Microbial Ecology

Responses of Nitrification and Ammonia-Oxidizing Bacteria to Reciprocal Transfers of Soil between Adjacent Coniferous Forest and Meadow Vegetation in the Cascade Mountains of Oregon

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Abstract

Despite the critical position of nitrification in N cycling in coniferous forest soils of western North America, little information exists on the composition of ammonia-oxidizing bacteria (AOB) in these soils, or their response to treatments that promote or reduce nitrification. To this end, an experiment was conducted in which a set of soil cores was reciprocally transplanted between adjacent forest (low nitrification potential) and meadow (high nitrification potential) environments, at two high-elevation (~1500 m) sites in the H.J. Andrews Experimental Forest located in the Cascade Mountains of Oregon. Half of the cores were placed in screened PVC pipe (closed) to prevent new root colonization, large litter debris inputs, and animal disturbance; the other cores were placed in open mesh bags. A duplicate set of open and closed soil cores was not transferred between sites and was incubated in place. Over the 2-year experiment, net nitrification increased in both open and closed cores transferred from forest to meadow, and to a lesser extent in cores remaining in the forest. In three of four forest soil treatments, net nitrification increases were accompanied by increases in nitrification potential rates (NPR) and 10- to 100-fold increases in AOB populations. In open cores remaining in the forests, however, increases in net nitrification were not accompanied by significant increases in either NPR or AOB populations. Although some meadow soil treatments reduced both net nitrification and nitrification potential rates, significant changes were not detected in most probable number (MPN)-based estimates of AOB population densities. Terminal restriction fragment profiles (T-RFs) of a PCR-amplified 491-bp fragment of the ammonia monooxygenase subunit A gene (*amo*A) changed significantly in response to some soil treatments, and treatment effects differed among locations and between years. A T-RF previously shown to be a specific biomarker of *Nitrosospira* cluster 4 (*Alu*390) was widespread and dominant in the majority of soil samples. Despite some treatments causing substantial increases in AOB population densities and nitrification potential rates, nitrosomonads remained undetectable, and the nitrosospirad AOB community composition did not change radically following treatment.

Introduction

Although ammonia-oxidizing bacteria (AOB) of the genus *Nitrosospira* are thought to play the major role in nitrification in most agricultural and grassland soils, the role of AOB in nitrification in coniferous forest ecosystems remains poorly understood. Several forest soil factors such as acidity, low NH_4^+ availability, high C availability, competition for NH_4^+ with plants and heterotrophic microbes, and the presence of allelochemical compounds are generally considered detrimental to AOB in these environments and to the production of NO_3^- [12]. Nonetheless, isotope dilution studies have shown that nitrification occurs at significant rates, and that NO_3^- production and consumption are tightly coupled in soils under coniferous forests in western North America [11, 40]. Although N cycling is normally tightly coupled in these soils, net N mineralization has been shown to increase substantially in response to disturbances such as clear cutting [37, 38], small changes in temperature [14], and long-term laboratory incubation of forest soil [13]. It

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remains uncertain, however, to what extent changes in net NO_3^- production are due to changes in AOB populations and their associative nitrifying activity, versus changes in rates of heterotrophic microbial NO_3^- assimilation responding to the status of labile carbon pools [13].

Recently, a picture has emerged of the composition of AOB across transects between high elevation meadow and coniferous forest sites in the Oregon Cascade Mountains [27]. Although AOB population sizes and nitrifying activities differed dramatically between adjacent meadow and forest environments, T-RFLP molecular profiles of ammonia monooxygenase subunit A (*amo*A) PCR-amplified from soil showed *Nitrosospira* cluster 4 to be widely distributed throughout both meadow and forest sites at two locations. In contrast, biomarkers associated with other *Nitrosospira* clusters were less widely distributed and generally comprised a smaller portion of the PCR-amplified *amo*A amplicons than did cluster 4 biomarkers. Most *Nitrosospira* isolates in culture collections worldwide belong to cluster 3, whereas cluster 4 isolates are rare and physiologically uncharacterized [31].

Although several studies have shown that AOB communities differ among differently managed agricultural and grassland soils [21, 42], and that community composition can change in response to N inputs [1, 16, 29], liming [2], and soil temperature [1], no studies have been conducted on AOB dynamics in coniferous forest soil ecosystems of the western USA.

The objective of this study was to examine the changes that occur in nitrification, and AOB community size and composition in response to experimental manipulation of high-elevation meadow and coniferous forest soils. We hypothesized that the microclimate changes associated with exposing high-elevation forest soils to meadow conditions, and vice versa, would promote differential changes in rates of N mineralization, NH_4^+ availability, and nitrification. Furthermore, we hypothesized that either allowing or preventing roots/mycorrhizae/soil animals from recolonizing soil cores would differentially affect the availability of NH_4^+ for AOB. Indeed, while plants and heterotrophic microbes are usually considered to be competitive with AOB for NH_4^+ and reduce nitrification, plants have also been shown to promote N mineralization and enhance the availability of NH_4^+ [28, 32, 41, 43]. Therefore, we designed a core transfer experiment in which open and closed cores were reciprocally transferred between adjacent meadow (high nitrification potential) and forest (low nitrification potential) sites. Because we had no background information to indicate how long it would take for treatment effects to manifest themselves in the field, sufficient cores were placed to allow sampling annually over a 2-year period.

Materials and Methods

Site Description. The study sites were located in the H.J. Andrews Experimental forest (44.2°N, 122.2°S) in the Cascade Mountains of Oregon, USA. At high elevations (~1500 m) on south-facing slopes, well-drained open areas of meadow vegetation are interspersed among coniferous forests. Two locations, hereafter referred to as Carpenter and Lookout, were chosen because of the close proximity of open areas and forest vegetation. Aspects and slopes were 210°SW/50% and 180°S/35% at Carpenter and Lookout, respectively. The soils at both sites are poorly developed, well drained, and rich in organic matter. Meadow soils ranged from pH 5.5 to 5.8 and are classified as lithic cryandepts; forest soils ranged from pH 5.0 to 5.2 and are classified as pachic haplumbrepts. Meadow vegetation consists primarily of grasses and forbs, with bracken fern also being a major component at Carpenter, and leguminous plants being a major component at Lookout. Douglas fir, silver fir, and grand fir were the dominant tree species at both sites, averaging about 50 y and 95 y old at Lookout and Carpenter, respectively. The experimental sites have been described in detail elsewhere [27, 33].

Experimental Setup. In September 2000, a 35 m × 35 m grid was established in each meadow and forest area at both Lookout and Carpenter. The grid consisted of 64 sample locations evenly spaced at 5-m intervals. Positions within the array were randomly assigned. Each grid accommodated 12 cores of each of four treatments: cores remaining in place (open and closed), hereafter abbreviated OR and CR, and cores transferred from one environment to the other (open and closed), hereafter abbreviated OT and CT. Prior to excavation, the organic surface layers of the forest and meadow soils were carefully scraped away to expose the mineral soil. Closed cores (6 × 15 cm) were collected in PVC pipe and either put back in place (CR) or transferred (CT). The upper end of the pipe was covered with 2-mm gauge window screen in an attempt to prevent inputs of large litter debris, and animal disturbance. Open cores were excavated in a similar fashion but were extruded from the pipe and placed into mesh bags constructed of window screen. The mesh bags were stapled shut and either put back in place (OR) or transferred (OT) into the assigned holes in the core field. Cores were recovered 1 and 2 years later. A set of background cores was also taken at each sampling.

Field Sampling. In September 2001 and 2002, soil cores were removed from their specific locations in the core field. Six cores were excavated from each treatment. To ensure that sufficient soil was available for the different assays carried out in the study, the six cores were

randomly grouped into three pairs of cores, and the soil from each pair was thoroughly mixed to produce three analytical replicates of each field treatment. Soil was brought back to the laboratory on the same day of sampling, stored overnight at 4°C, and sieved to <4.75 mm while field moist. Gravimetric water content was determined on subsamples of soil. In 2002, root material was recovered from the soil cores, washed, and dried at 65°C to determine treatment effects on root dry weight. In both years the following parameters were measured.

Mineralizable Nitrogen. Portions of soil (50 g) were added to mason jars and water contents adjusted to about two-thirds of water holding capacity. At weekly intervals over a 4-week period, portions of soil (10 g) were removed, extracted in 2 M KCl, and analyzed for NH_4^+ and NO_3^- with an autoanalyzer [27].

Nitrification Potential Rates (NPR). A shaken soil slurry procedure was followed as described elsewhere [15]. Soil slurries (1:10 w/v) were incubated at room temperature (~23°C). Aliquots were recovered at time intervals over 24–28 h and NO_3^- plus NO_2^- measured with an autoanalyzer. NO_2^- levels were below the limit of detection. Rates of NO_3^- formation were calculated using linear regression analysis.

Most Probable Number (MPN)-Based Determinations of the AOB Population Densities. A single composite soil sample was prepared from the three replicate soil samples of each field treatment. Of each composite sample, 5-g portions were diluted into 45-mL volumes of ammonia oxidizer growth medium lacking NH_4^+ [35]. Aliquots (1 mL) of soil dilutions (10^{-1} to 10^{-7}) were inoculated into four replicate tubes of growth medium containing 1 mM NH_4^+ and were incubated at room temperature in the dark. After 2, 3, and 4 months, aliquots were removed from each tube and assayed for NO_2^-. No further increases in the number of NO_2^- positive tubes were detected beyond 3 months of incubation. After scoring the NO_2^--positive tubes, population estimates were determined from published MPN tables after taking into account the soil water content [44]. In MPN-based estimates of population size, the estimate is multiplied or divided by a confidence factor (CF) to establish the upper and lower confidence intervals ($P = 0.05$) of the population density. The value of CF is influenced primarily by the dilution ratio (10-fold) and the number of replicates used per dilution ($n = 4$). In this study a CF of 3.8 was calculated [44].

Community Composition of AOB. DNA was extracted from soil (0.3 g) using a Fast DNA spinkit for soil (Bio101, Carlsbad, CA) and amplified by PCR with *amo*A primers [31]. The forward primer was fluorescently labeled with 6-Fam, and PCR products (*amo*A amplicons) were recovered from gels and purified using the Wizard DNA purification system (Promega, Madison, WI). Community fingerprints (i.e., TRFLP profiles) were determined by digesting 10 ng of *amo*A amplicon mixtures with three restriction enzymes (*Cfo*1, *Alu*1, and *Taq*1). Size and relative abundance of terminal restriction fragments (T-RFs) were quantified using Genescan version 3.5 (Applied Biosystems).

Statistical Analyses. Soil nitrogen pools and microbial activities were analyzed by analysis of variance (ANOVA) methods. The effect of disturbance was analyzed separately by comparing background with open remaining cores. The factorial arrangement of site (Lookout vs Carpenter), soil origin (forest vs meadow), and core type (background vs open remaining) was first analyzed using repeated measures ANOVA to examine the effect of sampling in different years. Significant interactions between year of sampling and one or more other treatment factors were found for all soil nitrogen pools and microbial activities. Therefore, years were analyzed using separate factorial ANOVA. These analyses also found significant interactions between site and the other factors, which led us to analyze each site separately for each year.

The treatment effects of site, soil origin, incubation location, and core type were analyzed using the data from the open and closed, remaining and transferred cores (i.e., background cores were excluded). The factorial arrangement of site, soil origin, incubation environment, and core type was first analyzed using repeated measures ANOVA to examine the effect of year of sampling. Significant interactions between year of sampling and one or more other treatment factors were found for all soil nitrogen pools and microbial activities. Therefore, years were analyzed individually using separate factorial ANOVA. Because of the complexity of this multiple factorial design, we examined only specific contrasts when the ANOVA showed significant ($p < 0.05$) interactions or main effects. These contrasts were the effect of site (Carpenter vs Lookout), soil origin (forest vs meadow), incubation location (forest vs meadow), or core type (open vs closed).

AOB community composition data were analyzed using PC-ORD version 4.01 (B. McCune and J.J.Mefford, PC-ORD for Windows: multivariate analysis of ecological data, 4.01ed, MjM Software, Gleneden Beach, OR, 1999), a multivariate statistical package. Multiresponse permutation procedures (MRPP) were used to test significant differences in the proportional abundance of T-RFs among treatments. MRPP is a nonparametric method for testing group differences, similar to multivariate analysis of variance (MANOVA). Unlike MANOVA, MRPP does not assume normal distribution, making it a more

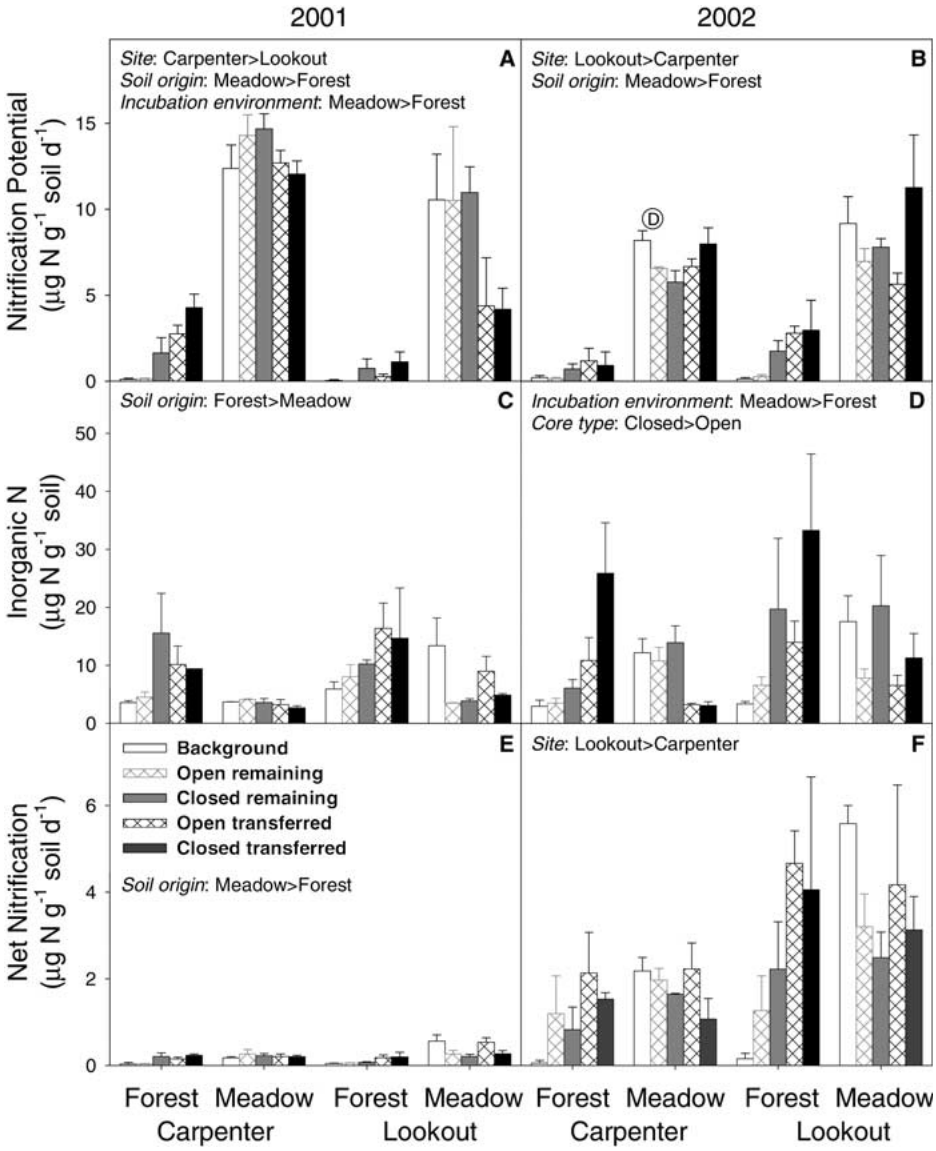

Figure 1. Treatment effects on N processes: (a) 2001 nitrification potential, (b) 2002 nitrification potential, (c) 2001 inorganic N concentration, (d) 2002 inorganic N concentration, (e) 2001 net nitrification, and (f) 2002 net nitrification. Significant main effects of site, soil origin, environment, and core type are listed within each of the panels ($p < 0.05$). A significant disturbance effect, designated by the circled D (Fig. 1B), occurred with 2002 nitrification potential in Carpenter Meadow ($p < 0.05$). Error bars represent standard errors of the mean, $n = 3$.

appropriate choice for community data. *P*-values and *A*-statistics were calculated by comparing real data to 1000 random iterations. *A*-statistics are a test of within group variability; for community data, *A*-statistics >0.1 are considered significant. Indicator species analysis was used in determining specific T-RFs that contributed to statistically significant treatment effects [26].

Results

Soil Properties and Activities

Temporal Changes. There was little year-to-year variation in inorganic N concentrations, net nitrification, and nitrification potentials in background cores (Fig. 1). This was also true for the nitrification potential of treated cores (Fig. 1 A,B). Although significant interactions between treatments and year of sample preclude statistical comparison, the twofold increases in inorganic N concentrations and eightfold increases in net nitrification of treated cores in 2002 compared to 2001 suggested that treatment effects developed with time (Fig. 1 C–F).

Disturbance Effects. The effect of simply taking a core was evaluated by comparing the background and open remaining cores. Of the 24 comparisons of soil properties and activities, only one significant effect was observed: the nitrification potential of the open remaining cores in Carpenter Meadow sampled in 2002 was lower than their associated background cores (Fig. 1B). Given a *p*-value of 0.05, this one significant difference might have occurred by chance. Therefore we conclude that the coring procedure used in establishing the treatments in this experiment had no statistically significant impact.

Site Differences. In 2001 only nitrification potential showed a significant difference between the two sites, being higher at Carpenter compared to Lookout (Fig. 1A). This site effect reversed itself in 2002, with nitrification potential and net nitrification being significantly higher at Lookout than Carpenter, a trend that was also observed for inorganic N (Fig. 1 B,F).

Soil Origin. The most common significant effect was that of soil origin. In general, nitrification potential and net nitrification were higher in meadow soils than forest soils, regardless of where they were incubated (Fig. 1 A,B,E,F). This effect was statistically significant in both years for nitrification potential and in 2001 for net nitrification. Curiously, the ranking of the two soil types switched for inorganic N concentrations, which were higher in the forest than meadow soil, significantly so in 2001 (Fig. 1 C). This was because of the higher NH_4^+ concentrations in the forest soils (data not shown).

Environment. Two significant effects of environment were observed. In 2001, nitrification potential was greater in soil incubated in the meadow than soil incubated in the forest and, in 2002, a similar environmental effect was observed for inorganic N (Fig. 1 A,D).

Core Type. We included the open vs closed core treatment to examine primarily the influence of plant roots on microbial activities and communities. No effect of core type was seen in 2001, but a significant effect was manifest in 2002, which may reflect the time needed for the open cores to become recolonized by roots. In the second year, there was a general affect of core type on inorganic N, with greater concentrations in closed compared to open cores (Fig. 1D). This may be caused by the accumulation of more inorganic N in the absence of plant N uptake. Nitrification potentials were also consistently higher in closed vs. open cores at the Lookout site in 2002, but this effect was not statistically significant (Fig. 1B).

Table 1. Treatment effects on MPN estimates of AOB population densities

Soil origin	Treatment	2001	2002
		$\times 10^3$ (cells/g soil)	
CF	CT	15.9	61.4
CF	OT	6.1	54.7
CF	CR	35.9	159.3
CF	OR	6.1	2.3
CF	Bk	1.6	0.2
CM	CT	ND	35.9
CM	OT	ND	21.3
CM	CR	ND	23.1
CM	OR	ND	11.2
CM	Bk	15.9	11.2
LF	CT	61.4	112.4
LF	OT	11.2	23.1
LF	CR	23.1	61.4
LF	OR	9.3	0.2
LF	Bk	6.1	0.1
LM	CT	ND	35.9
LM	OT	ND	23.1
LM	CR	ND	61.4
LM	OR	ND	23.1
LM	Bk	54.7	54.7

MPN values are divided into and multiplied by a confidence factor of 3.80 to establish lower and upper confidence limits ($P = 0.05$), respectively. ND: not determined.

AOB Population Densities. After 1 y, substantial increases in AOB population densities were measured in closed core treatments of forest soils at both locations (Table 1). Population densities in open cores remained similar to background. Further statistically nonsignificant increases occurred between year 1 and 2 in CR, OT, and CT treatments of both forest soils. Interestingly, in the case of open cores that remained in the forest, net nitrification increased substantially at both sites between years 1 and 2, yet neither NPR nor MPN population estimates increased. MPN estimates of AOB population densities of meadow soils remained stable over 2 y, despite net nitrification declining in several treatments.

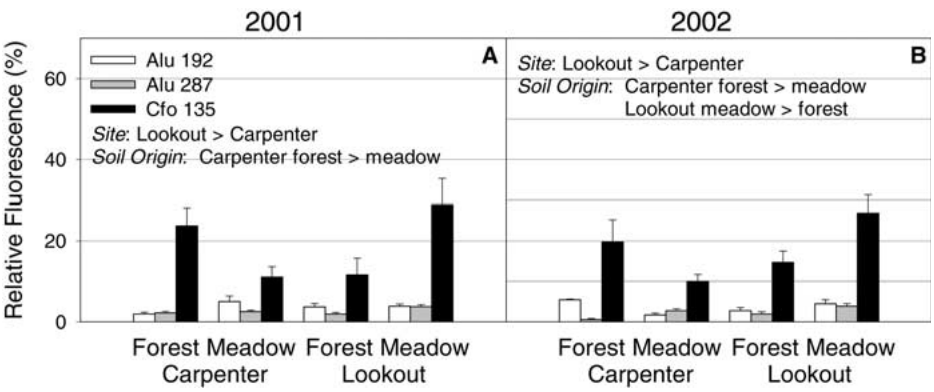

Figure 2. Representative T-RF abundances for (A) 2001 and (B) 2002. Values represent the mean of the four treatments grouped by soil origin. Error bars represent the standard error of the mean.

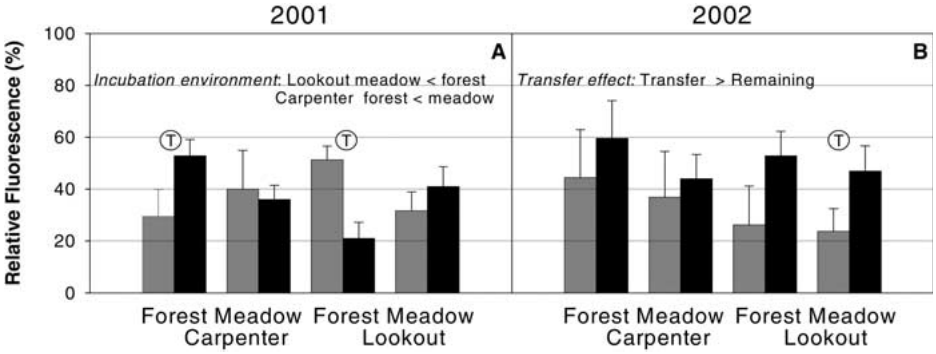

Figure 3. T-RF *Alu* 390 abundances in remaining (gray) and transferred (black) soil cores for (A) 2001 and (B) 2002. Significant transfer effects are represented by circled T. Values represent the mean of the three replicates of each treatment. Error bars represent standard error of the mean values.

AOB Population Composition. AOB composition was affected significantly by soil origin and specific treatments in specific years. After 1 y, site differences were observed between Lookout and Carpenter meadows ($p = 0.017$) and Carpenter meadow and forest ($p = 0.031$). The proportional abundances of *Alu* 287 and *Cfo* 135 were greater in Lookout meadow than Carpenter meadow (Fig. 2A), while the abundance of *Cfo* 135 was twice as large in Carpenter forest as Carpenter meadow (Fig. 2A). After 2 y, the proportional abundances of *Alu* 287 and *Cfo* 135 remained higher in Lookout than Carpenter meadows (Fig. 2B). Communities were markedly different across vegetation types at both sites during 2002. At Carpenter, forest and meadow differences were highly significant ($p < 0.001$) with *Cfo* 135 and *Alu* 192 being greater in the forest than meadow samples, whereas *Alu* 287 occurred in greater abundance in the meadow than forest. Similarly, communities differed between vegetation types at Lookout, where *Alu* 192, *Alu* 287, and *Cfo* 135 were all greater in meadow soil community profiles than in forest soil.

In addition to the soil origin differences, location of incubation also affected AOB community composition. At both sites, soils that were incubated in the meadow differed from those that were incubated in the forest (Carpenter, $p = 0.002$; Lookout, p value $= 0.004$). These differences occurred over the entire community, but can be observed specifically in *Alu* 390, where fragment abundance was greater in cores incubated in Carpenter meadow (Fig. 3A). In contrast, at Lookout the abundance of *Alu* 390 was greater in forest than meadow locations (Fig. 3A), and this particular T-RF was also a significant indicator species ($p = 0.003$).

Treatment effects were observed in particular years and sites. Table 2 lists *p*-values and *A*-statistics for significant treatment effects found using MRPP. Disturbance was assessed by comparing background cores to open remaining. Transfer differences refer to comparisons of remaining and transfer cores, and core effects compare open vs closed cores. After 1 y, disturbance and transfer effects were observed for Lookout forest soil. The transfer effect can be clearly seen for *Alu* 390, where the proportional abundance of this fragment was greater in cores that remained in the forest compared to those transferred to Lookout meadow (Fig. 3). A significant transfer effect also occurred in 2001 Carpenter forest soil. In this case, *Alu* 390 was greater in cores that were transferred to the adjacent meadow. In contrast, there was no evidence of transfer effects on AOB composition in either Lookout or Carpenter forest soils after 2 y. In the case of meadows, a significant transfer effect was observed in Lookout Meadow in 2002 that involved a higher abundance of *Alu* 390 in transferred than in remaining cores (Table 2).

Discussion

Our data clearly illustrate that after 2 y of exposing high-elevation (~1500 m) forest soils to changes in vegetation and microclimate, net nitrification rates and AOB population densities had increased to values that were similar to those measured in the neighboring meadow soils. The

Table 2. *P* values and *A*-statistics from MRPP analysis of AOB communities by soil origin[a]

Year	*Site*	*Disturbance*	*Transfer*	*Core*
2001	Lookout Forest	<0.001 (0.44)	0.001 (0.35)	—
	Carpenter Forest	—	0.029 (0.14)	—
2002	Lookout Forest	—	—	—
	Carpenter Forest	—	—	—
2001	Lookout Meadow		—	0.05 (0.08)
	Carpenter Meadow	—	—	—
2002	Lookout Meadow	—	0.020 (0.13)	—
	Carpenter Meadow	—	—	—

[a]See Materials and Methods for details of statistical analyses.

nitrification response time corresponded well with another study conducted at the H.J. Andrews Experimental Forest where large increases in soil solution NO_3^- (0 to 10 cm depth) appeared between 1 and 2 y after clear cutting a low elevation (~500 m) old-growth Douglas-fir stand [37, 38]. Furthermore, an independent reciprocal core transfer study conducted at higher latitude in the Alaskan tundra also showed changes in nitrification potentials, net nitrification, and N mineralization, 12 months after transfer of N-rich alder and N-poor poplar forest floors between sites [10].

Although there were only a few statistically significant treatment effects over the 2-y study, several distinct trends were apparent in the data. For example, after 2 y, nitrification potential and net nitrification rates had developed more extensively in soil cores that were transferred from forest to meadow than in cores that remained in the forest. We speculate that the microclimate of the south- and southwest-facing meadows played a major role in this effect. For example, it is well known that mean soil temperatures can be 4 to 5°C higher in clear-cut areas than under adjacent coniferous stands, and that daily surface soil temperature can fluctuate by as much as 10 to 15°C in clear cuts compared to 1 to 2°C in soil under mature stands of trees [8, 9]. Furthermore, other workers have shown that N mineralization in high elevation forest soils doubled in response to modest changes in temperature (~1 to 4°C) when soil cores were transferred from high to low elevation [14, 19]. A number of reports have appeared recently highlighting the sensitivity of microbial-driven processes (including nitrification) to small changes in soil temperature in various ecosystems [4, 17, 20, 36]. In the specific context of nitrification, several studies have shown that the temperature profiles of nitrification activity differ widely among soils from different places in the world [24, 25, 39]. A recent study has shown community changes in AOB composition of soil in response to temperature shifts [1]. Furthermore, ammonia oxidation by different *Nitrosospira* strains responded quite differently to increases in temperature between 15 and 30°C [18]. Additional work is needed to determine the temperature profiles of N mineralization, immobilization, and nitrification in our study system.

Although evidence was obtained that AOB population size, composition, and nitrification rate changed over time in response to some treatments, we gained no insight into the specific time of year when treatment effects might have manifested themselves. It is reasonable to speculate that the nitrification changes coincided with soil conditions immediately after snowmelt, when the combination of soil moisture and temperature are conducive for microbial activity and plant growth. Both microbial community structure and activities have been shown to change during the period between snow melt and the dry summer soil conditions at Niwot ridge in the Rocky Mountains of Colorado [22, 23, 34]. Other work from the same location has shown that significant microbial activity can occur during the winter months under the snow pack [5–7]. Although the elevations of our experimental sites are quite low compared to Niwot ridge, snow routinely covers the ground at this site from late November to mid June at 1500 m. We believe that further studies are warranted to examine in more detail at what season treatment effects on nitrification rates and AOB composition were manifested in this experimental system.

Our data raise interesting questions about the possible interactions between vegetation type and microclimate on nitrification and AOB composition. In particular, the detection of both disturbance and transfer effects on the AOB composition of Lookout forest soil, and a transfer effect on Carpenter forest AOB composition in year 1 is worthy of comment (Table 2). We hypothesize that the potential of both forest soils to mineralize N and increase available NH_4^+ was probably enhanced after their transfer to the exposed south-facing meadow environments. In addition, disturbance of the Lookout plant communities by soil coring and severing roots probably reduced the plant efficacy as an NH_4^+ sink, thereby promoting differential growth among AOB. Indeed, both a wide range of growth rates and growth/temperature responsiveness have been documented in AOB [3, 18, Taylor and Bottomley, unpublished observations]. At this time, however, our hypothesis must be considered a tentative one. Although some workers have shown increased contributions of *Nitrosomonas* spp. and/or *Nitrosospira* sp. cluster 3 to soil AOB communities in response to soil management styles that enhance nitrification [16, 21, 29], other workers found no detectable differences in AOB community composition in soils that differed in vegetation, N inputs, nitrification rates, and AOB population densities [30]. Similarly, we obtained no evidence for either the appearance of *amo*A biomarkers of *Nitrosomonas* spp., or for statistically significant changes occurring in relative abundances of biomarkers of *Nitrosospira* clusters 3 and 4 in enrichments of NO_2^- positive terminal soil dilutions from soil treatments where AOB population densities had increased by ~1000-fold above background. Although it would be interesting to physiologically compare isolates that had proliferated so extensively with isolates from treatments where no proliferation was observed, that remains easier said than done. Several studies have shown that only a small fraction (0.1–10%) of soil AOB proliferate under traditional enrichment conditions [21, 30].

Similar to our previous study [27], we consistently found differences in the AOB communities between soils recovered from forests and meadows. These trends were mirrored by differences in nitrification potentials; how-

ever, there were no correlations between indicator biomarkers, such as *Alu*281 and *Cfo*135, and nitrification potentials. This may be because many of the significant biomarkers were present at low abundances and therefore were not numerous enough to affect activity measurements. Although we routinely detected treatment-induced changes in T-RFs that were previously determined to be *amo*A biomarkers of *Nitrosospira* cluster 4 (*Alu*390), cluster 3 (*Alu*491), and of uncultured AOBs (*Alu*491/*Cfo*135) [27], these changes were not accompanied by elimination of any major T-RFs, nor did minor T-RFs ever achieve dominant status. Our data indicate these nitrosospirad communities are well adapted to nitrifying in this type of ecosystem regardless of the rate of N mineralization and whether or not NO_3^- production and consumption are effectively coupled.

Further studies are needed to examine the composition/function relationships of chemolithotrophic AOB with other microorganisms involved in nitrification and NO_3^- assimilation in coniferous forest soils.

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