ combined with the inhibition of NH_4^+ assimilation into proteins. The implication of this result is that microorganisms are likely a major sink for soil dissolved organic N (DON) in soils.

Keywords: Isotope dilution; Bronopol; Cycloheximide; Fungal:bacterial ratios; Forest soils; Nitrification; Ammonification; Nitrogen mineralization

1. Introduction

Fungal:bacterial ratios have been shown to differ in forest ecosystems of different vegetation types (Myers et al., 2001; Priha et al., 2001; Hackl et al., 2004; Grayston and Prescott, 2005) and between sites (Pennanen et al., 1999; Priha et al., 2001; Leckie et al., 2004). For instance, phospholipid fatty acid (PLFA) profiles showed that fungal:bacterial ratios varied between pure stands of western red cedar (*Thuja plicata*), western hemlock (*Tsuga heterophylla*), Douglas fir (*Pseudotsuga menziesii*), and Sitka spruce (*Picea sitchensis*) (Grayston and Prescott, 2005). Research has also shown that fungal:bacterial ratios vary in response to forest fertility, with the relative abundance of bacteria increasing in response to increased fertility (Pennanen et al., 1999) and increased N availability (Leckie et al., 2004; Högberg et al., 2007). The proportion of fungi in the microbial community may increase and fungi may be more active in soil with lower nutrient concentrations (Grønli et al., 2005; Wallenstein et al., 2006).

These variations in the abundance of fungi and bacteria may lead to differences in ecosystem processes such as N cycling. Researchers have hypothesized that the ability of fungi to span microsites and secrete exoenzymes that lead to the depolymerization of N-containing compounds may be key drivers in soil N cycling (Schimel and Bennett, 2004). Researchers have also observed that heterotrophic fungi and autotrophic bacteria may contribute to nitrification in acidic forest soils (Schimel et al., 1984; Hart et al.,

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1997). Similarly, both bacteria and fungi can assimilate NO_3^- (Merrick and Edwards, 1995; Marzluf, 1997), but in a soil system where fungi are able to explore more of the soil matrix and more effectively scavenge for NH_4^+, heterotrophic bacteria may preferentially access NO_3^- (Hart et al., 1994; Chen and Stark, 2000). The relative abundance of these two groups could, therefore, significantly impact N cycling rates.

In this study, we assessed bacterial and fungal contributions to N cycling by pairing ^{15}N isotope dilution with antibiotics to block protein synthesis. Although past research has made use of antibiotics that block protein synthesis to assess the relative population sizes (Anderson and Domsch, 1973; West, 1986; Velvis, 1997; Lin and Brookes, 1999; Bailey et al., 2003; Wallenstein et al., 2006) and functions (Landi et al., 1993) of soil bacteria and fungi, few studies have applied antibiotics to examine N cycling (Castaldi and Smith, 1998; Laughlin and Stevens, 2002; Tungaraza et al., 2003; Castaldi, 2005; Myrold and Posavatz, 2007). These novel isotope dilution experiments were conducted in low- and high-productivity forest soils under pure stands of Douglas fir and red alder (*Alnus rubra*). We hypothesized that at high-productivity sites, improved soil organic matter quality and availability of nutrients would lead to lower fungal:bacterial ratios and increased bacterial activity in N cycling processes. In contrast, at low-productivity sites, fungal populations would be higher and their relative contribution to N cycling would increase. We also hypothesized that the presence of red alder would increase gross N cycling rates (Hart et al., 1997), increase nitrification at both experimental sites, and enhance the contributions of bacteria relative to fungi.

2. Materials and methods

2.1. Site descriptions and collection

Two Oregon tree plantations, with differing soil properties (Table 1) were selected for this study. Sites were located in the Cascade Head Experimental Forest (Grotta et al., 2004) and the H.J. Andrews Experimental Forest (Radosevich et al., 2006). Cascade Head is located approximately 1.6 km from the Pacific Ocean in the Oregon coastal range at an elevation of 330 m. Soils are classified as Histic Epiaquands (Rhoades and Binkley, 1992) and native vegetation includes Sitka spruce, western hemlock, and red alder. The H.J. Andrews is located in the Cascade Mountains at an elevation of 800 m and soils are best classified as Haplumbrepts (Dyrness, 2005). Native vegetation at H.J. Andrews includes Douglas fir, western hemlock, and western red cedar.

Tree plantations were established at both sites after clear-cutting in 1985 (Radosevich et al., 2006). The plantations were originally established to test inter- and intra-specific interactions, and included experimental $27 \times 27\,m^2$ plots differing in the ratios of Douglas fir to red alder. For the purposes of this study, soils were collected from plots containing pure stands of Douglas fir and red alder, which were planted in the summers of 1986 and 1987 with $3 \times 3\,m^2$ spacing (Grotta et al., 2004).

Soils were sampled one month after red alder leaves emerged, in Spring 2005 and Spring 2006, when the trees were approximately 20 years old. Cascade Head was sampled two weeks prior to sampling at H.J. Andrews because of climatic differences. Ten soil cores ($3 \times 10\,cm^2$) were taken from each of three replicate plots per treatment. Five cores were placed in each of two sample bags and homogenized. The bags were transported on ice back to the lab and sampled for PLFA and DNA extraction. In 2005 the remaining soil was used in a $^{15}NO_3^-$ isotope dilution experiment and in 2006 the remaining soil was used in a $^{15}NH_4^+$ dilution experiment.

2.2. Soil properties

Background soil properties were determined in 2005 on samples of fresh soil. Total soil C and N were analyzed by combustion using an isotope ratio mass spectrometer

Table 1
Properties of Douglas-fir and red alder soils at each experimental site determined during 2005

	Total C ($g\,kg^{-1}$ soil)	Total N ($g\,kg^{-1}$ soil)	C:N ratio	NH_4^+ ($mg\,N\,kg^{-1}$ soil)	NO_3^- ($mg\,kg^{-1}$ soil)	DON ($mg\,kg^{-1}$ soil)	pH	Water content (%)	Microbial biomass ($mg\,C\,kg^{-1}$ soil)
Cascade Head									
Douglas fir	128±4	6.7±0.2	19.2±1.0	4.5±0.6	4.4±1.7	12.7±1.0	4.1±0.1	102±5	665±62
Red alder	144±18	9.2±1.6	17.1±3.9	3.4±0.8	8.0±2.2	17.8±1.5	3.6±0.0	113±2	820±123
H.J. Andrews									
Douglas fir	90±13	2.7±0.1	33.2±3.5	1.0±0.4	0.3±0.1	5.2±0.5	5.0±0.1	68±12	468±41
Red alder	82±21	3.4±0.3	24.0±4.3	2.6±0.8	3.2±0.7	8.3±1.0	5.1±0.1	63±20	435±4
p-Value									
Site	0.043	0.005	0.060	0.037	0.070	0.001	<0.001	0.033	0.030
Tree	NS	0.095	NS	NS	0.006	0.025	0.083	NS	NS

Values are mean±standard error ($n = 3$). Statistical significance for main effects of site and tree species is shown as a *p*-value (NS = $p > 0.10$); site-by-tree interactions were not significant ($p < 0.05$) for any soil property.

(IRMS) (PDZ Europa, England) on oven-dried samples. Inorganic N was extracted (20 g of fresh soil with 50 ml of 0.05 M K_2SO_4) and NH_4^+ and NO_3^- concentrations were determined colorimetrically using an autoanalyzer (Astoria Pacific, Portland, OR). Dissolved organic N (DON) was determined on unfumigated 0.05 M K_2SO_4 extracts. Soil pH was measured on slurries containing 10 g of soil in 60 ml of DI water using a pH electrode (Orion Research Inc., Beverly, MA). Microbial biomass (MB)-C was measured by drying 2 ml aliquots of unfumigated and fumigated extracts onto tin squares (Bruulsema and Duxbury, 1996), which were analyzed by IRMS (PDZ Europa, England). A correction factor of 0.38 (Vance et al., 1987; Joergensen, 1996) was used for MB-C.

2.3. Phospholipid fatty acid extraction

PLFAs were extracted using a modified Bligh-Dyer technique (Bligh and Dyer, 1959; White and Ringelberg, 1998) as described by Brant et al. (2006). Briefly, soils (2 g dry weight) were incubated overnight in a solution (2:1:0.8) of methanol, chloroform, and phosphate buffer. After filtering, the chloroform phase was separated and phospholipids were purified using Supelclean™ LC-Si solid phase extraction columns (Supelco, Bellefonte, PA). After derivatization, PLFAs were analyzed using an Agilent 6890 gas chromatograph (Agilent, Palo Alto, CA) equipped with an HP Ultra™-2 (5% phenyl-methylpolysiloxane) column and a flame ionization detector. PLFA concentrations were determined by comparing sample peaks to a 13:0 FAME standard.

Fungal:bacterial ratios were calculated by dividing the mol% of the fungal phospholipid marker 18:2ω6,9 by the sum of mol% values for a set of bacterial phospholipids that included: i15:0, a15:0, i16:0, 16:1ω9, 16:1ω5, 17:1ω9, i17:0, a17:0, cy17:0, 18:1ω7, and cy19:0 (Blume et al., 2002).

2.4. DNA extractions and quantitative-PCR amplification (Q-PCR)

Two 0.5-g samples of fresh soil were extracted from each plot using an MOBio PowerSoil™ DNA isolation kit (MoBio Laboratories, Carlsbad, CA), according to manufacturer's instructions except that a Bio101 FastPrep instrument was used to lyse cells (Bio 101, Carlsbad, CA). The MOBio bead beating tubes were shaken for 45 s on the FastPrep. DNA in extracts was quantified using a NanoDrop™ ND-1000 UV-visible spectrophotometer (NanoDrop Technologies, Wilmington, DE) and diluted to 25 ng μl^{-1}. The two extracts from each plot were used in a composite template by combining 25 µl of each 25 ng μl^{-1} dilution.

A brilliant SYBR Green™ Q-PCR Core Reagent Kit (Stratagene, Jolla, CA) and an ABI 7500 Sequence Detection System (Applied Biosystems, Foster City, CA) were used for all Q-PCR assays. Two microliters of a 1.25 ng μl^{-1} dilution was used with 16S rRNA primers (Eub338 and Eub518) for general bacteria and ITS primers (5.8S and ITS1f) for general fungi as described previously (Fierer et al., 2005). The sequence for ITS1f was as follows: CTTGGTCATTTAGAGGAAGTAA (N. Fierer, personal communication). Each soil DNA extract and standard was run in triplicate under the following conditions: 50 °C 2 min, 95 °C 10 min, 40 cycles at 95 °C 30 s, 53 °C 30 s, 72 °C 30 s, and a final disassociation stage of 95 °C 15 s and 60 °C 20 s. SYBR green was quantified during the 72 °C elongation step. 16S rRNA clones of the bacterium *Pseudomonas aeruginosa* and ITS clones of the fungus *Haematanectria haematocoeca* were plasmid purified and used to generate a standard curve. Plasmid concentrations ranged from 5.0×10^{-1} to 5.0×10^{-7} ng DNA. Standard curves from each run were analyzed to ensure r^2 values >0.95, efficiency values between 95% and 105%, and disassociation curves containing a single peak.

2.5. ^{15}N isotope dilution experiments

Soils used in the isotope dilution incubations were sieved to <4 mm. The two replicate sample bags from each plot were combined resulting in 12 soil samples. A 5 g sample was removed and dried at 105 °C overnight to determine water content (Table 1). The remaining soil was air dried for 24 h at 20 °C to reduce water content for isotope and antibiotic addition and then stored in sealed plastic bags at 25 °C. A second 5 g sample was dried overnight at 105 °C to determine water content after air-drying.

Laboratory incubations were carried out to determine net N mineralization, MB C and N, and gross N transformation rates. Aqueous solutions containing $^{15}NO_3^-$ or $^{15}NH_4^+$ and antibiotics to block bacterial or fungal protein synthesis were added to air-dried soils, rewetting them to field moisture. $^{15}NO_3^-$ or $^{15}NH_4^+$ solutions (99 atom%) were added at concentrations of 0.6 mg NO_3^--N kg^{-1} soil or 0.02 mg NH_4^+-N kg^{-1} soil, to label the soil pools to 2–4 atom% ^{15}N. Bronopol (Shepherd et al., 1988; Bailey et al., 2003) was used to block bacterial protein synthesis and cycloheximide (Velvis, 1997; Castaldi, 2005) was used to block fungal protein synthesis. After preliminary experiments to assess antibiotic effectiveness (respiration and preliminary isotope dilution experiments were conducted), antibiotics were added at a final concentration of 2 g kg^{-1} soil.

After solutions were added, soil samples were thoroughly homogenized and 35 g soil added to canning jars (475 ml). Jars were sealed and incubated at 25 °C. At time 0 (3 h after addition of solution), half the jars were destructively sampled; the remaining jars were sampled after an additional 48 h incubation. Portions of soil (20 g) were extracted with 0.05 M K_2SO_4 and additional 10 g portions of soil were chloroform fumigated for 48 h and extracted with 0.05 M K_2SO_4. Aliquots of fumigated and unfumigated samples were later dried to determine MB-C.

Unfumigated soil extracts were used to measure net and gross N transformations. Net rates were determined by measuring NH_4^+ and NO_3^- colorimetrically using an autoanalyzer (Astoria Pacific, Portland, OR). Soil extracts were prepared for ^{15}N analysis by sequential diffusion (Hart et al., 1994) and ^{15}N abundance was measured by IRMS (PDZ Europa, England). Net N mineralization was calculated as the difference in NH_4^+ and NO_3^- concentrations during the 48 h incubation and nitrification was calculated as the difference in NO_3^- concentrations. Gross rates were calculated using the equations of Kirkham and Bartholomew (1954). When no significant change in pool sizes occurred during the incubation, the mean concentrations of NH_4^+ and NO_3^- were used in the equation for equal rates of production and consumption. In other cases, the equation for unequal rates was used.

2.6. Statistical analysis

PLFA data were analyzed using ChemStation™ software (Agilent, Palo Alto, CA) and exported to Microsoft Excel (Microsoft, Seattle, WA). Q-PCR data were analyzed using ABI Sequence Detection System software version 1.4 (Applied Biosystems, Foster City, CA).

Statistical analyses were performed using SAS 9.1 (SAS Institute Inc., Cary, NC). Data for background soil characteristics, PLFA, Q-PCR, and gross rates of the water controls were analyzed by two-way analysis of variance (ANOVA). Where interactions occurred, contrasts were used to test specific treatment differences. The effects of antibiotics on gross rate data were analyzed by three-way ANOVA. Where interactions occurred, data were analyzed separately by antibiotic treatment. Repeated measures ANOVA was performed on net N mineralization and net nitrification data to test for interactions with year of sampling (Ramsey and Schafer, 2002). Because interactions with year were found, separate ANOVA was done for each year and contrasts were used to test for the effect of antibiotic treatments. Linear regression analysis was also performed to determine if fungal:bacterial ratios measured by PLFA and Q-PCR were correlated. p-values $\leqslant 0.05$ were considered significant, but p-values between 0.05 and 0.10 are reported.

3. Results

3.1. Soil properties

Soil properties including soil C and N varied significantly between the two experimental sites; in particular, N content was substantially higher in Cascade Head than H.J. Andrews soils. Although soil C did not vary between Douglas fir and red alder, N varied ($p = 0.095$) with red alder soils containing an average of $6.3\,g\,N\,kg^{-1}$ soil across both sites and Douglas fir soils containing an average of $4.7\,g\,N\,kg^{-1}$ soil (Table 1). C:N ratios ($p = 0.060$) and NH_4^+ concentrations varied between sites, but not between vegetation types. NO_3^- concentrations varied significantly between red alder ($5.6\,mg\,N\,kg^{-1}$ soil) and Douglas fir ($2.4\,mg\,N\,kg^{-1}$ soil) across both sites, and were higher ($p = 0.070$) in Cascade Head ($6.2\,mg\,N\,kg^{-1}$ soil) than H.J. Andrews soils ($1.8\,mg\,N\,kg^{-1}$ soil). DON varied significantly both between sites and between vegetation types, with red alder averaging $13\,mg\,N\,kg^{-1}$ soil compared to $8.9\,mg\,N\,kg^{-1}$ soil in Douglas-fir plots. Soil pH was significantly lower at Cascade Head (pH 3.8) compared to H.J. Andrews (pH 5.1). Soil water content was significantly higher in Cascade Head, compared to H.J. Andrews soils, where andic soil properties increased soil water holding capacity. Cascade Head soils contained significantly more MB-C in comparison to H.J. Andrews soils (Table 1).

3.2. Fungal:bacterial ratios

The PLFA 18:2ω6,9, a fungal marker, varied significantly between sites with more fungal PLFA in H.J. Andrews soils (Table 2). Significant site by tree interactions were observed in the amount of bacterial PLFAs, with Cascade Head Douglas-fir soils containing the highest amount of bacterial PLFA and H.J. Andrews Douglas-fir soils containing the lowest. PLFA-derived fungal:bacterial ratios differed significantly between the two sites, with a mean of 0.04 at Cascade Head and a mean of 0.15 at H.J. Andrews, but did not vary by tree type.

Fungal ITS copy numbers ranged almost four-fold, with the lowest number in Cascade Head Douglas-fir plots and the highest in Douglas-fir soils at H.J. Andrews (Table 2). Cascade Head soil had two-fold higher bacterial 16S rRNA genes compared to H.J. Andrews soils. As with fungal: bacterial ratios determined by PLFA extraction, ratios calculated from fungal and bacterial gene copies were significantly higher in H.J. Andrews compared to Cascade Head soils. No effect of tree type was observed. Ratios determined by PLFA and by Q-PCR were highly correlated ($r^2 = 0.86$).

3.3. Net N-cycling rates

During both years, net N mineralization was higher in Cascade Head versus H.J. Andrews soils and higher in red alder compared to Douglas-fir soils (Figs. 1A and B). Bronopol significantly increased net N mineralization in Cascade Head Douglas-fir soils during 2006 and in Cascade Head red alder soils in 2005 and 2006, but had no significant effect on H.J. Andrews soils (Figs. 1A and B). Cycloheximide increased net N mineralization in red alder soils at H.J. Andrews in 2006.

Net nitrification was significantly higher in soils at Cascade Head and in red alder compared to Douglas-fir soils. Cycloheximide additions had no effect on net nitrification for either site or tree type in either year (Figs. 1C and D). Bronopol had no effect on net nitrification in soils from the H.J. Andrews during either year; however, net nitrification decreased with bronopol

Table 2
Fungal and bacterial biomass for both Douglas-fir and red alder soils at each experimental site

	PLFA			Q-PCR		
	Fungi (mol%)	Bacteria (mol%)	F:B ratio	Fungi (gene copies g^{-1} soil)	Bacteria (gene copies g^{-1} soil)	F:B ratio
Cascade Head						
Douglas fir	2.3 ± 0.3	50.0 ± 1.0^{a}	0.043 ± 0.006	$2.8 \pm 0.56 \times 10^{8}$	$2.8 \pm 0.31 \times 10^{10}$	0.010 ± 0.001
Red alder	1.9 ± 0.2	41.3 ± 1.8^{bc}	0.043 ± 0.003	$3.9 \pm 0.85 \times 10^{8}$	$3.8 \pm 0.73 \times 10^{10}$	0.011 ± 0.002
H.J. Andrews						
Douglas fir	7.5 ± 1.5	38.8 ± 1.7^{c}	0.197 ± 0.046	$9.9 \pm 3.2 \times 10^{8}$	$1.8 \pm 0.08 \times 10^{10}$	0.053 ± 0.010
Red alder	4.5 ± 0.3	46.4 ± 0.4^{ab}	0.097 ± 0.007	$3.2 \pm 0.67 \times 10^{8}$	$1.7 \pm 0.39 \times 10^{10}$	0.027 ± 0.009
p-Value						
Site	0.003	NA	0.006	NS	0.012	0.024
Tree	NS	NA	NS	NS	NS	NS

Values are mean ± standard error ($n = 3$). Statistical significance for main effects of site and tree species is shown as a *p*-value (NS = $p > 0.10$). If the site-by-tree interaction was significant ($p < 0.05$), lower case letters are used to designate treatment differences. NA = not applicable.

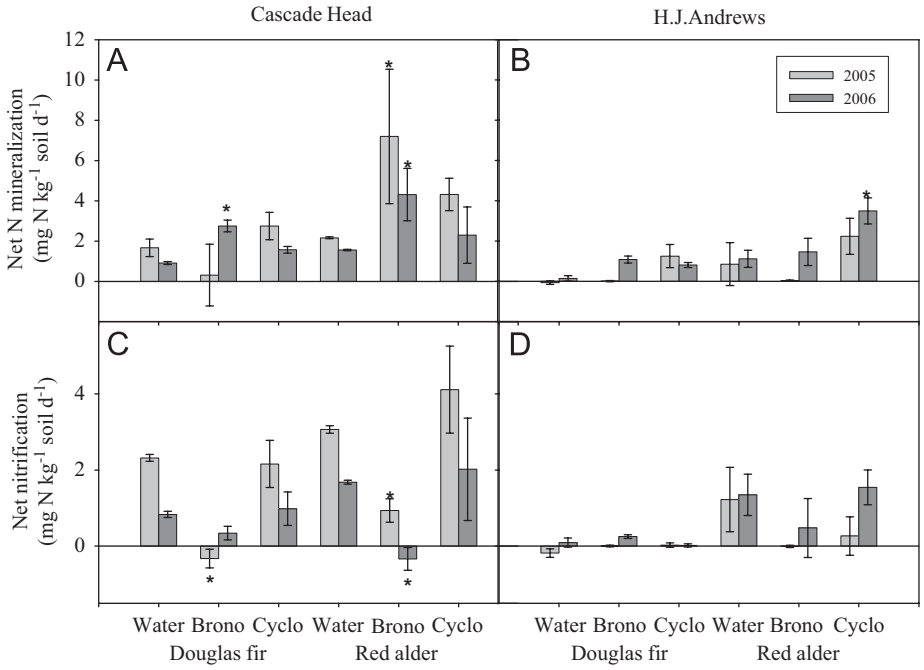

Fig. 1. Net N mineralization and net nitrification for 2005 and 2006. Panels A and B show the net change in $NH_4^+ + NO_3^-$ concentrations during the 48-h incubations for both Douglas fir and red alder across all three antibiotic treatments: control (water), bronopol (brono), and cycloheximide (cyclo). Panels C and D show net change in NO_3^- concentrations. Panels A and C represent data from Cascade Head and panels B and D show H.J. Andrews results. Each bar is the mean of three field replicates with error bars showing standard error ($n = 3$). Significant effects of an antibiotic are shown by a *($p < 0.05$).

additions in both tree types at Cascade Head in 2005 and also decreased in red alder soil during 2006.

3.4. Gross N-cycling rates

There were no significant differences in gross ammonification rates between sites or tree types in water treatments. In Cascade Head soils ammonification rates increased significantly in response to bronopol additions, but no significant change occurred in H.J. Andrews soils (Table 3). Cycloheximide significantly increased ammonification in soils at both sites and tree types. Gross NH_4^+ consumption did not vary based on site or tree type in water controls, but bronopol caused significant decreases in gross NH_4^+ consumption across all treatments. Although bronopol addition led to a significant main effect, gross NH_4^+ consumption did not decrease in H.J. Andrews Douglas-fir soils (Table 3). Cycloheximide consistently increased NH_4^+ consumption, however the trend was not statistically significant compared to water controls.

Gross nitrification in water controls was significantly higher in Cascade Head soils ($2.91\ mg\ N\ kg^{-1}\ soil\ d^{-1}$)

Table 3
Gross N-cycling rates for Douglas-fir and red alder soils at Cascade Head and H.J. Andrews

	Antibiotic treatment	Ammonification	NH_4^+ consumption ($mg\,N\,kg^{-1}\,soil\,d^{-1}$)	Nitrification ($mg\,N\,kg^{-1}\,soil\,d^{-1}$)	NO_3^- Consumption ($mg\,N\,kg^{-1}\,soil\,d^{-1}$)
Cascade Head					
Douglas fir	Control	0.84 ± 0.41	0.84 ± 0.41	2.71 ± 0.12	0.21 ± 0.15
	Bronopol	2.10 ± 0.20*	−0.04 ± 0.10	−0.21 ± 0.23*	0.13 ± 0.06
	Cycloheximide	1.26 ± 0.55	1.26 ± 0.55	1.73 ± 0.46	−0.30 ± 0.88
Red alder	Control	0.49 ± 0.14	0.49 ± 0.14	3.11 ± 0.11	0.04 ± 0.21
	Bronopol	4.24 ± 0.96*	−0.27 ± 0.25	0.17 ± 0.18*	−0.76 ± 0.28
	Cycloheximide	1.27 ± 0.05	0.99 ± 0.05	2.97 ± 0.76	−1.14 ± 0.39
H.J. Andrews					
Douglas fir	Control	0.47 ± 0.32	0.47 ± 0.32	0.594 ± 0.11	0.59 ± 0.11
	Bronopol	1.19 ± 0.81	0.53 ± 0.71	0.04 ± 0.03*	0.04 ± 0.03
	Cycloheximide	0.81 ± 0.13	0.81 ± 0.13	0.41 ± 0.04	0.41 ± 0.04
Red alder	Control	1.20 ± 0.39	1.20 ± 0.39	1.22 ± 0.71	1.22 ± 0.71
	Bronopol	0.50 ± 0.45	−0.36 ± 0.61	0.11 ± 0.25*	0.11 ± 0.25
	Cycloheximide	3.40 ± 1.09	1.44 ± 1.25	0.45 ± 0.55	0.45 ± 0.55
p-Value					
Antibiotic	Bronopol	NA	0.049	NA	0.004
	Cycloheximide	0.036	NS	0.103	0.047

Rates are mean ± standard error ($n = 3$). Statistical significance for main effects of the antibiotics is shown as a *p*-value (NS = $p > 0.10$). If interactions involving antibiotics were significant ($p < 0.05$).
*Indicates a significant effect of the antibiotic ($p < 0.05$). NA = not applicable.

compared to H.J. Andrews soils ($0.90\,mg\,N\,kg^{-1}\,soil\,d^{-1}$) (Table 3), but did not vary significantly between Douglas-fir and red alder soils. Bronopol blocked gross nitrification in Cascade Head and H.J. Andrews soils whereas cycloheximide had no significant effect on nitrification. NO_3^- consumption was higher in H.J. Andrews compared to Cascade Head soils ($p = 0.087$) but did not vary between tree types in water controls. Both antibiotics significantly decreased NO_3^- consumption with a decrease of 81% in bronopol treatments and a decrease of 78% in cylcohexamide treatments (Table 3).

4. Discussion

Previously, researchers at Cascade Head Experimental Forest reported significant differences in soil N cycling (Hart et al., 1997; Binkley et al., 1992) and C substrate utilization (Selmants et al., 2005) in the presence of red alder compared to Douglas fir in tree stands older than 50 years. Based upon these findings, we hypothesized that increased N inputs from red alder would lead to significant variations in soil properties and MB, but we found Douglas-fir and red alder soils to be surprisingly similar. The strong similarity between Douglas-fir and red alder soils at Cascade Head may have been due to the inherently high soil nutrient status of coastal andisols. Our work and other research in the Oregon Coast Range (Perakis et al., 2006) has shown that even pure stands of Douglas fir contain soil N values exceeding the threshold for N limitation, and Radosevich et al. (2006) found that mixed stands of Douglas fir and red alder at Cascade Head did not increase Douglas fir production over monocultures.

Although we observed few significant differences in soil properties or fungal:bacterial ratios, NO_3^- and DON concentrations were higher in red alder soils. We also found that net and gross nitrification increased in the presence of red alder in agreement with previous research (Hart et al., 1997). These results show that the presence of red alder does affect some N cycling processes and may also impact sub-populations of the microbial community, such as those involved in nitrification (Boyle et al., in review).

A number of factors including landscape position and understory vegetation have been shown to alter soil pH, microbial community composition, and N cycling at different forest sites with the same dominant tree species (Prescott et al., 2000; Bengtson et al., 2006). H.J. Andrews soils contained lower concentrations of organic and mineral N than soils at Cascade Head (Table 1), and production and consumption of mineral N appeared to be tightly coupled at the H.J. Andrews (Fig. 1 and Table 3). Availability of N may have limited MB (Table 1), and in agreement with our hypothesis, provided a competitive advantage for fungi in these N-limited soils (Table 2). Our findings support other research that found relative fungal biomass to decrease with increasing soil fertility and N availability (Bardgett et al., 1996; Myers et al., 2001; Pennanen et al., 2001; Grayston and Prescott, 2005; Högberg et al., 2007). Nilsson and Wallander (2003) found that ectomycorrhizal fungal biomass decreased when N fertilizer was added under Norway spruce (*Picea abies*) and other researchers have suggested that increased N availability may lead to a decrease in decomposer fungi (Hammel, 1997; Sinsabaugh et al., 2005). We observed

lower fungal PLFA in Cascade Head soils compared to H.J. Andrews soils and an increase in 16S rRNA copy number. Both PLFA and Q-PCR fungal:bacterial ratios were lower in Cascade Head soils, suggesting that high nutrient availability may have resulted in lower fungal contributions to MB.

These observed variations in fungal:bacterial ratios highlight the need to better understand the contributions of fungi and bacteria to N cycling processes. Although using antibiotics comes with inherent difficulties, pairing them with ^{15}N isotope dilution techniques provided evidence for different roles of fungi and bacteria in the soil N cycle under different vegetation types and nutrient status.

The high sensitivity of gross nitrification rates to bronopol combined with insensitivity to cycloheximide suggest that bronopol-sensitive prokaryotes were primarily responsible for nitrification in all soil types, despite acidic soil conditions, and represent the primary sink for NH_4^+. This prokaryotic community included ammonia-oxidizing bacteria, but we do not know the potential contributions of archaea in these processes. Although cultured representatives of archaea are insensitive to cycloheximide (Madigan et al., 2003; Briones et al., 2005), to our knowledge the effect of bronopol on archaea has not been tested. We were able to amplify archaeal *amoA* from Cascade Head soils (Boyle et al., in review), so it will be important to determine their contribution in future studies. When bronopol was added, gross nitrification was completely inhibited at both sites (Table 3). Bronopol also decreased gross NH_4^+ consumption rates, which is consistent with prokaryotes being responsible for the majority of nitrification in soils at both sites (Table 3).

Although it has been widely reported that NO_3^- assimilation may play an important role in N retention within temperate forests (Davidson et al., 1992; Hart et al., 1994, 1997), we did not observe enrichment of the MB-N pool when NO_3^- was added (data not shown). At Cascade Head, NO_3^- consumption was not significantly different than zero (Table 3). NO_3^- consumption rates were higher at H.J. Andrews, although significantly greater than zero only in Douglas-fir soils (Table 3). Presumably these results to some extent reflect in situ NH_4^+-rich conditions, which may repress NO_3^- assimilation and explain the high NO_3^- concentrations observed in background soils (Table 1) (Rice and Tiedje, 1989). In spite of measurable NO_3^- consumption rates in H.J. Andrews soils, we did not observe abiotic fixation, dissimilatory NO_3^- reduction to ammonia (DNRA), or enrichment of the MB-N pool (data not shown). We did not measure denitrification, but the aerobic incubation conditions and low denitrification potentials previously measured at these locations (Vermes and Myrold, 1992), suggest that denitrification was unlikely to account for NO_3^- consumption rates. Our ability to trace ^{15}N assimilation into biomass may have been hindered by the low concentrations of ^{15}N we used in our experiments and by a fast turnover of N in the MB (Herman et al., 2006) which could have diluted the pool too quickly to be measured. Remarkably, bronopol had no effect on NH_4^+ consumption in Douglas-fir soils at the H.J. Andrews but stopped NO_3^- consumption implying that in these low N systems fungi may assimilate NH_4^+ while bacteria assimilate NO_3^-. This is consistent with the results of Myrold and Posavatz (2007) who found bacteria to dominate in NO_3^- assimilation potential assays of a soil from the H.J. Andrews.

The general lack of significant NO_3^- assimilation suggests that microorganisms in these forest soils rely on either NH_4^+ or organic N as their N source. Furthermore, gross nitrification rates were large enough to account for the measured consumption of NH_4^+, and are consistent with the findings of Booth et al. (2005) who report that at mineralizing conditions $<1\,mg\ NH_4^+\text{-}N\,kg^{-1}$ soil d^{-1} the bulk of NH_4^+ goes to nitrification. These results suggest that heterotrophic bacteria and fungi in forest systems may rely primarily on organic N for building biomass (Fierer et al., 2001; Schimel and Bennett, 2004).

Through the excretion of extracellular enzymes, microorganisms mediate the decomposition of complex polymers into N-containing monomers, such as nucleic acids, amino acids, or amino sugars. These small organic N molecules may be taken up and directly incorporated into MB or deaminated through the action of intracellular enzymes to produce NH_4^+. The NH_4^+ produced may then be incorporated into biosynthetic pathways or released from the cell resulting in ammonification (Myrold and Bottomley, 2007). Presumably, even after antibiotics were added, preexisting extracellular and intracellular enzymes remained active; however, microbial protein biosynthesis should have stopped. The net result would be the release of NH_4^+ into the soil, which would be measured as enhanced ammonification. Cycloheximide stimulated ammonification at both sites, suggesting that fungi were universally important in utilizing organic N. The effect of bronopol was only observed at the N-rich Cascade Head site, where it stimulated ammonification more than cycloheximide did, which is consistent with the higher relative amounts of bacteria at this site. Future studies using ^{15}N-amino acids will help us to better understand the role of organic N in these systems.

In conclusion, our data showed that fungal:bacterial ratios were significantly lower in the high-productivity site compared to the low-productivity site. The presence of red alder increased gross and net nitrification at both sites, and in all treatments these processes appeared to be mediated by prokaryotes. These results also suggest that the main sink for NH_4^+ in these soils may be autotrophic nitrifiers. In most cases, the addition of antibiotics significantly increased ammonification suggesting that when protein synthesis was blocked DON utilization switched from assimilation to mineralization. These observations highlight the importance of organic N to heterotrophic forest communities and emphasize the need for future investigations of the dynamics of DON pools.

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