RESEARCH ARTICLE

# Effects of long-term fertilization of forest soils on potential nitrification and on the abundance and community structure of ammonia oxidizers and nitrite oxidizers

Sophie Wertz, Adam K.K. Leigh & Sue J. Grayston

Department of Forest Sciences, University of British Columbia, Vancouver, BC, Canada

**Correspondence:** Sophie Wertz, Agriculture and Agri-Food Canada, Potato Research Centre, PO Box 20280, 850 Lincoln Rd, Fredericton, New Brunswick E3B 4Z7, Canada. Tel.: +1 506 452 4033; fax: +1 506 452 3316; e-mail: sophie.wertz@agr.gc.ca

Received 25 May 2011; revised 2 September 2011; accepted 6 September 2011.
Final version published online 10 October 2011.

DOI: 10.1111/j.1574-6941.2011.01204.x

Editor: Tillmann Lueders

**Keywords**
lodgepole pine; spruce; nitrogen fertilizer; potential nitrification; DGGE; quantitative PCR.

## Abstract

Forest fertilization in British Columbia is increasing, to alleviate timber shortfalls resulting from the mountain pine beetle epidemic. However, fertilization effects on soil microbial communities, and consequently ecosystem processes, are poorly understood. Fertilization has contrasting effects on ammonia-oxidizing bacteria and archaea (AOB and AOA) in grassland and agricultural ecosystems, but there are no studies on AOB and AOA in forests. We assessed the effect of periodic (6-yearly application 200 kg N $ha^{-1}$) and annual (*c.* 75 kg N $ha^{-1}$) fertilization of lodgepole pine and spruce stands at five long-term maximum productivity sites on potential nitrification (PN), and the abundance and diversity of AOB, AOA and *Nitrobacter* and *Nitrospira*-like nitrite-oxidizing bacteria (NOB). Fertilization increased AOB and *Nitrobacter*-like NOB abundances at some sites, but did not influence AOA and *Nitrospira*-like NOB abundances. AOB and *Nitrobacter*-like NOB abundances were correlated with PN and soil nitrate concentration; no such correlations were observed for AOA and *Nitrospira*-like NOB. Autotrophic nitrification dominated (55–97%) in these forests and PN rates were enhanced for up to 2 years following periodic fertilization. More changes in community composition between control and fertilized plots were observed for AOB and *Nitrobacter*-like NOB than AOA. We conclude that fertilization causes rapid shifts in the structure of AOB and *Nitrobacter*-like NOB communities that dominate nitrification in these forests.

## Introduction

A catastrophic, climate-induced outbreak of the mountain pine beetle is currently occurring in interior British Columbia (B.C.), Canada, with a record 16.3 million hectares of pine forest showing signs of attack (B.C. Ministry of Forests and Range, 2010). As an intervention strategy, large-scale application of fertilizers to forest soils in B.C. has been implemented to accelerate growth of existing stands and shorten rotation times, to overcome future projected timber shortfalls.

Forest fertilization has variable effects on soil microbial biomass in the short term (Prescott *et al.*, 1992; Hart & Stark, 1997; Forge & Simard, 2001); but in the long term, it often results in decreased microbial biomass and activity (Ohtonen, 1992; Smolander *et al.*, 1994; Périé & Munson, 2000). A few studies, however, have investigated the effect of forest fertilization on microbial community structure (Ohtonen, 1992; Forge & Simard, 2001; Frey *et al.*, 2004); shifts in microbial community composition could potentially lead to unpredictable alterations in critical ecosystem processes, such as nutrient cycling (Schimel, 1995; Balser *et al.*, 2002). One approach to study the effects of fertilization on soil microorganisms would be to monitor changes in the total microbial community and indeed, in agricultural systems, changes in total bacterial community composition in soil amended with N fertilizers have been described (Peacock *et al.*, 2001; Enwall *et al.*, 2007). However, the vast diversity of microorganisms in soil, many with similar functional capabilities,







FEMS MICROBIOLOGY ECOLOGY

means changes in microbial community structure do not necessarily lead to changes in function. Therefore, a better approach is to study the fertilization effects on phylogenetically constrained groups with specific functions, such as autotrophic nitrifiers that are key players in the nitrogen cycle (Prosser, 1989).

Autotrophic nitrification is a two-step oxidation in the conversion of ammonia to nitrate, both steps performed by distinct groups of microorganisms. The first step, oxidation of ammonia to nitrite, is carried out by ammonia-oxidizing bacteria (AOB) and recently discovered ammonia-oxidizing archaea (AOA) (Prosser, 1989; Könneke *et al.*, 2005). The second step, oxidation of nitrite to nitrate, is performed by nitrite-oxidizing bacteria (NOB) (Prosser, 1989). *Nitrobacter* is, to date, the only NOB genus isolated from soils (Pan, 1971; Bock *et al.*, 1983). However, the presence of 16S rRNA sequences belonging to another NOB genus, *Nitrospira*, has been detected in edaphic environments (Freitag *et al.*, 2005; Attard *et al.*, 2010). Some fungi and heterotrophic bacteria are also able to nitrify and have been shown to be the major nitrifiers in some forest ecosystems (Pederson *et al.*, 1999; Jordan *et al.*, 2005).

Most research studying the effects of environmental parameters on nitrifiers has focused on ammonia oxidizers (AOB, and to a lesser extent AOA), as the first step of autotrophic nitrification is often assumed to be rate-limiting (e.g. Webster *et al.*, 2005; Le Roux *et al.*, 2008; Nicol *et al.*, 2008). However, accumulation of nitrite has been shown to occur in soil at high ammonia concentrations, and high pH, through inhibition of nitrite oxidoreductase by $NH_3$ (Prosser & Cox, 1982; Monreal *et al.*, 1986). In addition, nitrite oxidation is more inhibited than ammonia oxidation by steam sterilization of soil (Roux-Michollet *et al.*, 2008) or drought (Gelfand & Yakir, 2008), highlighting the need to assess concurrently the responses of ammonia-oxidizer and nitrite-oxidizer communities to environmental conditions.

Recent studies on agricultural and grassland soils have revealed contrasting effects of N fertilizers on AOB and AOA (Shen *et al.*, 2008; Di *et al.*, 2009, 2010; Jia & Conrad, 2009; Wang *et al.*, 2009). Indeed, addition of fertilizers increased AOB abundance and/or induced changes in AOB community composition, while AOA abundance and/or community structure were unaffected. In addition, nitrification activity was correlated with AOB abundance, but not AOA abundance, suggesting that AOB dominated nitrification in these N-amended soils (Shen *et al.*, 2008; Di *et al.*, 2009, 2010). No study, however, has examined the effect of N fertilization on AOB and AOA in forest environments. Regarding nitrite oxidizers, N fertilizers may affect *Nitrobacter* NOB and *Nitrospira* NOB differently. Studies on NOB from waste-water treatment plants revealed that *Nitrobacter*-like NOB was more dominant under high nitrite concentrations and had lower nitrite affinity than *Nitrospira*-like NOB (Kim & Kim, 2006; Nogueria & Melo, 2006; Blackburne *et al.*, 2007). Only one study has addressed the effect of N fertilization on the diversity of soil NOB communities and demonstrated that the composition of *Nitrospira*, but not the *Nitrobacter*, community was changed following fertilization of grasslands (Freitag *et al.*, 2005). The objective of our study was to determine the potential activity, abundance, and diversity of nitrifying communities in soils from stands of lodgepole pine and interior spruce at five sites subjected to long-term fertilization. The sites were located in different biogeoclimatic zones in British Columbia and had different soil types. At each site, soils from unfertilized control stands and stands receiving two fertilization regimes (periodic and annual) were analyzed. Periodic fertilization corresponded to application of 200 kg N $ha^{-1}$ every 6 years and was initiated at different years between sites. Annual fertilization comprised 25–50% the amount of added N of the periodic application. We determined whether periodic and annual fertilization affected potential nitrification (PN) and the abundance (quantitative real-time PCR, qPCR) and composition (PCR-DGGE) of AOB, AOA, *Nitrobacter*-like NOB and *Nitrospira*-like NOB similarly. For the periodic fertilization regime, we examined over what time period since fertilization, effects on nitrifier activity, abundance, and composition were observed. We also used acetylene inhibition to determine the contribution of autotrophs to PN in these forests. We predicted an increase in the abundance of AOB and *Nitrobacter*-like NOB under both fertilization regimes, contrasting with no or negative impact of fertilizers on the abundance of AOA and *Nitrospira*-like NOB. We also predicted that the periodic fertilization would have a more pronounced effect on PN and the abundance of soil nitrifiers than annual fertilization and that the effect of periodic fertilization on nitrifiers and PN would decline over time. Finally, we investigated whether changes PN rates, and in key environmental factors influencing nitrifiers (i.e. soil ammonium, nitrate, pH), were correlated with changes in nitrifier community abundance and composition. We hypothesized variation in PN rates following fertilization would be related to changes in the abundance and community structure of AOB and *Nitrobacter*-like NOB, but not AOA and *Nitrospira*-like NOB.

## Materials and methods

### Forest sites and soil sampling

The studied sites were part of the 'maximum productivity' field installations (EP 866.13) established in the

1990s by the B.C. Ministry of Forests and Range (Brockley & Simpson, 2004), located within three major biogeoclimatic zones in the interior of B.C. (Meidinger & Pojar, 1991). Two lodgepole pine sites and three interior spruce sites (*Picea glauca* [Moench] Voss and *Picea engelmannii* Parry, or naturally occurring hybrids of these species) were sampled, and detailed site descriptions are given in Table 1. The lodgepole pine sites were Crater Lake (CL, established 1996) and McKendrick Pass (MP, established 1995). The spruce sites comprised Lodi Lake (LL, established 1995), Crow Creek (CC, established 1994), and Hand Lake (HL, established 1999). Each site contained three replicate plots (0.164 ha) of three different fertilization treatments: control (unfertilized), periodic, and annual. Fertilization was undertaken in the spring (May) following establishment (stand ages ranged from 9 to 15 years) and was a mixture of sources of N, S, B, P, K, and Mg (Brockley & Simpson, 2004). Periodic fertilization treatment was applied every 6 years and contained (kg $ha^{-1}$) N (200), S (50), B (1.5), P (100), K (100), and Mg (25). Fertilizer N is supplied as monoammonium phosphate and urea (Brockley & Simpson, 2004). Years of periodic fertilizer application differed between sites (Table 1). In the annual fertilization regime, fertilizers were applied to maintain foliar N at 1.3%, which represented around 25–50% of periodic amount of N (R.P. Brockley, pers. commun.). Monoammonium phosphate, ammonium nitrate, and urea are the sources of N (Brockley & Simpson, 2004).

Soil samples from the five sites were collected from 17 to 21 June 2009 (3 weeks after annual fertilization at all sites and the periodic fertilization at Crater Lake). At each site, soil was sampled in the three replicates plots per treatment. In each plot, 10 cores of 5 cm diameter and 15 cm deep were taken randomly and divided into organic and mineral soil layers. For each layer, the 10 subsamples were pooled and then sieved to 2 mm. Soils were stored for a few days at 4 °C before measurements of soil pH and PN activity. For measurements of ammonium and nitrate concentrations and molecular analysis, subsamples of soil were immediately stored at −20 °C after sieving.

## Soil physicochemical properties

Soil ammonium and nitrate were extracted from subsamples (6 g) of frozen soil, shaken for 45 min with 30 mL of 1 M KCl. The extracts were filtered and analyzed with a Lachat QuickChem FIA+ (8000 series) instrument (Hach Company, Loveland, CO).

Soil pH was measured after shaking samples (10 g) in 20 mL of 10 mM $CaCl_2$.

**Table 1.** Site descriptions

| Site | Tree species | Latitude | Longitude | Year of establishment | Stand age at establishment | Years of periodic fertilization | BEC subzone/ variant* | Site series† | Soil type‡ |
|---|---|---|---|---|---|---|---|---|---|
| Crater Lake (CL) | Pl | 52°50′ | 123°44′ | 1996 | 15 | 1997, 2003, 2009 | MSxv | 01, 04 | Orthic Humo-Ferric Podzol |
| McKendrick Pass (MP) | Pl | 54°49′ | 126°48′ | 1995 | 9 | 1996, 2002, 2008 | ESSFmc | 01, 04 | Eluviated Dystric Brunisol |
| Lodi Lake (LL) | Sx | 53°22′ | 112°06′ | 1995 | 11 | 1996, 2002, 2008 | SBSwk1 | 01 | Eluviated Dystric Brunisol |
| Crow Creek (CC) | Sx | 54°20′ | 126°17′ | 1994 | 10 | 1995, 2001, 2007 | SBSmc2 | 01 | Orthic Humo-Ferric Podzol or Eluviated Dystric Brunisol |
| Hand Lake (HL) | Sx | 54°24′ | 122°53′ | 1999 | 14 | 2000, 2006 | SBSmk1 | 01 | Orthic Dystric Brunisol |

*Biogeoclimatic Ecosystem Classification (BEC) (Meidinger & Pojar, 1991).
†Soil and vegetation types (Banner *et al.*, 1993; DeLong, 2000).
‡Soil Classification Working Group (1998).

## Potential nitrification (PN)

PN assays were performed under aerobic and nonlimiting N substrate conditions, on duplicate samples of soil (2 g) using a soil-slurry method (modified from Hart *et al.*, 1994). To remove initial nitrate, soil samples were shaken in potassium phosphate buffer (1 mM; pH 7.2) for 30 min and collected by centrifugation. Washed soil samples were placed in 125-mL Erlenmeyer flasks and resuspended in 20 mL of the same buffer containing $NH_4Cl$ (200 μg N $g^{-1}$ soil). Erlenmeyer flasks were sealed with rubber stoppers. For half of the samples, 1% (v/v) acetylene was injected into headspaces to block autotrophic ammonia oxidation. Soil slurries were shaken at 20 °C for 72 h. $NH_3$ is the preferred form for uptake for ammonia oxidizers because it can diffuse passively across the cell membrane while $NH_4^+$ requires active transport (Burton & Prosser, 2001). Thus, the pH of the soil slurries was adjusted to 7 with NaOH every 24 h to insure the presence of $NH_3$ in the solution. At 0, 24, 48, and 72 h, aliquots of slurries were centrifuged, supernatants filtered (0.2 μm), and nitrate concentrations determined using Szechrome NAS reagent (Polysciences, Inc., Warrington, PA). PN activity was expressed as the production of nitrate over time.

## DNA extraction and quantitative PCR

DNA was extracted from frozen soil (0.4 g) as described by Dandie *et al.* (2007) and quantified using a picogreen kit (Invitrogen, Burlington, ON, Canada). qPCR was used to measure the abundance of the following communities: AOB, AOA, *Nitrobacter*-like and *Nitrospira*-like NOB. Primers used to target bacterial and archaeal *amoA* genes were, respectively, amoA-1F and amoA-2R (Rotthauwe *et al.*, 1997) and CrenamoA23f and CrenamoA616r (Tourna *et al.*, 2008). *Nitrobacter*-like *nxrA* genes were amplified using primers F1nxrA and R2nxrA (Wertz *et al.*, 2008). 16S rRNA genes of *Nitrospira* were amplified using primers Nspra675f and Nspra746r (Graham *et al.*, 2007), recently used for soil samples by Attard *et al.* (2010). Thermal-cycling conditions were given as follows: 95 °C for 5 min followed by (i) 10 cycles of 94 °C for 30 s, 30 s at specific annealing temperature and elongation at 72 °C for 1 min and followed by (ii) 30 cycles of 92 °C for 30 s, 30 s at specific annealing temperature, and elongation at 72 °C for 1 min. Annealing temperatures were 59 °C for amoA-1F and amoA-2R primers, 57 °C for CrenamoA23f and CrenamoA616r, 58 °C for F1nxrA and R2nxrA, and 64 °C for Nspra675f and Nspra746r. Reactions were conducted using an Applied Biosystems ABI PRISM 7000 thermal cycler (Applied Biosystems, Streetsville, ON, Canada) and SYBR Green detection (Applied Biosystems Power Sybr Green master mix), 0.2 mg $mL^{-1}$ of BSA and 0.4 μM of primers. Standard curves were obtained using three replicates of serial dilutions of linearized plasmids containing cloned bacterial *amoA*, archaeal *amoA*, *Nitrobacter*-like *nxrA*, and *Nitrospira*-like 16S rRNA sequences. Two independent qPCR were run for all samples. Melting curve analysis at the end of all qPCR runs and agarose gel running of qPCR products were performed to check for amplification and specificity of the products. No, or low, inhibitory effects (< 3%) of soil DNA extracts on qPCR amplification were detected when $10^5$ copies of the standards were spiked with the soil DNA.

## DGGE analyses

Community structures of AOB, AOA, and *Nitrobacter*-like NOB were characterized by PCR-DGGE targeting, respectively, the 16S rRNA genes of AOB, archaeal *amoA*, and *Nitrobacter*-like *nxrA*. No molecular tool targeting specifically *Nitrospira*-like NOB is available, to date, to assess the diversity of these microorganisms. 16S rRNA genes of AOB were amplified using a nested-PCR approach (Freitag & Prosser, 2003) with 0.2 μM of primers CTO189f and CTO654r (Kowalchuck *et al.*, 1997) for the first PCR step and primers 357f-GC (containing a GC clamp) and 518r (Muyzer *et al.*, 1993) for the second PCR step. Amplification of archaeal *amoA* genes was performed using 0.4 μM of primers CrenamoA23f and CrenamoA616r; no GC clamp was attached on the primers (Tourna *et al.*, 2008). *Nitrobacter*-like *nxrA* sequences were amplified using two PCR steps and 0.4 μM of primers as described by Wertz *et al.* (2008). Briefly, primary amplification was performed using the same primers as for qPCR. PCR products were then loaded on agarose gels, and gel slices containing the *nxrA* PCR products were excised, crushed in 200 μL of sterile $H_2O$, and incubated at 4 °C overnight to elute the DNA. Second amplification was then carried out from the eluted material with same primers containing a GC clamp added at the 5′ end of the forward primer (Wertz *et al.*, 2008). Thermal-cycling conditions were as described for qPCR, except that an extension step of 10 min at 72 °C was added at the end of the runs. Annealing temperatures were the same as for qPCR for amplifications of archaeal *amoA* and *Nitrobacter*-like *nxrA* sequences. For amplification of 16S rRNA genes of AOB, an annealing temperature of 55 °C was used.

PCR products were loaded onto 8% polyacrylamide gels containing a gradient of 30–60% denaturant, 100% denaturing solution being defined as 7 M urea and 40% formamide. Gels were run for 16 h at 75 V in 1× TAE buffer at 60 °C using the D-code Universal Mutation

Detection System (Bio-Rad, Mississauga, ON, Canada). Gels were stained with Sybr Green (Invitrogen) and then imaged using a UV source system (Typhoon Variable Mode Imager; GE Healthcare, Baie D'Urfe, QC, Canada) and corresponding software (Typhoon Scanner Control v5.0; GE Healthcare). DGGE banding profiles from all soil samples were analyzed using Gel CompariII software (Applied Maths, Kortrijk, Belgium) to obtain matrices consisting of the position and relative intensity of each DNA band.

### Statistical analyses

Because periodic treatment was initiated at different years between sites (Table 1) and because the numbers of pine and spruce sites were different, data from each site were analyzed individually.

One-way analysis of variance (anova) and Tukey's test were performed to determine, for each soil layer (organic and mineral), whether soil ammonium, nitrate, pH, PN, and nitrifier abundances differed between fertilization treatments or between organic and mineral soil layers. anova and Tukey's test were also carried out to determine, for each soil layer under a particular treatment, differences between PN levels measured in the presence or absence of acetylene.

Pearson's test was used to determine whether (i) PN was significantly correlated with soil ammonium, nitrate, or pH and (ii) nitrifier abundances were significantly correlated with PN, soil ammonium, nitrate, or pH.

Two types of data matrices were constructed from DGGE profiles: (i) a matrix consisting of the relative intensity of each DNA band (i.e. ratios of the intensity of each band vs. the total band intensity) and (ii) a binary matrix consisting of the presence/absence of each DNA band. Matrices were then analyzed using PRIMER-E Ltd software (Plymouth, UK). Rank similarity matrices were computed and used to construct nonmetric multidimensional scaling (MDS) representations of similarities in community genetic structure among soil samples. Two-way analysis of similarity (anosim) was performed to test the significance of the treatment effects and of the soil layer effects on the nitrifier community structures.

To determine the correlations between nitrifier community structure and PN, soil ammonium, nitrate or pH, spearman correlation coefficients were computed using Ginkgo software (http://biodiver.bio.ub.es/ginkgo/Ginkgo.htm).

## Results

### PN and environmental variables

Sampling took place 3 weeks after annual fertilization of the five sites and periodic fertilization at Crater Lake, 1 year after periodic fertilization at McKendrick Pass and Lodi Lake, and 2 and 3 years after periodic fertilization at Crow Creek and Hand Lake, respectively (Table 1). PN was, in general, significantly higher in organic than mineral soil layers (data not shown). At Crater Lake, which had received periodic fertilization just 3 weeks before sampling (previous application was 6 years ago), no differences in PN rates were observed between treatments (Fig. 1). PN was higher in organic soil layers in forest stands receiving periodic fertilization than in those stands that had received annual fertilization or were unfertilized, at sites that had been fertilized 1 or 2 years prior to sampling (McKendrick Pass, Lodi Lake, and Crow Creek) (Fig. 1). In contrast, at the Hand Lake site (3 years since periodic fertilization (Table 1), PN activity was similar in the periodic and unfertilized stands, but highest in the stands receiving annual fertilization (Fig. 1).

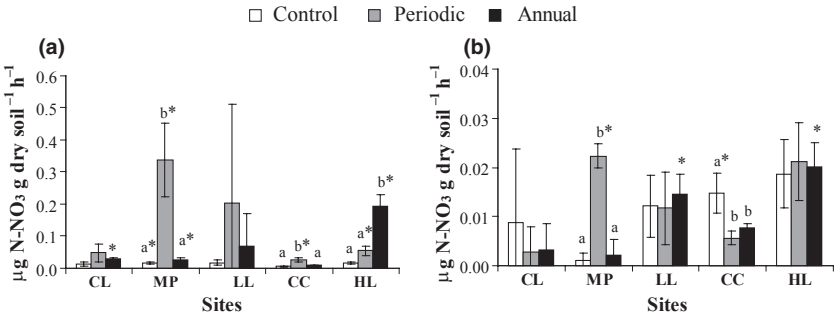


**Fig. 1.** PN in organic (a) and mineral (b) soil layers of plots from Crater Lake (CL), McKendrick Pass (MP), Lodi Lake (LL), Crow Creek (CC), and Hand Lake (HL) sites. Time since last periodic fertilization was 3 weeks for CL, 1 year for MP and LL, 2 years for CC, and 3 years for HL. Letters indicate, for each site, significant differences ($P < 0.05$) between control plots (unfertilized) and fertilized plots (periodic and annual treatments). *When PN measured without acetylene was significantly higher than PN measured in the presence of acetylene (acetylene being used to block autotrophic nitrification).

In treatment plots exhibiting high PN rates and low variability in activity within replicates, the contribution of autotrophic microorganisms to PN was significantly higher than heterotrophs (results of acetylene inhibition tests, Fig. 1) and ranged from 54.6% to 96.9% of the total PN activity.

Concentrations of soil ammonium and nitrate and soil pH are presented in Table S1. Fertilization had weak and inconsistent effects, or no effect, on soil pH (Table S1). PN rate was strongly and positively correlated with soil nitrate concentration at all sites ($R^2$ ranged from 0.83 to 0.97, $P = 0.0001$) except Crater Lake (weak correlation of $R^2 = 0.28$, $P = 0.01$). No correlations were observed between PN and soil ammonium concentration or soil pH, except a weak correlation between PN and ammonium at Hand Lake ($R^2 = 0.23$, $P = 0.04$) and a moderate relation between PN and soil pH at McKendrick Pass ($R^2 = 0.45$, $P = 0.001$).

## Abundance of nitrifier communities

Amplification of archaeal *amoA* sequences and *Nitrobacter*-like *nxrA* sequences by qPCR could not be obtained for samples from all sites (Fig. 2).

Abundances of archaeal *amoA* genes and *Nitrospira*-like 16S rRNA genes did not differ between fertilization regimes in either organic (Fig. 2) or mineral soil layers (data not shown). However, fertilization induced some changes in bacterial *amoA* and *Nitrobacter*-like *nxrA* copy numbers (Fig. 2). At the McKendrick Pass site, similar to PN, abundance of bacterial *amoA* gene was the highest under the periodic fertilization regime in both organic (Fig. 2) and mineral layers (data not shown). At the Hand Lake site, bacterial *amoA* and *Nitrobacter*-like *nxrA* genes in organic soils were more abundant under the annual fertilization treatment, where PN was also the highest, compared to the other treatments (Fig. 2). AOA/AOB *amoA* gene ratios ranged from 0.03 to 110.6 and were < 1 in organic soils that had received periodic and annual fertilization at Lodi Lake and annual fertilization at Hand Lake.

Abundances of archaeal *amoA* genes did not vary between soil layers (data not shown). Bacterial *amoA* gene, *Nitrobacter*-like *nxrA*, and *Nitrospira*-like 16S rRNA gene copy numbers differed inconsistently between soil layers in 33%, 11%, and 13% of the plots, respectively (data not shown).

No correlations were observed between archaeal *amoA* and *Nitrospira*-like 16S rRNA gene copy numbers and

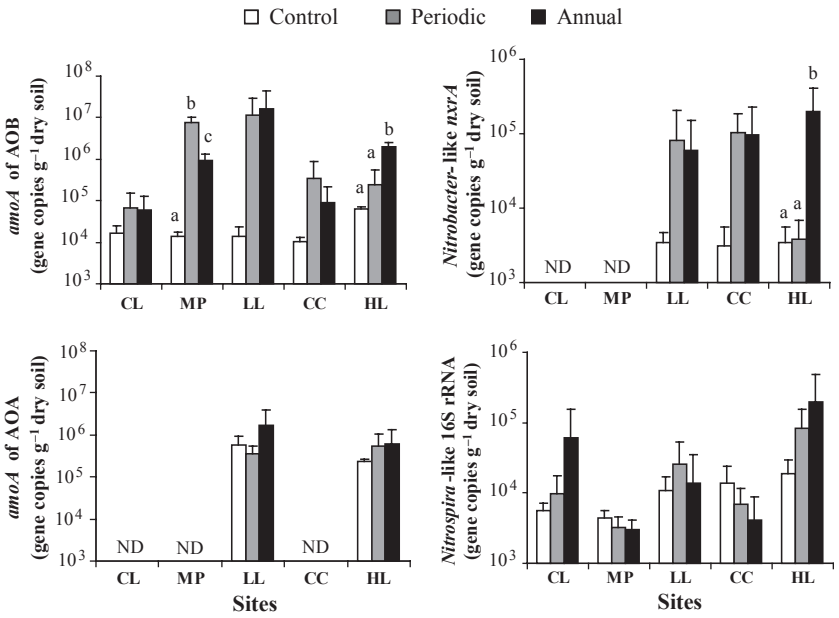


**Fig. 2.** Abundances of *amoA* of AOB, *amoA* of AOA, *Nitrobacter*-like *nxrA*, and *Nitrospira*-like 16S RNA genes in organic soil of plots from Crater Lake (CL), McKendrick Pass (MP), Lodi Lake (LL), Crow Creek (CC), and Hand Lake (HL) sites determined by qPCR. Slopes, efficiencies (E), and $R^2$: *amoA* of AOB gene copy numbers: slope = −3.35 to −3.56, E = 90.7–98.7, $R^2$ = 0.994–0.999; *amoA* of AOA gene copy numbers: slope = −3.69 to −3.80, E = 82.6–86.4, $R^2$ = 0.990–0.998; *Nitrobacter*-like *nxrA* gene copy numbers: slope = −3.32 to −3.38, E = 97.5–99.9, $R^2$ = 0.990–0.995; *Nitrospira*-like 16S RNA gene copy numbers: slope = −3.50 to −3.80, E = 82.9–91.9, $R^2$ = 0.996–0.999. Time since last periodic fertilization was 3 weeks for CL, 1 year for MP and LL, 2 years for CC, and 3 years for HL. Letters indicate, for each site, significant differences ($P < 0.05$) between control plots (unfertilized) and fertilized plots (periodic and annual treatments). ND, gene copies not detected by qPCR.

PN, soil ammonium, nitrate, or pH (data not shown). In contrast, abundances of bacterial *amoA* or *Nitrobacter*-like *nxrA* genes were positively correlated with PN and soil nitrate concentration at all sites except Crow Creek (Table 2). At McKendrick Pass, the abundance of bacterial *amoA* genes was correlated with soil pH (Table 2).

### Nitrifier community structure

Comparisons of community structures were carried out by taking into account (i) in a first analysis the relative intensities of the different bands (Figs 3, S1 and 4) and (ii) in a second analysis the presence/absence of bands (data not shown). Both analyses revealed similar results in terms of fertilization, soil layer, site effects, correlation with PN or edaphic parameters excepted for *Nitrobacter* community structure in Crater Lake.

Differences in AOB community structure between control and fertilized treatments (periodic and/or annual) were observed at all sites excepted Hand lake (Fig. 3). Interestingly, at the Crater Lake site, AOB in plots that had just received the periodic fertilization 3 weeks before sampling exhibited different community structure than the unfertilized control and annually fertilized plots (Fig. 3).

AOA community structure varied between treatments only at Lodi Lake (difference between control and periodic fertilization, $R = 0.25$, $P = 0.04$, Fig. S1).

Community composition of *Nitrobacter*-like NOB differed between the unfertilized and periodic fertilization treatments at the three sites (McKendrick Pass, Lodi Lake, and Crow Creek) where periodic fertilization was applied 1 or 2 years previously (Fig. 4). Differences in *Nitrobacter* community structure between unfertilized and annual fertilization regimes were also observed at these sites (Fig. 4). In contrast, no effect of fertilization on *Nitrobacter* community structure was observed at Hand Lake (last periodic fertilization 3 years ago) (Fig. 4). For Crater Lake (no periodic fertilization for 6 years before recent application), no differences in community composition were observed from analysis, taking into account the relative intensities of the DGGE bands (Fig. 4), while differences between unfertilized plots and plots under periodic and annual treatments were observed when only the presence/absence of bands was compared ($R = 0.39$, $P = 0.008$).

At all sites, the community composition of AOA, AOB, or *Nitrobacter*-like NOB did not differ between soil layers (Figs 3, S1 and 4). However, nitrifier community structure varied greatly between sites (i.e. 74% to 80% of pairwise site comparisons showed significant differences in community structures).

Only a few erratic correlations were observed between nitrifier community structure and PN rates or edaphic parameters (Table 3).

## Discussion

### Effects of timing and intensity of fertilization on PN and nitrifier abundance and community structure

We examined the effect of two forest fertilization regimes, differing in N concentration and frequency of application, on PN and nitrifier abundance and diversity. The periodic fertilization regime consisted of a large N (200 kg $ha^{-1}$) application every 6 years, and the annual fertilization regime consisted of a yearly application of N at 25–50% the concentration of the periodic fertilization. The last periodic fertilizations were applied 3 weeks, or 1, 2 or 3 years before sampling, depending on the site (Table 1). A major goal of this study was, therefore, to

**Table 2.** Pearson coefficients $R^2$ and significance for correlations of community abundance (AOB or *Nitrobacter*-like NOB) with PN, soil ammonium, nitrate, and pH in Crater Lake (CL), McKendrick Pass (MP), Lodi Lake (LL), Crow Creek (CC), and Hand Lake (HL)

| | $R^2$ coefficients ($P$ values) for samples from sites | | | | |
|---|---|---|---|---|---|
| | CL | MP | LL | CC | HL |
| AOB abundance vs. | | | | | |
| PN | **0.22 (0.04)** | **0.51 (0.001)** | **0.59 (0.0002)** | **0.25 (0.03)** | **0.61 (0.0001)** |
| Ammonium | 0.005 (0.78) | 0.001 (0.87) | 0.01 (0.74) | 0.002 (0.85) | 0.19 (0.07) |
| Nitrate | **0.56 (0.0003)** | **0.6 (0.0002)** | **0.75 (0.00004)** | 0.19 (0.06) | **0.65 (0.0005)** |
| pH | 0.04 (0.39) | **0.57 (0.0002)** | 0.06 (0.34) | 0.03 (0.46) | 0.07 (0.27) |
| *Nitrobacter* abundance vs. | | | | | |
| PN | ND | ND | **0.51 (0.001)** | 0.14 (0.12) | **0.41 (0.004)** |
| Ammonium | ND | ND | 0.0002 (0.95) | 0.02 (0.54) | 0.05 (0.34) |
| Nitrate | ND | ND | **0.62 (0.0001)** | 0.1 (0.19) | **0.44 (0.003)** |
| pH | ND | ND | 0.05 (0.38) | 0.09 (0.22) | 0.18 (0.08) |

ND, not determined. Significant values are shown in bold.

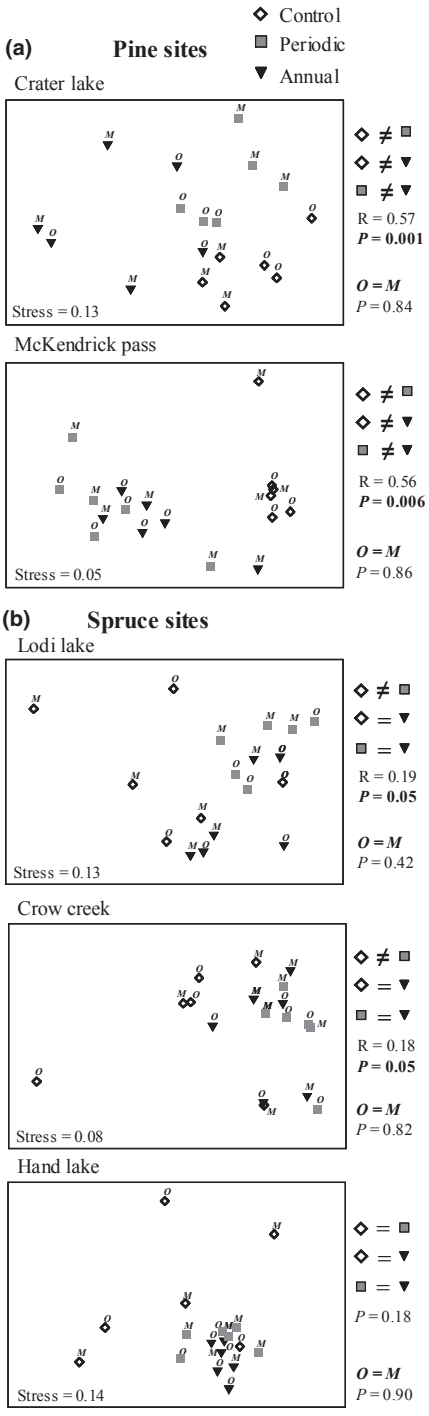


**Fig. 3.** MDS representation of AOB community structure in plots from Crater Lake (CL), McKendrick Pass (MP), Lodi Lake (LL), Crow Creek (CC), and Hand Lake (HL) sites. Similarities or differences in community structure between fertilization treatments (control, periodic, and annual) and soil layers [organic (O) and mineral (M)] are indicated. Time since last periodic fertilization was 3 weeks for CL, 1 year for MP and LL, 2 years for CC, and 3 years for HL.

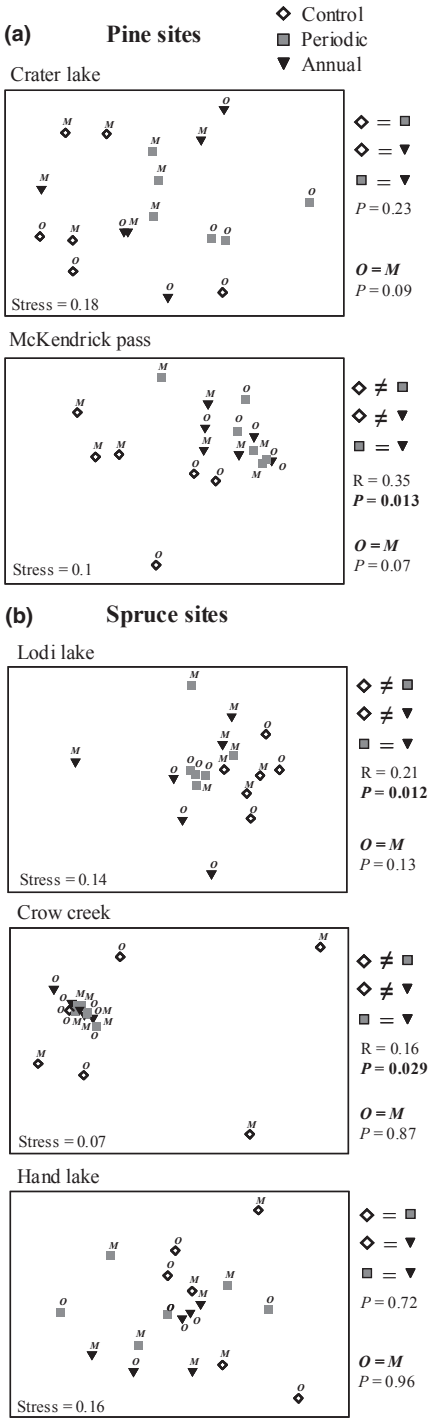


**Fig. 4.** MDS representation of *Nitrobacter*-like NOB community structure in plots from Crater Lake (CL), McKendrick Pass (MP), Lodi Lake (LL), Crow Creek (CC), and Hand Lake (HL) sites. Similarities or differences in community structure between fertilization treatments (control, periodic, and annual) and soil layers [organic (O) and mineral (M)] are indicated. Time since last periodic fertilization was 3 weeks for CL, 1 year for MP and LL, 2 years for CC, and 3 years for HL.

**Table 3.** Spearman coefficients ρ and significance for correlations of nitrifier community structure with PN, soil ammonium, nitrate, and pH in Crater Lake (CL), McKendrick Pass (MP), Lodi Lake (LL), Crow Creek (CC), and Hand Lake (HL)

| | ρ coefficients (*P* values) for samples from sites | | | | |
|---|---|---|---|---|---|
| | CL | MP | LL | CC | HL |
| AOB community structure vs. | | | | | |
| PN | −0.02 (0.49) | 0.08 (0.23) | 0.13 (0.2) | −0.07 (0.32) | −0.11 (0.22) |
| Ammonium | −0.07 (0.25) | **0.17 (0.04)** | 0.1 (0.18) | **0.21 (0.03)** | **0.2 (0.02)** |
| Nitrate | −0.11 (0.22) | **0.5 (0.002)** | **0.37 (0.02)** | 0.13 (0.19) | **0.29 (0.003)** |
| pH | −0.04 (0.39) | 0.09 (0.12) | 0.07 (0.26) | **0.22 (0.03)** | −0.06 (0.39) |
| AOA community structure vs. | | | | | |
| PN | −0.08 (0.36) | 0.27 (0.08) | **0.23 (0.04)** | 0.008 (0.45) | −0.007 (0.57) |
| Ammonium | **0.24 (0.04)** | −0.17 (0.09) | 0.12 (0.12) | 0.05 (0.33) | −0.03 (0.4) |
| Nitrate | 0.18 (0.1) | 0.12 (0.12) | 0.16 (0.85) | 0.03 (0.42) | **0.2 (0.03)** |
| pH | 0.15 (0.12) | 0.01 (0.4) | 0.05 (0.29) | −0.03 (0.42) | −0.17 (0.09) |
| *Nitrobacter* community structure vs. | | | | | |
| PN | −0.03 (0.42) | 0.26 (0.08) | −0.07 (0.36) | −0.03 (0.43) | −0.14 (0.14) |
| Ammonium | −0.03 (0.39) | −0.01 (0.48) | 0.24 (0.03) | 0.24 (0.06) | 0.08 (0.17) |
| Nitrate | 0.06 (0.28) | 0.18 (0.06) | −0.06 (0.42) | **0.42 (0.03)** | −0.33 (0.4) |
| pH | 0.1 (0.12) | −0.02 (0.47) | 0.04 (0.38) | **0.49 (0.001)** | 0.12 (0.22) |

Significant values are shown in bold.

assess how long after application of fertilizers any effects on nitrifier communities last. In forest stands at sites that had received periodic fertilization 1–2 years prior to sampling (i.e. McKendrick Pass, Lodi Lake and Crow Creek), soil PN was enhanced compared to unfertilized stands (significant effect for McKendrick Pass and Crow Creek sites). This increase in PN was always associated with a shift in community structure of AOB and *Nitrobacter*-like NOB at the three sites and a shift in the AOA community structure at Lodi Lake. Abundances of AOB and *Nitrobacter*-like NOB also tended to be higher in soils that had received the periodic fertilization treatment compared to unfertilized controls at these sites, but this was significant only for AOB at McKendrick Pass, probably due to the high variation in abundance data. These findings match well with the previous studies in grasslands and agricultural systems, which have also shown an increase in nitrification or PN rates correlating with an increase in AOB abundance and change in community structure following fertilization (Shen *et al.*, 2008; Di *et al.*, 2009, 2010; Jia & Conrad, 2009). In contrast, at Hand Lake where the last periodic fertilization was 3 years before sampling, PN, nitrifier abundance, and diversity in soils receiving this treatment did not differ from the control. This may indicate an elasticity of nitrifier communities between 2–3 years after fertilization. Le Roux *et al.* (2008) similarly reported a resilience of PN and AOB abundance 12–18 months after cessation of simulated grazing (inducing a reduction in ammonia availability to the levels of ungrazed soils). The more rapid changes in nitrifier communities compared to our study are probably due to a lower N input in the grazing study.

Interestingly, at the site that had only received the periodic fertilization treatment 3 weeks prior to sampling (Crater Lake), although there were no changes in PN and nitrifier abundance between this treatment and the unfertilized controls, the community structure of AOB was already affected by the periodic fertilization treatment. This suggests that a shift in AOB community structure is required before enhancement of nitrification. This is in agreement with Webster *et al.* (2005) who showed a delay of 20–35 days preceding nitrification in natural grasslands following application of urea. They suggested that the delay in nitrification was associated with the presence of nitrifying bacteria that are sensitive to high ammonia concentrations, *Nitrosospira* cluster 3a, and that an increase in relative abundance of high ammonia-tolerant strains belonging to *Nitrosospira* cluster 3b was required to increase nitrification. Le Roux *et al.* (2008) showed that application of simulated grazing (i.e. treatment comprising urine input) led first to changes in AOB community structure, with subsequent increases in AOB abundance and PN activity. However, in contrast to Webster *et al.* (2005), changes in AOB diversity in their study did not correspond to similar shifts between *Nitrosospira* cluster 3a and 3b.

In contrast to the periodic fertilization regime, annual fertilization did not induce increases in soil PN with the exception of the Hand Lake site. The Hand Lake site was the last established and had received annual fertilization for only 10 years, and the other four sites had received 13–15 years of fertilization, so there may have been acclimation to the low annual nitrogen application at the older sites. Higher rates of soil PN and AOB abundance with increasing concentrations of ammonium amendment have

been reported in previous studies (Avrahami & Conrad, 2003; Okano *et al.*, 2004). At Crater Lake and McKendrick Pass, while no differences in soil PN and nitrifier abundances were observed between control and annual fertilization regimes, community composition of AOB varied between these treatments. Community structure of *Nitrobacter*-like NOB also differed between control and annual fertilization regimes at McKendrick Pass, Lodi Lake, and Crow Creek. This demonstrates that communities of AOB and *Nitrobacter*-like NOB with different compositions can exhibit similar PN rates, which is in accordance with the presence of functional redundancy within these communities, as observed by Wertz *et al.* (2006, 2007).

Nitrifier community composition varied greatly between the five sites differing in tree species, soil type, and biogeoclimatic zones. Similar changes between forest sites and tree type have been reported for AOA and AOB (Boyle-Yarwood *et al.*, 2008), suggesting an influence of forest vegetation, soil, and climate on the diversity of nitrifiers. However, in our study, no such differences between sites were observed in terms of nitrifier abundance or activity.

In plots where PN was increased by fertilization, the contribution of autotrophic microorganisms to the activity was higher than heterotrophs (accounting for 54.6–96.9% of the total activity). In previous studies, nitrification in forest soils has been shown to be dominated either by autotrophic (e.g. Martikainen, 1984) or by heterotrophic microorganisms (e.g. Pederson *et al.*, 1999; Jordan *et al.*, 2005), but the dominance of autotrophs or heterotrophs were not correlated with pH or N availability.

PN levels were generally lower in mineral than organic soil layers. These differences with depth are consistent with other studies (e.g. Clays-Josserand *et al.*, 1988; Di *et al.*, 2010) and are probably related to differences in N availability. However, the community structure of AOA, AOB, and *Nitrobacter*-like NOB did not differ between the two soil layers. AOA abundance did not change between the two horizons, and abundances of AOB, *Nitrobacter*, and *Nitrospira*-like NOB did not vary, or changed inconsistently, between organic and mineral layers. In contrast, some studies have shown a decrease in AOB abundance and an increase in AOA abundance and/or AOA/AOB ratio with depth (Leininger *et al.*, 2006; Di *et al.*, 2010). However, greater soil depths (e.g. > 15 cm), likely incorporating a greater range of environmental conditions, were analyzed in these previous studies.

## Comparison of fertilization effects on AOB, AOA, *Nitrobacter*- and *Nitrospira*-like NOB

The effects of fertilization on AOB and *Nitrobacter*-like NOB communities differed from AOA and *Nitrospira*-like NOB communities. The abundances of AOA and *Nitrospira*-like NOB in soil were not responsive to fertilization. In contrast, AOB and *Nitrobacter*-like NOB tended to be more abundant in soils receiving fertilization treatments compared to unfertilized controls. The community structure of AOA was affected by fertilization only at the Lodi Lake site. On the contrary, significant changes in AOB community composition with application of N fertilizers were observed at four sites and at three sites for *Nitrobacter*-like NOB. Similar contrasting responses to N amendments between AOB and AOA communities have been reported in recent studies from agricultural and grassland soils (as noted in the introduction). The impact of N fertilization on soil NOB diversity has been previously investigated only in one study (Freitag *et al.*, 2005) by targeting 16S rRNA genes of these organisms. In contrast to our study, the authors showed no changes in the diversity of *Nitrobacter* community between long-term fertilized (amount of N similar to that of our periodic regime) and unfertilized grassland soils. The close similarity of 16S sequences among *Nitrobacter* genus (Orso *et al.*, 1994) might explain the absence of observed changes in this previous study. Interestingly, the diversity of *Nitrospira* community was reported to be more diverse under long-term fertilization compared to unfertilized management (Freitag *et al.*, 2005).

Abundances of AOB and *Nitrobacter*-like NOB were positively correlated with PN at all sites (except Crow Creek), with no such correlations observed for AOA and *Nitrospira*-like NOB. Similarly, Di *et al.* (2009, 2010) and Jia & Conrad (2009) demonstrated that nitrification rates were correlated with AOB, but not AOA abundances in N-amended grassland and agricultural soils. A strong positive correlation between *Nitrobacter*-like NOB abundances and potential nitrite oxidation contrasting with a weak and negative relation between *Nitrospira*-like NOB abundances and the activity, in soils under tillage or no tillage, were reported by Attard *et al.* (2010). In our study, nitrification assays were measured under high N concentration and at pH neutral (to insure ammonia availability); thus, some nitrifiers may not be active under these conditions but may contribute to nitrification *in situ*. Indeed, Stopnisek *et al.* (2010) showed that AOA dominate ammonia oxidation in an organic acidic forest peat soil characterized by low soil ammonium concentration. The authors also demonstrated that AOA abundance was not influenced by ammonium amendment, suggesting that AOA oxidized preferentially ammonia produced at low rates from mineralization of organic matter. However, in our study, a strong correlation was observed between PN and soil nitrate concentration *in situ* in all sites except Crow Creek. In addition, in the same sites, abundances of AOB and *Nitrobacter*-like

NOB, not AOA and *Nitrospira*-like NOB, were also correlated with soil nitrate concentration. This suggests that changes in PN in response to N fertilization reflect changes in nitrification activity *in situ* and that AOB and *Nitrobacter*-like NOB play a more important role in nitrification than AOA and *Nitrospira*-like NOB following fertilization in these sites. Some AOA and *Nitrospira*-like NOB strains may not solely oxidize ammonia or nitrite as energy sources, but may also, or mostly, grow by mixotrophic and/or heterotrophic metabolism. Indeed, assimilation of organic carbon has also been demonstrated for *Nitrospira*-like bacteria of waste-water treatments plants (Daims *et al.*, 2001) and suggested for the putative AOA *Crenarchaeum symbosium* (Hallam *et al.*, 2006).

Changes in PN rates were not significantly correlated with overall changes in nitrifier community structure, but were correlated with abundances of AOB and *Nitrobacter*-like bacteria. The only exception was a weak correlation between AOA composition and PN at the Lodi Lake site. Attard *et al.* (2010) demonstrated that potential nitrite oxidation was linked to *Nitrobacter*-like community structure, but to a lesser extent than the abundance of these organisms. The absence of coupling between variations in overall nitrifier community structure and PN may indicate the presence of functional redundancy or that only a small portion of the nitrifier community was active or responded to N fertilization.

In conclusion, periodic application of 200 kg N $ha^{-1}$ fertilizer to lodgepole pine and interior spruce stands increased PN rates for up to 2 years following fertilizer application, while annual fertilization did not induce any effect on PN except at Hand Lake. Some variations in PN, nitrifier abundance, and community structure were observed with time since periodic fertilizer application, with no impact of the periodic fertilization regime detected 3 years following fertilization, possibly indicating resilience of the nitrifier community. Fertilization increased AOB and *Nitrobacter*-like NOB abundances at some sites, but did not influence AOA and *Nitrospira-like* NOB abundances. AOB and/or *Nitrobacter*-like NOB abundances were correlated with PN at all sites; no such correlations were observed for AOA and *Nitrospira*-like NOB. Reasons for these differences (tolerance or sensitivity to high N conditions, degree of contribution to nitrification or implication in other ecosystem processes) still need to be elucidated. Approaches targeting functional gene transcripts and/or DNA stable isotope probing ($^{13}CO_2$ assimilation) could help to understand the contributions of AOB, AOA, *Nitrobacter*, and *Nitrospira* communities to nitrification activity and to identify active nitrifiers within these communities.

## Acknowledgements

Funding for this study was provided by a Natural Sciences and Engineering Research Council Strategic Grant to Melanie Jones (PI) for the project entitled 'Potential of forest fertilization to alleviate effects of climate change-induced insect infestation'. We are grateful to Rob Brockley of the British Columbia Ministry of Forests and Range for providing access to the 'maximum productivity' installations and providing detailed site information.

## References

Attard E, Poly F, Commeaux C, Laurent F, Terada A, Smets BF & Le Roux X (2010) Shifts between *Nitrospira*- and *Nitrobacter*-like nitrite oxidizers underlie the response of soil potential nitrite oxidation to changes in tillage practices. *Environ Microbiol* **12**: 315–326.

Avrahami S & Conrad R (2003) Patterns of community change among ammonia oxidizers in meadow soils upon long-term incubation at different temperatures. *Appl Environ Microbiol* **69**: 6152–6164.

Balser TC, Kinzig AP & Firestone MK (2002) Linking soil microbial communities and ecosystem functioning. *The Functional Consequences of Biodiversity: Empirical Process and Theoretical Extensions* (Kinzig AP, Pacala SW & Tilman D, eds), pp. 265–293. Princeton University Press, Princeton, NJ.

Banner A, MacKenzie W, Haeussler S, Thomson S, Pojar J & Trowbridge R (1993) *A Field Guide to Site Identification and Interpretation for the Prince Rupert Forest Region*. B.C. Min. For., Victoria, B.C. Land Manage. Handb. 26, http://www.for.gov.bc.ca/hfd/pubs/Docs/Lmh/Lmh26.htm.

B.C. Ministry of Forest and Range (2010) Mountain Pine Beetle. Beetle Facts. http://www.for.gov.bc.ca/hfp/mountain_pine_beetle/facts.htm.

Blackburne R, Vadivelu VM, Yuan Z & Keller J (2007) Kinetic characterisation of an enriched *Nitrospira* culture with comparison to *Nitrobacter*. *Water Res* **41**: 3033–3042.

Bock E, Sundermeyer-Klinger H & Stackebrandt E (1983) New facultative lithoautotrophic nitrite-oxidizing bacteria. *Arch Microbiol* **136**: 281–284.

Boyle-Yarwood SA, Bottomley PJ & Myrold DD (2008) Community composition of ammonia-oxidizing bacteria and archaea in soils under stands of red alder and Douglas fir in Oregon. *Environ Microbiol* **10**: 2956–2965.

Brockley RP & Simpson DG (2004) *Effects of Intensive Fertilization on the Foliar Nutrition and Growth of Young Lodgepole Pine and Spruce Forests in the Interior of British Columbia (E.P. 886.13). Establishment and Progress Report*. B.C. Ministry of Forests, Victoria, Tech. Rep. 018.

Burton SAQ & Prosser JI (2001) Autotrophic ammonia oxidation at low pH through urea hydrolysis. *Appl Environ Microbiol* **67**: 2952–2957.

Clays-Josserand A, Lensi R & Gourbiere F (1988) Vertical distribution of nitrification potential in an acid forest soil. *Soil Biol Biochem* **20**: 405–406.

Daims H, Nielsen JL, Nielsen PH, Schleifer KH & Wagner M (2001) *In situ* characterization of *Nitrospira*-like nitrite-oxidizing bacteria active in wastewater treatment plants. *Appl Environ Microbiol* **67**: 5273–5284.

Dandie CE, Miller MN, Burton DL, Zebarth BJ, Trevors JT & Goyer C (2007) Nitric-oxide reductase-targeted real-time PCR quantification of denitrifier populations in soil. *Appl Environ Microbiol* **73**: 4250–4258.

DeLong C (2000) *A Field Guide for Site Identification and Interpretation of Ecosystems of the Northwest Portion of the Prince George Forest Region*. B.C. Min. For., Victoria, B.C. Draft field guide insert (update for Land Manage. Handb., 21). http://www.for.gov.bc.ca/hfd/pubs/Docs/Lmh/Lmh21.htm.

Di HJ, Cameron KC, Shen JP, Winefield CS, O'Callaghan M, Bowatte S & He JZ (2009) Nitrification driven by bacteria and not archaea in nitrogen–rich grassland soils. *Nat Geosci* **2**: 621–624.

Di HJ, Cameron KC, Shen JP, Winefield CS, O'Callaghan M, Bowatte S & He JZ (2010) Ammonia-oxidizing bacteria and archaea grow under contrasting soil nitrogen conditions. *FEMS Microbiol Ecol* **72**: 386–394.

Enwall K, Nyberg K, Bertilsson S, Cederlund H, Stenström J & Hallin S (2007) Long-term impact of fertilization on activity and composition of bacterial communities and metabolic guilds in agricultural soil. *Soil Biol Biochem* **39**: 106–115.

Forge TA & Simard SW (2001) Short-term effects of nitrogen and phosphorus fertilizers on nitrogen mineralization and trophic structure of the soil ecosystem in forest clearcuts in the southern interior of British Columbia. *Can J Soil Sci* **81**: 11–20.

Freitag TE & Prosser JI (2003) Community structure of ammonia-oxidizing bacteria within anoxic marine sediments. *Appl Environ Microbiol* **69**: 1359–1371.

Freitag TE, Chang L, Clegg CD & Prosser JI (2005) Influence of inorganic nitrogen management regime on the diversity of nitrite-oxidizing bacteria in agricultural grassland soils. *Appl Environ Microbiol* **71**: 8323–8334.

Frey SD, Knorr M, Parrent JL & Simpson RT (2004) Chronic nitrogen enrichment affects the structure and function of the soil microbial community in temperate hardwood and pine forests. *For Ecol Manage* **196**: 159–171.

Gelfand I & Yakir D (2008) Influence of nitrite accumulation in association with seasonal patterns and mineralization of soil nitrogen in a semi-arid pine forest. *Soil Biol Biochem* **40**: 415–424.

Graham DW, Knapp CW, van Vleck ES, Bloor K, Lane TB & Graham CE (2007) Experimental demonstration of chaotic instability in biological nitrification. *ISME J* **1**: 385–393.

Hallam SJ, Mincer TJ, Schleper C, Preston CM, Roberts K, Richardson PM & DeLong EF (2006) Pathways of carbon assimilation and ammonia oxidation suggested by environmental genomic analyses of marine *Crenarchaeota*. *PLoS Biol* **4**: 520–536.

Hart SC & Stark JM (1997) Nitrogen limitation of the microbial biomass in an old-growth forest soil. *Ecoscience* **4**: 91–98.

Hart SC, Stark JM, Davidson EA & Firestone MK (1994) Nitrogen mineralization, immobilization, and nitrification. *Methods of Soil Analysis. Part 2. Microbiological and Biochemical Properties* (Weaver RW, Angle S, Bottomley P, Bezdicek D, Smith S, Tabatabai A & Wollum A, eds), pp. 985–1018. Soil Science Society of America, Madison, NJ.

Jia Z & Conrad R (2009) *Bacteria* rather than *Archaea* dominate microbial ammonia oxidation in an agricultural soil. *Environ Microbiol* **11**: 1658–1671.

Jordan FL, Cantera JJL, Fenn ME & Stein LY (2005) Autotrophic ammonia-oxidizing bacteria contribute minimally to nitrification in a nitrogen-impacted forested ecosystem. *Appl Environ Microbiol* **71**: 197–206.

Kim DJ & Kim SH (2006) Effect of nitrite concentration on the distribution competition of nitrite-oxidizing bacteria in nitratation reactor systems and their kinetic characteristics. *Water Res* **40**: 887–894.

Könneke M, Bernhard AE, De La Torre JR, Walker CB, Waterbury JB & Stahl DA (2005) Isolation of an autotrophic ammonia-oxidizing marine archaeon. *Nature* **437**: 543–546.

Kowalchuck GA, Stephen JR, De Boer W, Prosser JI, Embley TM & Woldendorp JW (1997) Analysis of ammonia-oxidizing bacteria of the β-subdivision of the class *Proteobacteria* in coastal sand dunes by denaturing gradient gel electrophoresis and sequencing of PCR-amplified 16S ribosomal DNA fragments. *Appl Environ Microbiol* **63**: 1489–1497.

Le Roux X, Poly F, Currey P, Commeaux C, Hai B, Nicol GW, Prosser JI, Schloter M, Attard E & Klumpp K (2008) Effects of aboveground grazing on coupling among nitrifier activity, abundance and community structure. *ISME J* **2**: 221–232.

Leininger S, Urich T, Schloter M, Schwark L, Qi J, Nicol GW, Prosser JI, Schuster SC & Schleper C (2006) Archaea predominate among ammonia-oxidizing prokaryotes in soils. *Nature* **442**: 806–809.

Martikainen PJ (1984) Nitrification in two coniferous forest soils after different fertilization treatments. *Soil Biol Biochem* **16**: 577–582.

Meidinger D & Pojar J (1991) *Ecosystems of British Columbia*. B.C. Ministry of Forests, Victoria, B.C. Special Report Series 6.

Monreal C, McGill WB & Nyborg M (1986) Spatial heterogeneity of substrates: effects on hydrolysis, immobilization and nitrification of urea-N. *Can J Soil Sci* **66**: 499–511.

Muyzer G, De Waal EC & Uitterlinden AG (1993) Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Appl Environ Microbiol* **59**: 695–700.

Nicol GW, Leininger S, Schleper C & Prosser JI (2008) The influence of soil pH on the diversity, abundance and transcriptional activity of ammonia oxidizing archaea and bacteria. *Environ Microbiol* **10**: 2966–2978.

Nogueria R & Melo LF (2006) Competition between *Nitrospira* spp. and *Nitrobacter* spp. in nitrite-oxidizing bioreactors. *Biotechnol Bioeng* **95**: 169–175.

Ohtonen R (1992) Soil microbial community response to silvicultural intervention in coniferous plantation ecosystems. *Ecol Appl* **2**: 363–375.

Okano Y, Hristova KR, Leutenegger CH, Jackson LE, Denison RF, Gebreyesus B, Lebauer D & Scow KM (2004) Application of real-time PCR to study effects of ammonium on population size of ammonia-oxidizing bacteria in soil. *Appl Environ Microbiol* **70**: 1008–1016.

Orso S, Gouy M, Navarro E & Normand P (1994) Molecular phylogenetic analysis of 17 *Nitrobacter* spp. *Int J Syst Bacteriol* **44**: 83–86.

Pan PHC (1971) Lack of distinction between *Nitrobacter agilis* and *Nitrobacter winogradskyi*. *J Bacteriol* **108**: 1416–1418.

Peacock AD, Mullen MD, Ringelberg DB, Tyler DD, Hedrick DB, Gale PM & White DC (2001) Soil microbial community responses to dairy manure or ammonium nitrate applications. *Soil Biol Biochem* **33**: 1011–1019.

Pederson H, Dunkin KA & Firestone M (1999) The relative importance of autotrophic and heterotrophic nitrification in a conifer forest soil as measured by $^{15}N$ tracer and pool dilution techniques. *Biogeochemistry* **44**: 135–150.

Périé C & Munson AD (2000) Ten-year responses of soil quality and conifer growth to silvicultural treatments. *Soil Sci Soc Am J* **64**: 1815–1826.

Prescott CE, Corbin JP & Parkinson D (1992) Immobilization and availability of N and P in the forest floors of fertilized Rocky Mountain coniferous forests. *Plant Soil* **143**: 1–10.

Prosser JI (1989) Autotrophic nitrification in bacteria. *Adv Microbial Physiol* **30**: 125–181.

Prosser JI & Cox DJ (1982) Nitrification. *Experimental Microbial Ecology*. Blackwell, Oxford, pp. 178–193.

Rotthauwe JH, Witzel KP & Liesack W (1997) The ammonia monooxygenase structural gene *amoA* as a functional marker: molecular fine-scale analysis of natural ammonia-oxidizing populations. *Appl Environ Microbiol* **63**: 4704–4712.

Roux-Michollet D, Czarnes S, Adam B, Berry D, Commeaux C, Guillaumaud N, Le Roux X & Clays-Josserand A (2008) Effects of steam disinfestation on community structure, abundance and activity of heterotrophic, denitrifying and nitrifying bacteria in an organic farming soil. *Soil Biol Biochem* **40**: 1836–1845.

Schimel J (1995) Ecosystem consequences of microbial diversity and community structure. *Arctic and Alpine Biodiversity: Patterns, Causes, and Ecosystem Consequences* (Chapin FS & Korner C, eds), pp. 239–254. Springer-Verlag, Berlin.

Shen JP, Zhang LM, Zhu YG, Zhang JB & He JZ (2008) Abundance and composition of ammonia-oxidizing bacteria and ammonia-oxidizing archaea communities of an alkaline sandy loam. *Environ Microbiol* **10**: 1601–1611.

Smolander A, Kurka A, Kitunen V & Mälkönen E (1994) Microbial biomass C and N, and respiratory activity in soil of repeatedly limed and N- and P-fertilized Norway spruce stands. *Soil Biol Biochem* **26**: 957–962.

Soil Classification Working Group (1998) *The Canadian System of Soil Classification*. Agriculture and Agri-Food Canada, Ottawa, Ont. Publ. 1646.

Stopnisek N, Gubry-Rangin C, Höfferle S, Nicol GW, Mandic-Mulec I & Prosser JI (2010) Thaumarchaeal ammonia oxidation in an acidic forest peat soil is not influenced by ammonium amendment. *Appl Environ Microbiol* **76**: 7626–7634.

Tourna M, Freitag TE, Nicol GW & Prosser JI (2008) Growth, activity and temperature responses of ammonia-oxidizing archaea and bacteria in soil microcosms. *Environ Microbiol* **10**: 1357–1364.

Wang Y, Ke X, Wu L & Lu Y (2009) Community composition of ammonia-oxidizing bacteria and archaea in rice field soil as affected by nitrogen fertilization. *Syst Appl Microbiol* **32**: 27–36.

Webster G, Embley MT, Freitag TE, Smith Z & Prosser JI (2005) Links between ammonia oxidizer species composition, functional diversity and nitrification kinetics in grassland soils. *Environ Microbiol* **7**: 676–684.

Wertz S, Degrange V, Prosser JI, Poly F, Commeaux C, Freitag TE, Guillaumaud N & Le Roux X (2006) Maintenance of soil functioning following erosion of microbial diversity. *Environ Microbiol* **8**: 2162–2169.

Wertz S, Degrange V, Prosser JI, Poly F, Commeaux C, Guillaumaud N & Le Roux X (2007) Decline of soil microbial diversity does not influence the resistance and resilience of key soil microbial functional groups following a model disturbance. *Environ Microbiol* **9**: 2211–2219.

Wertz S, Poly F, Le Roux X & Degrange V (2008) Development and application of a PCR-denaturing gradient gel electrophoresis tool to study the diversity of *Nitrobacter*-like *nxrA* sequences in soil. *FEMS Microbiol Ecol* **63**: 261–271.

## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Fig. S1.** MDS representation of AOA community structure in plots from Crater Lake (CL), McKendrick Pass (MP), Lodi Lake (LL), Crow Creek (CC) and Hand Lake (HL) sites.

**Table S1.** Ammonium and nitrate concentrations and pH of organic and mineral soil layers from unfertilized plots (control treatment) and fertilized plots (periodic and annual treatments) in Crater Lake (CL), McKendrick Pass (MP), Lodi Lake (LL), Crow Creek (CC) and Hand Lake (HL).

Please note: Wiley-Blackwell is not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article