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SOIL MICROBIOLOGY

Soil Microbial Communities Associated with Douglas-fir and Red Alder Stands at High- and Low-Productivity Forest Sites in Oregon, USA

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Abstract Communities of archaea, bacteria, and fungi were examined in forest soils located in the Oregon Coast Range and the inland Cascade Mountains. Soils from replicated plots of Douglas-fir (*Pseudotsuga menziesii*) and red alder (*Alnus rubra*) were characterized using fungal ITS (internal transcribed spacer region), eubacterial 16S rRNA, and archaeal 16S rRNA primers. Population size was measured with quantitative (Q)-PCR and composition was examined using length heterogeneity (LH)-PCR for fungal composition, terminal restriction fragment length (T-RFLP) profiles for bacterial and archaeal composition, and sequencing to identify dominant community members. Whereas fungal and archaeal composition varied between sites and dominant tree species, bacterial communities only varied between sites. The abundance of archaeal gene copy numbers was found to be greater in coastal compared to montane soils accounting for 11% of the prokaryotic community. *Crenarchaea* groups 1.1a-associated, 1.1b, 1.1c, and 1.1c-associated were putatively identified. A greater abundance of *Crenarchaea* 1.1b indicator fragments was found in acidic (pH 4) soils with low C:N ratios under red alder. In coastal soils, 25% of fungal sequences were putatively identified as basidiomycetous yeasts belonging to the genus *Cryptococcus*. Although the function of these yeasts in soil is not known, they could significantly contribute to decomposition processes in coastal soils distinguished by rapid tree growth, high N content, low pH, and frequent water-saturation events.

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Introduction

The soil microbial community is a complex assemblage of archaea, bacteria, and fungi, the composition of which may impact the rate of terrestrial nutrient cycling. Although all three major groups of organisms are routinely identified in a variety of soil ecosystems, few studies have examined the phylogenetic diversity of more than one group in a single study [8, 25, 31, 58], and even fewer studies include all three [15, 35, 41, 57]. Fierer et al. [15] reported comparable diversity in all three groups among different ecosystem types, suggesting that the poorly characterized soil archaea are genotypically diverse and likely involved in a number of important ecosystem processes.

There is a need for more studies that examine the effect of soil and environmental variables on soil fungal, bacterial, and archaeal communities. These factors include dominant vegetation type [30, 33, 44], nutrient availability [11, 21, 53, 66], and soil pH [34, 54]. Whereas it is possible that soil and environmental properties equally affect the composition of all three domains of the soil microbiota, reports in the literature suggest that some factors differentially shape microbial groups. For example, at the continental scale, pH has been shown to strongly correlate to bacterial communities [34], whereas soil nutrient status has been shown to correlate to fungal composition [34, 64]. Reports on soil archaea are mixed; Oline et al. [46] were unable to link archaeal community composition to any environmental correlate, and yet Nicol et al. [45] reported correlations between crenarchaeal communities, pH, and vegetation type.

Forests of the Pacific Northwest region, United States, west of the Cascade Mountain Range crest are dominated by Douglas-fir (*Pseudotsuga menziesii*). This coniferous species is important to the economy of the region and also to the structure of forest ecosystems. In these forests, the most extensively studied soil microorganisms have been fungi able to form mycorrhizal associations with Douglas-fir. Fungal species of *Cenococcum*, *Rhizopogan*, *Russula*, and *Piloderma* [12, 20, 26] are the most commonly reported types of ectomycorrhizal fungi, but estimates place the number of fungal species able to form mycorrhizal associations with Douglas-fir as high as 2000 [60]. Although it is presumed that ectomycorrhizae dominate forest fungal communities, few studies have characterized bulk soil using molecular methods [1]. Even less is known about the prokaryotic communities. Some studies suggest that the bacterium, *Pseudomonas fluorescens*, may serve as a helper bacterium in the ectomycorrhizal colonization process [17] but this species would likely comprise a small fraction of the diverse prokaryotic community.

The hardwood species, red alder (*Alnus rubra*) is also a common tree species in Pacific Northwest forests, especially in moist locations, such as coastal sites and in riparian areas. Like Douglas-fir, red alder is also capable of forming mycorrhizal symbioses. Miller et al. [39] found that only one fungal species *Thelephora terrestris was* able to colonize both Douglas-fir and red alder, even when the trees were grown in the same soil, suggesting that the dominant tree species will likely have a dramatic effect on the fungal diversity. Miller et al. [39] also found that red alder was less likely to form ectomycorrhizal associations when Douglas-fir was present. Red alder is also an N-fixing species, forming root nodules that house populations of the actinomycete *Frankia*. Jeong and Myrold [28] reported increased populations of *Frankia* in the presence of host plants compared to conifers, suggesting that the presence of red alder could alter bacterial composition.

Although direct impacts of these two different tree species on the soil microbial community is likely profound, a number of other variables will also influence composition. For instance, the diversity of understory vegetation can number in the hundreds of species and tends to vary with precipitation; the shrub Salal (*Gaultheria shallon*) is common in drier, less productive sites, while the Western Sword Fern (*Polystichum munitum*) is common in moist locations [24]. Microbial communities will likely vary in response to changes in the understory plant populations, and although outside of the scope of this study, the distribution of and predation by soil animals undoubtedly helps to shape microbial community composition as well. Differences in soil properties also impact particular groups of the microbial community. For example, nodulation of red alder roots depends on soil properties including pH and nitrate (NO_3^-) concentration. Martin et al. [37] reported a reduction in nodulation under increasingly acidic conditions.

In this study, we examined the general communities of fungi, bacteria, and archaea to determine if these groups differed phylogenetically between forests stands in different ecoregions and planted with different tree species. We hypothesized that the dominant members of the fungal, bacterial, and archaeal communities would differ between coastal and inland mountain forests and between planted stands of Douglas-fir and red alder. Given previous findings, we hypothesized that the fungal and bacterial communities would vary largely based on tree species [28, 34, 64], but other factors including soil C, soil N, and pH would also correlate with community composition of all three groups. Lastly, we looked at correlations among the three communities, examining the extent to which the composition of these groups may be linked to the same environmental factors.

Materials and Methods

Site Description and Sample Collection

Experimental plots of planted Douglas-fir and red alder were established in 1984–1986 at sites in the Oregon Coast Range (Cascade Head) and in the Cascade Mountains (HJ Andrews) [49]. Soils were sampled from the two experimental tree plantations in the spring of 2006 when trees were approximately 20-years-old. Cascade Head Experimental Forest is located in an ecoregion of high aboveground net primary productivity (1.25 kg C m^{-2} $year^{-1}$) [62], 1.6 km from the Pacific Ocean at an elevation of 330 m (45°03′44″ N/123° 57′14″ W). The weathering of basalt headlands has created a Histic Epiaquand [50] with high soil N content and a pH of 4 (Table 1) [5]. In 1998, tree height averaged 13.5 m for red alder and 8.5 m for Douglas-fir [49]. HJ Andrews Experimental Forest in the West Cascades ecoregion is located at an elevation of 800 m (44°13′59″ N/122°10′34″ W). Here soils are primarily classified as inceptisols [49] and aboveground net primary productivity is lower (0.63 kg C m^{-2} $year^{-1}$) [62]. Concentrations of total C, total N, and NH_4^+ were significantly lower in HJ Andrews soils compared to Cascade Head, but pH was higher (pH 5; Table 1). In 1998, tree height was found to be significantly lower at HJ Andrews, averaging 7.5 m for red alder and 5 m for Douglas-fir [49].

Previous work at these sites indicated differences in the fungal:bacterial ratio (Table 1) [5] and ammonia–monooxygenase (*amoA*) gene composition in ammonia-oxidizing bacteria and archaea [6]. HJ Andrews soil had a significantly greater fungal:bacterial ratio than Cascade

Table 1 Selected soil characteristics for the four experimental treatments (n=3) [5]

Soil characteristics	Cascade Head		HJ Andrews	
	Red alder	Douglas-fir	Red alder	Douglas-fir
Total C(g kg^{-1} soil)	144±18	128±4	82±21	90±13
Total N(g kg^{-1} soil)	9.2±1.6	6.7±0.2	3.4±0.3	2.7±0.1
NH_4^+ (mg N kg^{-1} soil)	3.4±0.8	4.5±0.6	2.6±0.8	1.0±0.4
NO_3^- (mg N kg^{-1} soil)	8.0±2.2	4.4±1.7	3.2±0.7	0.3±0.1
pH	3.6±0.0	4.1±0.1	5.1±0.1	5.0±0.1
Water content (%)	113±2	102±5	63±20	68±12
F:B ratio (PLFA)	0.011±0.002	0.010±0.001	0.027±0.009	0.053±0.010

Head (Table 1). Bacterial *amoA* community structure differed between the two sites, but did not differ between Douglas-fir and red alder stands. Archaeal *amoA* could only be amplified from Cascade Head soil and differed between the two tree species.

Plots were organized into randomized blocks, with each plot measuring 27×27 m. Soil was only collected from plots that were planted exclusively with Douglas-fir or red alder. Ten soil cores (3 cm diameter×10 cm depth) were taken from each of three replicate plots per treatment. The organic layer was removed so that only mineral soil was sampled. Cores within plots were homogenized and soils were transported on ice to the lab for DNA extraction.

DNA Extractions and PCR Amplification

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

Quantitative PCR

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. Q-PCR was used to quantify the number of fungal ITS, bacterial 16S, and archaeal 16S rRNA sequences. Briefly, 2 µl of a 1.25 ng μl^{-1} dilution was combined with ITS primers (5.8S and ITS1F) for fungi or 16S rRNA primers (Eub338 and Eub518) for bacteria. PCR conditions have been described previously [5, 16]. 16S rRNA primers A915-for (AGG AAT TGG CGG GGG AGC AC) and Arc 1059r (GCC ATG CAC CWC CTC T) were used to quantify the archaeal community [67]. Each soil DNA extract and standard was run in triplicate. An ITS clone of the fungus *Haematonectria haematococca* was used as a standard for fungi, a 16S rRNA clone of the bacterium *Pseudomonas aeruginosa* was used for bacteria, and a 16S rRNA clone of *Sulfolobus solfataricus* were used for archaea. 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 to affirm that disassociation curves contained a single dominant peak.

T-RFLP and LH-PCR Profiles

Approximately 100 ng of DNA was used in each conventional PCR reaction. The fungal internal transcribed spacer region (ITS) was amplified with primers ITS1F and ITS4 [18] as previously described [2]. Eubacterial 16S rRNA was amplified using primers 16S 8-F [13] and 16S 907-R [40] as described by Hackl et al. [21]. Archaeal 16S rRNA was amplified using primers Ar3f [19] and Ar927r [29]. ITS1F, 16S 8-F, and Ar3f contained FAM labels for sequence detection. Eubacterial and archaeal 16S PCR products were cleaned using a Qiaquick™ PCR Purification kit (Qiagen Inc., Valencia, CA) and restricted using three enzymes. PCR products for eubacterial 16S rRNA were restricted using enzymes CfoI, MspI, and RsaI, and products from archaeal 16S rRNA were restricted using AluI, MspI, and RsaI. Restriction enzymes were selected based on in silico digestion of ~100 sequences per gene, representing a cross section of microbial phyla, downloaded from the NCBI website (data not shown). Restriction products were purified using illustra Sephadex™ columns (GE Healthcare, Piscataway, NJ). The fungal ITS PCR products were not restricted and were instead analyzed for length heterogeneity (LH-PCR). Both terminal restriction fragment length polymorphism (T-RFLP) and LH-PCR profiles were generated by Oregon State University's Center of Genome Research and

Biocomputing using an ABI 3100 capillary DNA sequencer (Applied Biosystems, Foster City, CA).

Cloning and Sequencing

Clones were generated using a Topo TA cloning™ kit for sequencing (Invitrogen, Carlsbad, CA) according to manufacturer's instructions. For each of the three genes one PCR reaction was performed per plot, products from the three replicate plots were combined, and a single cloning reaction was used for each site × treatment (n=12). For the fungal and archaeal libraries 24 clones were selected from each cloning reaction resulting in 96 clones per gene. For the bacterial library 35 clones were selected from each cloning reaction resulting in 140 clones per gene. Clones were plasmid purified using QIAprep™ Spin Miniprep kit (Qiagen, Carlsbad, CA) and sequenced by the High Throughput Genomics Unit (Dept. of Genome Science, University of Washington, Seattle, WA).

Statistical and Phylogenetic Analysis

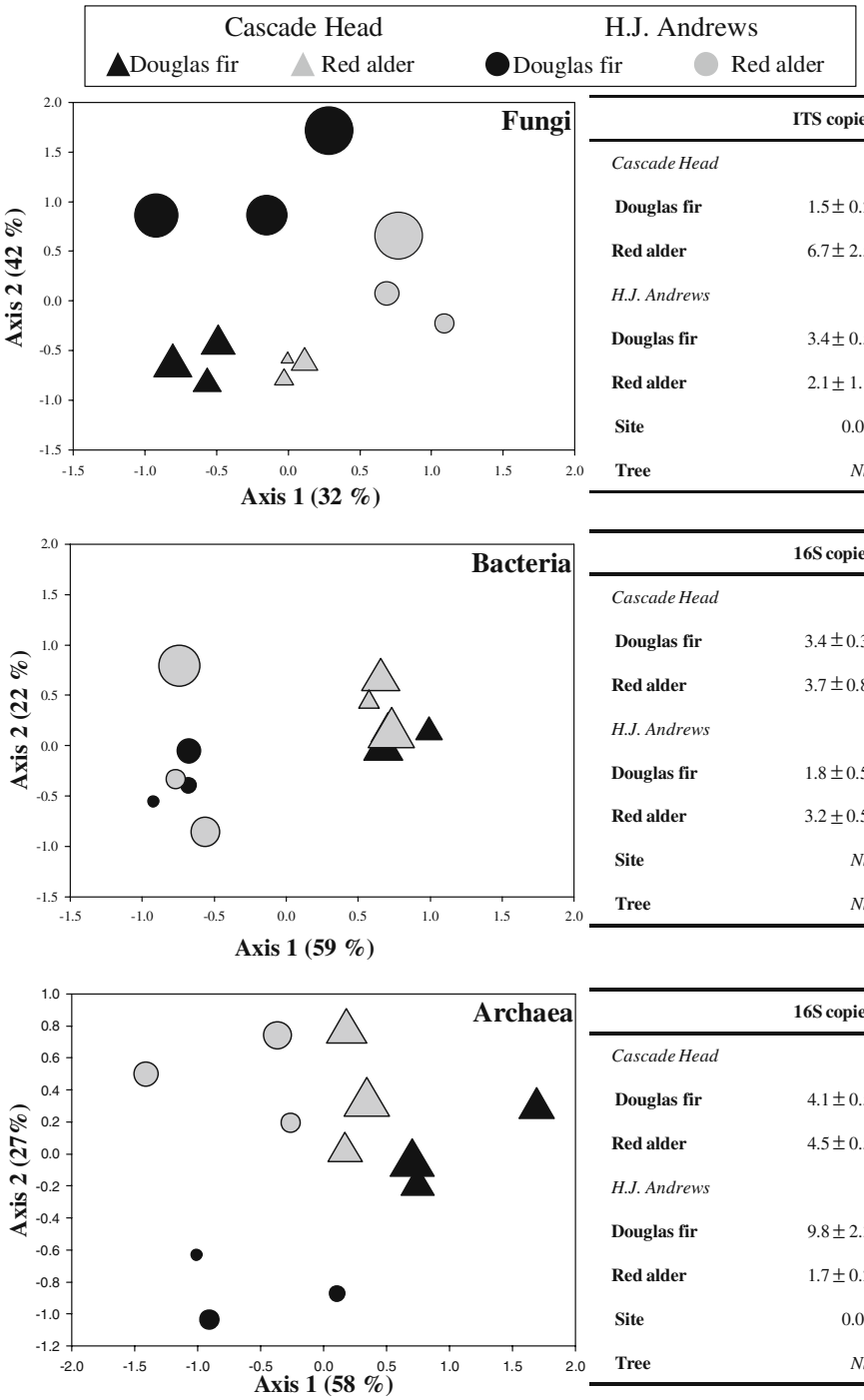
T-RFLP and LH-PCR profiles were analyzed using GenoTyper version 3.7 (Applied Biosystems, Foster City, CA). T-RFLP and LH-PCR profiles were further analyzed according to methods described previously [4]. Community composition was investigated using PC-ORD Multivariate Analysis of Ecological Data version 4.06 (MjM software, Gleneden Beach, OR). Non-metric multidimensional scaling (NMS) was used to visualize community compositional variation by the Relative Sørensen measure and NMS constrained to two-dimensions with a stress threshold <10 [38]. Blocked multi-response permutation procedures (MRBP) were used to determine differences between site and tree species [38]. MRBP randomizes each row of a distance matrix and compares it to the original matrix to test the likelihood of treatment variations. Results include A statistics which are a measure of within-group variability and p values (an A statistic=1 indicates no variability within sample groups) [38]. Indicator species analysis was performed to determine which fragments contributed to treatment differences [38]. Indicator species analysis identifies the members of each community that occur in treatments at a relatively higher rate than could occur by chance (random distribution is calculated using a Monte Carlo test). Finally, Mantel tests were used to test for significant correlations between community data sets [38]. Here, distance matrices are calculated for each of the communities and tested against a random distribution to determine if two communities are more highly correlated than could occur by chance.

DNA sequences were aligned using ClustalX version 1.81 [59] and alignments were edited using Bioedit sequence alignment editor version 7.0.5 [23]. Identification of sequences was made using the NCBI Blast search engine. Identification of 16S sequences was confirmed using the software and information from the Ribosomal Database Project [9]. All sequences were analyzed using Mallard Version 1.02 (Cardiff University, Boston, MA) to ensure that no chimeras or other sequencing anomalies occurred. In cases where Mallard flagged potential chimeric sequences, the first and second halves of each sequence were blasted to test for the same match. When the two halves had different matches, they were excluded from the data set. Phylogenetic trees were constructed using Mr. Bayes Version 3.1 [27, 51] and confirmed using Phylip Version 3.2 [14]. Mr. Bayes was run using an omega variation model (M3), a codon model that calculates the likely rate of variation at each site. The model was run for 1 million generations to ensure convergence at a stable value [22]. Sequences were further analyzed by calculating richness estimates and examining the numbers of operational taxonomic units (OTUs) using the DOTUR program [55]. All sequences were submitted to Genbank and assigned the following acquisition numbers: fungi GU366661–GU366750, bacteria GU366751–GU366879, and archaea GU366880–GU366972.

Results and Discussion

Fungal Community Composition

Fungal community fingerprints clustered based on site and dominant tree species (Fig. 1). MRBP statistics indicated that fungal profiles differed significantly between sites (A statistic=0.1 and p=0.009), and also separated by tree species (A statistic=0.0 and p=0.009). There were significantly greater numbers of ITS copies g^{-1} soil in HJ Andrews' soils compared to Cascade Head, but gene copy numbers did not differ between tree species (Fig. 1). Although a significant difference in ITS copies g^{-1} soil might be indicative of differences in fungal population size, the magnitude of this difference is hard to discern because the number of ITS regions can vary from several hundred to a few thousand copies per genome across different fungal lineages [52]. Community profiles were also examined for the presence of indicator fragments, in other words, those LH-PCR peaks differing significantly between sites or tree species. Profiles contained a single indicator fragment for tree species, which was significantly higher in red alder compared to Douglas-fir soils. Unfortunately, this fragment was not identified through sequencing. Five indicator fragments were found between the two sites. One fragment was not identified through sequencing, but the others are described below.



	ITS copies g^{-1} soil
Cascade Head	
Douglas fir	$1.5 \pm 0.2 \times 10^8$
Red alder	$6.7 \pm 2.5 \times 10^7$
H.J. Andrews	
Douglas fir	$3.4 \pm 0.5 \times 10^8$
Red alder	$2.1 \pm 1.1 \times 10^8$
Site	0.055
Tree	*NS*

	16S copies g^{-1} soil
Cascade Head	
Douglas fir	$3.4 \pm 0.3 \times 10^{10}$
Red alder	$3.7 \pm 0.8 \times 10^{10}$
H.J. Andrews	
Douglas fir	$1.8 \pm 0.5 \times 10^{10}$
Red alder	$3.2 \pm 0.5 \times 10^{10}$
Site	*NS*
Tree	*NS*

	16S copies g^{-1} soil
Cascade Head	
Douglas fir	$4.1 \pm 0.5 \times 10^9$
Red alder	$4.5 \pm 0.5 \times 10^9$
H.J. Andrews	
Douglas fir	$9.8 \pm 2.3 \times 10^8$
Red alder	$1.7 \pm 0.2 \times 10^9$
Site	0.003
Tree	*NS*

Figure 1 Non-metric multidimensional scaling ordinations of fungal LH-PCR and bacterial and archaeal T-RFLP profiles. Each point represents an experimental plot coded by treatment and sized to show the relative size of the population as determined by Q-PCR. Tables give the mean gene copy numbers for each treatment (n=3) and ANOVA results for population differences between sites and tree species (*NS* not significant)

A clone library was constructed to look for dominant fungal lineages and to help in phylogenetic identification of indicator fragments. A total of 96 clones were sequenced. Four sequences were excluded because of chimeras and other anomalies, leaving 92 clones for analysis: 24 from Cascade Head red alder, 23 from Cascade Head Douglas-fir, 22 from HJ Andrews red alder, and 23 from HJ Andrews Douglas-fir. At a 99% similarity threshold there were 69 unique OTUs representing 27 fungal families. These families included four families of ascomycetes that had greater than four sequenced representatives: *Helotiaceae*, *Hyaloscyphaceae*, *Pyronemataceae*, and *Trichocomaceae* (Table 2). Sequences were analyzed in silico for fragment lengths and matched to LH-PCR peaks. The indicator fragment putatively identified as belonging to the families *Pyronemataceae* and *Trichocomaceae* was significantly higher in HJ Andrews' than Cascade Head soils (p=0.002; Table 2). Fungi in the family *Pyronemataceae* are known to have diverse trophic strategies [48] and sequences matched both mycorrhizal and saprobic environmental isolates. Fungi in the family *Trichocomaceae*

Table 2 Dominant fungal groups identified through cloning and sequencing. LH-PCR fragments were determined in silico and matched to ITS LH-PCR profiles to estimate relative abundance of each family within a treatment

		Relative Fluorescence			
	LH-PCR	Cascade Head		HJ Andrews	
Fungal groups	(bp)	Red alder	Douglas-fir	Red alder	Douglas-fir
Ascomycetes					
Helotiaceae[a]	593	1.9±1.0	0	0	13.0±3.2
Hyaloscyphaceae	600	1.7±1.0	6.6±2.8	0	0
Pyronemataceae, *Trichocomaceae*	627	0	0	1.7±0.9	14.8±3.1
Basidiomycetes					
Atheliaceae	667	12.6±4.3	0	0	0
Hygrophoraceae[a]	593	1.9±1.0	0	0	13.0±3.2
Filobasidiales	683	8.3±3.3	14.5±1.8	0	0.6±0.6
Tremellales	553	16.7±2.5	19.5±5.2	2.2±1.2	3.0±0.1

Relative fluorescence values are the mean ± SE (n=3)

[a] Sequences matching *Helotiaceae* and *Hygrophoraceae* both had lengths of 593 bp

included commonly described saprobes including *Penicillium* and *Aspergillus*. Fungal sequences putatively identified in *Helotiaceae* and *Hygrophoraceae* had fragment lengths of approximately 593 bp and could not be distinguished using the LH-PCR methods.

LH-PCR fragment lengths of 553 bp (p=0.004) and 683 bp (p=0.004) were identified as indicator species for Cascade Head soils (Table 2). These fragments were putatively identified to match two species of *Cryptococcus*. One fragment most closely matched *Cryptococcus terriocola*, which belongs to the order *Filobasidiales* and the second most closely matched *Cryptococcus podzolicus* belonging to the order *Tremellales*. Twenty-five percent of the clones recovered from Cascade Head soils matched these two *Cryptococcus* species and fungal profiles contained a mean relative fluorescence of 29±3% of these two indicator peaks.

Our findings suggest that basidiomycetous yeast may represent a large portion of the fungal community in Cascade Head soils. The andic soil at Cascade Head is a nutrient-rich matrix, low in pH, and typically well-drained in spite of large annual rainfall. Previous studies have shown that basidiomycetous yeasts are ubiquitous in soils [3, 7, 10, 63]. Vishniac [63] reported a high abundance of *C. podzolicus* in tropical cloud forest soils (pH of 4.8), and observed that a correlation existed between the abundance of *C. podzolicus*, increased annual rainfall, and net primary productivity. These conditions may favor populations of basidiomycetous yeast that likely function in organic matter decomposition. Recently, using 454 sequencing Buée et al. [7] reported *Cryptococcus* as the second most dominant sequence identified in temperate hardwood forests. Maksimova and Chernov [36] used culturing techniques to study soil yeast diversity and reported the dominance of *C. terriocola* and *C. podzolicus* in forest soil mineral layers under bilberry (*Vaccinium myrtillus*)-spruce (*Picea abies*), birch (*Betula* sp.), and alder (*Alnus* sp.). The role of these aerobic, unicellular fungi in soil ecosystems is unclear. Shubakov [56] reported that *C. podzolicus* cultures are able to degrade xylose and other polymeric carbohydrates.

Bacterial Community Composition

Cascade Head bacterial communities differed from HJ Andrew communities (Fig. 1), as confirmed by MRBP statistics (A statistic=0.4 and p<0.001,). Profiles did not separate by dominant tree species (A statistic=−0.1 and p=0.506). Similarly, bacterial 16S gene copy numbers g^{-1} soil did not differ between sites or tree species, but tended to be lower in Douglas-fir plots at HJ Andrews in comparison to the other three treatments (Fig. 1).

Several fragments were identified using indicator species analysis that significantly differed between Cascade Head and HJ Andrews communities. These included six terminal restriction fragments (T-RFs) that were dominant in at least one soil type and were identified through sequencing. A total of 132 clones were used in bacterial sequence analysis (eight sequences were excluded because of possible chimeras). Six clones were matched to each indicator T-RF. Indicator fragments for each of these bacterial groups were summed and the means for each treatment were compared (Fig. 2). Although it is doubtful that our sequencing could identify all T-RFs belonging to members of *Acidobacteria*, *α-proteobacteria*, and *δ-Proteobacteria*, we chose to use these T-RFs as putative indicators of these major bacterial groups.

CfoI 277 and CfoI 293-294 were found in all soil profiles and averaged 11±1% and 7±1% respectively across all soils. These TR-Fs were found in four and seven clones, respectively, all matching the phylum *Acidobacteria*. The relative fluorescence of putatively identified *Acidobacteria* peaks did not vary between the two sites, but made up a significantly greater proportion of bacterial

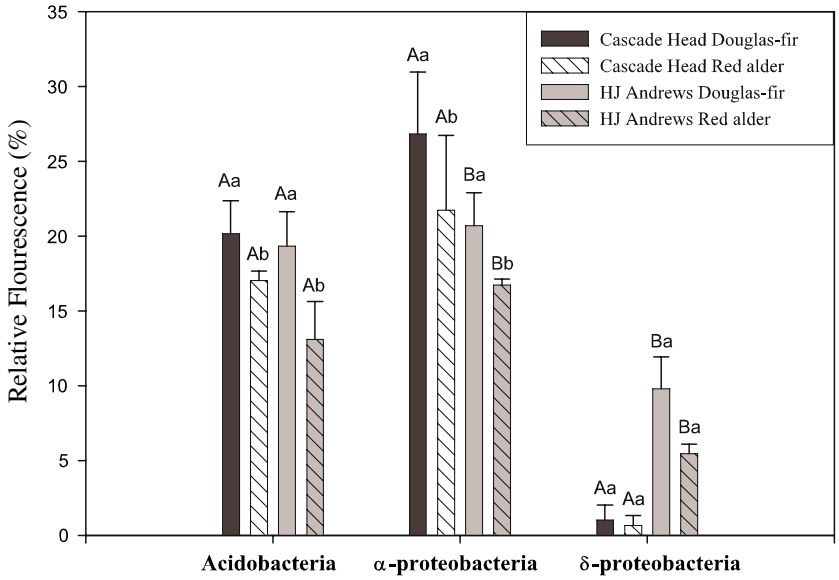


Figure 2 Relative fluorescence of bacterial indicator fragments across the four experimental treatments. Indicators include: acidobacterial T-RFs CfoI 277and CfoI 293–294; α-proteobacterial T-RFs MspI 146, RsaI 419, and RsaI 421–422; and δ-proteobacterial indicators RsaI 471–472. *Bars* represent treatment means (n=3) of the sum of group indicator fragments with SE bars. *Capital letters* represent site differences and *lowercase letters* represent differences in dominant vegetation type (p<0.05)

profiles in Douglas-fir compared to red alder soil (p=0.033; Fig. 2).

A large majority of sequences matching *α-proteobacteria* belonged to the order *Rhizobiales*, with sequences clustering into the families *Bradyrhizobiaceae* and *Hyphomicrobiaceae*. Few sequences could be matched to a lower taxonomic level, but four sequences had >90% similarity to species of *Bradyrhizobium*. Surprisingly, *Bradyrhizobium* was the only N-fixing bacteria identified in clone libraries, even though red alder is a host for *Frankia*. Host plant species for *Bradyrhizobium* were present at both sites (i.e., species of *Trifolium* and *Lathyrus*), but no attempt was made to quantify or characterize the presence of these understory species. Three T-RFs matched members of the subphylum *α-proteobacteria* and were present in most soil types: MspI 146 (7±3%), RsaI 419 (5±1%), and RsaI 421–422 (12±1%). The sum of the *α-proteobacterial* indicators varied significantly between the two sites (p=0.032) and was nearly significant between the dominant tree species (p=0.058), with the largest proportion found in the Douglas-fir treatments at Cascade Head.

RsaI 471 was present in HJ Andrews' soils and averaged 8±1% of the total relative fluorescence. RsaI 471 was significantly greater in HJ Andrews soils compared to Cascade Head soils (p=0.008; Fig. 2). Four clones containing this TR-F matched environmental clones of *δ-proteobacteria*. Phylogenetic analysis revealed no close associations to cultured representatives of *δ-proteobacteria* (data not shown). The closest known sequences belonged to the order *Myxococcales* (43% sequence similarity). The high diversity [65] and varied functionality within the *δ-proteobacteria*, including complex cell communication and sulfate reduction, make it impossible to assign a putative function to the current group of uncultured bacteria.

Archaeal Community Composition

T-RFLP profiles for the archaeal 16S separated in ordination space both by site and by tree species (Fig. 1). MRBP analysis separated the communities statistically by site (A statistic=0.2 and p=0.003) and by tree species (A statistic=0.2 and p=0.007). Even more dramatic than compositional shifts, however, were significant differences found in the archaeal 16S gene copy numbers between the two sites. Cascade Head soils contained approximately fourfold more archaeal 16S copy numbers g^{-1} soil compared to HJ Andrews' soils, with archaeal 16S accounting for 11±1% of the total prokaryotic population (Fig. 1). Kemnitz et al. [32] reported a similar percentage in acidic forest soils with mixed deciduous tree species, whereas researchers studying agricultural soils reported a much lower proportion (<1%) [47].

All 16S archaeal clones were putatively placed into four subgroups of Crenarchaeota: 1.1a-associated, 1.1b, 1.1c, and 1.1c-associated (Fig. 3) [42]. The majority of clones clustered within the *Crenarchaea* 1.1c. In silico analysis of sequences revealed good agreement in T-RFs for each group. 1.1a-associated sequences all contained cut site AluI 223; all 1.1b sequences contained RsaI

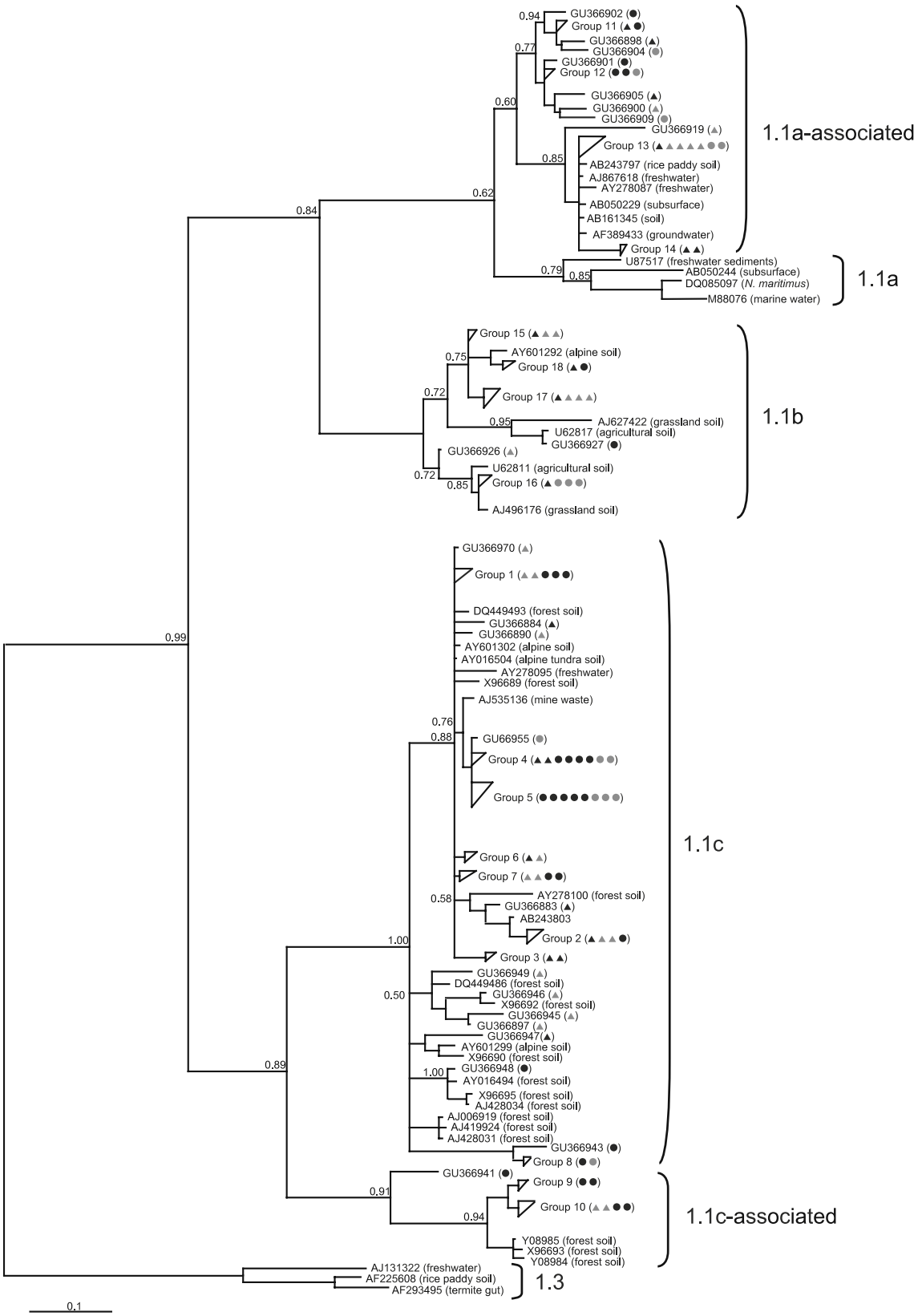


Figure 3 Archaeal 16S rRNA tree constructed using Bayesian analysis. Numbers show the probability of clade assignments. Cluster assignments are made based on Nicol et al. [42] with sequences from this study appearing in bold. When a single sequence represents an OTU the clone number is given, in all other cases group assignments 1–18 show OTUs with multiple sequences. *Triangles* represent clones from Cascade Head, circles from the HJ Andrews, *black symbols* represent Douglas-fir, and *gray symbols* denote red alder clones

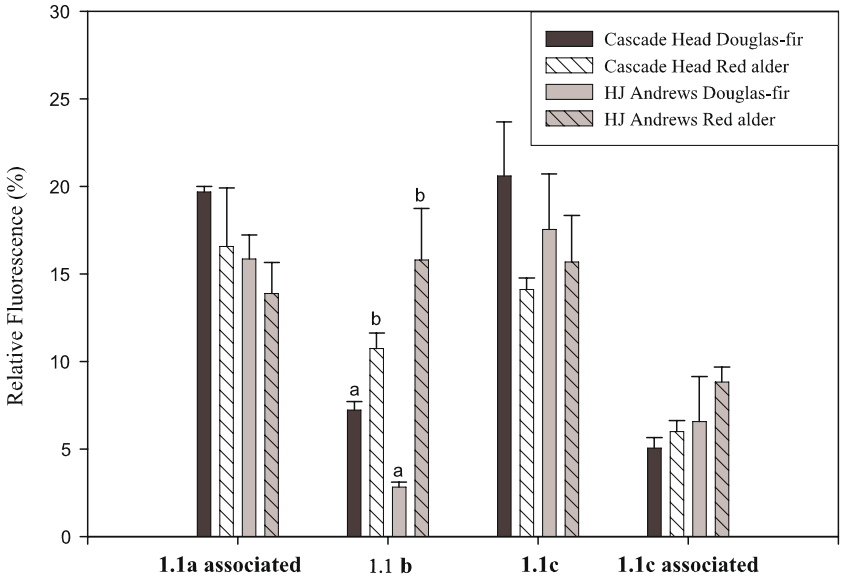


Figure 4 Relative fluorescence of archaeal indicator fragments across the four experimental treatments. Indicators are as follows: *1.1a-associated* AluI 223, *1.1b* RsaI 260–262, *1.1c* RsaI 90, and *1.1c-associated* RsaI 290. *Bars* represent treatment means (n=3) with SE bars. *Lowercase letters* represent differences in dominant vegetation type (p<0.05)

260-262; 17 clones putatively identified as 1.1c contained RsaI 90, and all 1.1c-associated sequences contained RsaI 290.

Indicator fragments for *Crenarchaea* 1.1a-associated and 1.1c made up the largest fractions of relative fluorescence (16±1% and 17±1%, respectively), but did not significantly differ between sites or by tree species. *Crenarchaea* 1.1c have been shown previously to dominate acidic, forest ecosystems [32, 42], but have not been linked to specific functions. *Crenarchaea* 1.1b indictor T-RFs were significantly greater in red alder (13±2%) compared to Douglas-fir soil (5±1%; Fig. 4). Previously, we reported a similar difference in ammonia-oxidizing archaeal composition (based on *amoA* T-RFLP) between red alder and Douglas-fir [6]. Although archaeal *amoA* was linked to the *crenarchaea* 1.1b group in a soil fosmid [61], the extent to which *amoA* genes are distributed among the *Crenarchaea* is unclear.

Compositional and Environmental Correlations

A set of standard measurements that included C and N content, C:N ratio of microbial biomass, and pH were taken for each soil plot (Table 1) [5]. These soil properties are not an exhaustive list and cannot capture the complexity of microhabitats experienced by soil microorganisms. Rather, these select properties are meant to provide information on bulk soil characteristics that may contribute to microbial community differences. For each microbial community, an NMS distance matrix was created that could be correlated to soil properties (Table 3). Fungal community composition significantly correlated with all soil properties measured (Table 3). The most significant correlations were found between composition and pH and gravimetric water content (Table 3). Because of dramatic differences in soil properties between the two sites and a number of co-variables, we cannot definitively determine which of these factors, if

Table 3 Correlations between community profiles of fungi, bacteria, and archaea and soil properties

Soil property[a]	Fungi		Bacteria		Archaea	
	R	*p* value	*R*	*p* value	*R*	*p* value
Total soil C (49–176 g kg^{-1} soil)	−0.70	0.012	−0.54	NS	0.12	NS
Total soil N (2.6–12.3 g kg^{-1} soil)	−0.77	0.003	−0.57	NS	0.00	NS
NO_3^- –N (0.7–12.2 mg kg^{-1} soil)	−0.64	0.025	−0.42	NS	−0.20	NS
NH_4^+ –N (0.5–5.4 mg kg^{-1} soil)	−0.67	0.018	−0.87	<0.001	−0.16	NS
Soil C:N ratio (9.2–40.1)	0.62	0.033	0.64	0.025	0.18	NS
Microbial Biomass C:N (11.9–24.4)	0.62	0.030	0.75	0.005	−0.29	NS
pH (3.6–5.3)	0.89	<0.001	0.68	0.014	−0.10	NS
Gravimetric H_2O (53–120%)	0.89	<0.001	−0.79	0.002	0.02	NS

R the Pearson correlation coefficient with two sided *p* values compared to a random distribution (N=12). *NS* not significant, *p* value>0.05

[a] Soil properties have been published previously [5], the range across all experimental treatments are given in parentheses

Table 4 Mantel test results of correlations between community profiles of fungi, bacteria, and archaea. Distances matrices were calculated for each set of profiles and compared. *p* values were determined by Monte Carlo tests

Comparison	R^2	*p* value
Fungal ITS x bacterial 16S	0.56	<0.001
Fungal ITS x archaeal 16S	0.48	<0.001
Bacterial 16S x archaeal 16S	0.36	0.003

any, had the greatest effect on fungal composition. It is possible that different fractions of the fungal community (i.e., filamentous vs. yeast forms) will be preferentially affected by a variety of environmental factors, although to our knowledge no studies have addressed this question.

Bacterial composition was also significantly correlated to a number of soil properties (Table 3); the strongest correlation was to soil NH_4^+ concentration. Bacterial composition was not significantly correlated to soil C, N, or NO_3^- concentrations, however (Table 3). Similar to previous reports [34, 54], bacterial community composition was correlated to pH.

Unlike fungal and bacterial composition, no correlations could be found between archaeal community composition and any of the measured soil parameters (Table 3). Oline et al. [46] observed a similar lack in correlations between archaeal composition and soil properties. Given the significant differences we observed between archaeal composition at the two experimental sites and under the different vegetation types, this result was surprising. The lack of correlation between archaeal composition and bulk soil properties underscores the need to better understand microhabitat structure, especially in the interest of studying these yet uncultured microorganisms.

Using the distance matrices for each of the community profiles, we also examined data for correlations between the community structures of major groups of microorganisms. All three microbial groups were significantly correlated (Table 4). The correlations between the community structure of fungi, bacteria, and several soil properties suggest the composition of both communities is in part determined by the same soil factors. Interestingly, although the structure of the archaea did not correlate to these soil properties they did correlate to bacteria and to a slightly greater extent to the fungal communities. It has recently been suggested that the ability to carry out ammonia oxidation may be widely distributed across the Group 1 *Crenarchaea* [43]. Therefore, we also compared the 16S archaeal T-RFLP profiles to those of the archaeal *amoA* from Cascade Head [6], but found that the profiles did not correlate (p=0.489). The *Crenarchaea* 1.1c and 1.1c-associated, which have not been linked to nitrification genes, accounted for 20–25% of the archaeal abundance (Fig. 4) and may have masked any correlation between the *amoA* T-RFLPs and groups such as the *Crenarchaea* 1.1b. The results may indicate that only a subpopulation of the *Crenarchaea* possesses *amoA*. An alternative conclusion may be that 16S and archaeal *amoA* gene families do not have congruent tree topology, although Nicol et al. [43] compared 16S archaeal phylogenetic trees to archaeal *amoA* trees and reported good agreement between the two genes.

Conclusion

We observed microbial community compositional and abundance differences between the two ecoregions, dominant vegetation types, and soil properties. All three communities differed between the two experimental sites suggesting that environmental factors help to shape unique communities of fungi, bacteria, and archaea. In the case of fungi and bacteria, these factors include concentrations of mineral N, soil pH, and water content. Presumably, archaeal compositional differences also arise from differences in soil properties, but we were unable to uncover the main drivers for these community memberships. We eagerly await isolation of the first pure culture of soil-borne *Crenarchaea* to study the phenotypes and gain a better understanding of their niche.

In the N-rich, acidic soils at Cascade Head our community characterization revealed a fungal community with a high abundance of unicellular, basidiomycetous yeast. Future research will focus on these microorganisms in an attempt to learn more about their distribution and functional significance. A relatively large population of archaea was also found in the soils at Cascade Head. From previous research, we know that the genes for archaeal ammonia oxidation are present at Cascade Head [6] and that archaea are likely to play an important role in soil N cycling under acidic conditions [43]. With archaeal populations of 10^9 gene copies g^{-1} soil and representing 11% of the prokaryotic population, they are also likely to be important in soil C cycling. Greater abundance of *Crenarchaea* 1.1b under red alder compared to Douglas-fir also suggests a linkage between this group and the dominant tree species. These observations make Cascade Head and HJ Andrews interesting soil ecosystems that could yield valuable insights into the functional diversity of forest soil archaea.

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