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Rapid and dissimilar response of ammonia oxidizing archaea and bacteria to nitrogen and water amendment in two temperate forest soils

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ABSTRACT

Biochemical processes relevant to soil nitrogen (N) cycling are performed by soil microorganisms affiliated with diverse phylogenetic groups. For example, the oxidation of ammonia, representing the first step of nitrification, can be performed by ammonia oxidizing bacteria (AOB) and, as recently reported, also by ammonia oxidizing archaea (AOA). However, the contribution to ammonia oxidation of the phylogenetically separated AOA versus AOB and their respective responsiveness to environmental factors are still poorly understood. The present study aims at comparing the capacity of AOA and AOB to momentarily respond to N input and increased soil moisture in two contrasting forest soils. Soils from the pristine Rothwald forest and the managed Schottenwald forest were amended with either NH_4^+–N or NO_3^-–N and were incubated at 40% and 70% water-filled pore space (WFPS) for four days. Nitrification rates were measured and AOA and AOB abundance and community composition were determined via quantitative PCR (qPCR) and terminal restriction length fragment polymorphism (T-RFLP) analysis of bacterial and archaeal *amoA* genes.

Our study reports rapid and distinct changes in AOA and AOB abundances in the two forest soils in response to N input and increased soil moisture but no significant effects on net nitrification rates. Functional microbial communities differed significantly in the two soils and responded specifically to the treatments during the short-term incubation. In the Rothwald soil the abundance and community composition of AOA were affected by the water content, whereas AOB communities responded to N amendment. In the Schottenwald soil, by contrast, AOA responded to N addition. These results suggest that AOA and AOB may be selectively influenced by soil and management factors.

1. Introduction

Soil microorganisms involved in biochemical processes such as nitrification are important regulators of N cycling in soil. Ammonia oxidizers perform the first step in nitrification, the oxidation of NH_4^+ to NH_2OH, which is catalysed by the ammonia monooxygenase enzyme, and the subsequent formation of NO_2^-. Recent molecular biology methods target ammonia oxidizers via the *amoA* functional marker gene, coding for the α-subunit of the ammonia monooxygenase enzyme (Junier et al. 2010).

The oxidation of ammonia was long believed to be exclusively accomplished by a monophyletic group of bacteria, until recent metagenomic analysis revealed the existence of a novel group of ammonia oxidizers. Treusch et al. (2005) demonstrated that besides bacteria members of the Crenarchaeota phylum within the archaea domain also possess *amoA* homologues, potentially enabling them to perform ammonia oxidation. Archaeal ammonia oxidizers are ubiquitous, appeared to thrive under a wide range of growth conditions (Erguder et al. 2009) and in many soils dominate in number over their bacterial counterparts (Leininger et al. 2006). Nevertheless, it is still unknown if archaeal ammonia oxidizers (AOAs) are truly equivalent in function to their bacterial counterparts (AOBs), whether they have different growth requirements and physiologies and consequently respond differently to environmental conditions and management (Francis et al. 2007; Nicol and Schleper 2006; Prosser and Nicol 2008).

Previous studies have demonstrated that AOB populations may undergo changes in activity and population size in response to fertiliser application (Mendum and Hirsch 2002). Corresponding adaptations of AOB communities to fertilisation were delayed in time (Mendum et al. 1999; Avrahami et al. 2003). In various Chinese soils the impact of ammonium fertilisation on both AOA and AOB communities after 40 days was insignificant (Shen et al. 2008a). However, soil Crenarchaeota have been shown to vary in composition between soil types (Nicol et al. 2007) and AOA populations

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Table 1
Site characteristics and soil properties of Rothwald (R) and Schottenwald (S). Soil organic carbon (C_{org}), soil total nitrogen (N_{tot}), soil water-filled pore space (WFPS), above sea level (a.s.l.), temperature (Temp.), precipitation (Ppt.).

	Rothwald[a]	Schottenwald[b]
Soil type	Chromic cambisol	Dystric cambisol
Geology	Dolomite	Sandstone
pH ($CaCl_2$)	5.3	4.4
C_{org} (%)	16.0	3.8
N_{tot} (%)	0.94	0.24
C:N	17.1	16.0
Initial WFPS (%)	35	22
Elevation (m a.s.l.)	1035	370
Average annual Temp. (°C)	5.5	9.0
Mean annual Ppt. (mm)	1759	465

[a] Data from Hackl et al. (2004).
[b] Data from Kitzler et al. (2006).

differed among plant communities (Boyle-Yarwood et al. 2008). We hypothesise that bacterial versus archaeal ammonia oxidizers may be specifically affected by abiotic factors, since recent studies revealed a different response of AOA and AOB to soil pH (Nicol et al. 2008), salinity (Santoro et al. 2008), N fertilisation (Shen et al. 2008) and manure application (Schauss et al. 2009), soil moisture and temperature (Szukics et al. 2010).

To examine this hypothesis, the present study investigates the capacity of AOA and AOB to respond to short-term increases in soil water content and mineral nitrogen supply in two temperate forests with contrasting management histories and distinct soil characteristics. The *Rothwald* virgin forest represents one of few forest ecosystems in the temperate zone which have never been managed or used for wood production (Hackl et al. 2004). In contrast, the managed *Schottenwald* forest is situated in direct vicinity of Vienna (Vienna Woods) and is subject to nitrogen deposition resulting from traffic and industry (Kitzler et al. 2006). As the two forest sites selected for this study are characterized by major differences in N cycling and soil chemistry, our aim was to study whether the ammonia oxidizing communities prevalent in these soils also responded differently to changes in soil environmental conditions. To this aim abundances of bacterial and archaeal *amoA* genes were measured in combination with nitrification rates. We furthermore examined whether ammonia oxidizing communities in the two forest soils differed in their structural composition and tested their responsiveness to short-term changes in soil N supply at different soil moisture levels.

2. Methods

2.1. Soil sampling

Experimental sites were set-up in the Rothwald virgin forest (47°46′N 15°07′E), a spruce-fir-beech forest situated in a remote valley at the eastern border of the Austrian Alps (Lower Austria) and in the Schottenwald beech forest (48°14′N 16°15′E), located in close vicinity of the Vienna city area. Soil samples from the Rothwald (R) and the Schottenwald (S) forests were collected in June 2005. Soil and site characteristics are listed in Table 1. At each site 10 soil samples were taken from the mineral layer (0–10 cm depth) at 5 m intervals along transects of 50 m length. Soil samples were sieved to 5 mm and the 10 individual samples from each site were combined to a composite sample by mixing thoroughly.

2.2. Soil incubation experiment

Eighteen stainless steel cylinders (7.5 cm diameter, 5 cm height) were filled with approximately 120 g homogenised fresh Rothwald and Schottenwald soil, respectively. The water contents of nine soil cores each were adjusted to either 40% ("40") or 70% ("70") water-filled pore space (WFPS) by wetting with a syringe. Of the nine cores adjusted to a specific water level, three soil cores were amended with 0.1 mg $(NH_4)_2SO_4$–N g^{-1} dry soil (NH_4), three soil cores were amended with 0.1 mg KNO_3–N g^{-1} dry soil (NO_3) and three control cores were treated with distilled water only (oN). The amended soil cores and the control cores were incubated for four days at 20 °C with periodic readjustment of the water content. After four days, soil cores were sampled destructively for replicated determination of the nitrification rate. Subsequently, soils were stored at −20 °C for DNA extraction.

2.3. Nitrification measurement

In nitrification assays, $NaClO_3$ (0.234 M), serving as an inhibitor of NO_2^- oxidation (Belser and Mays 1980), was applied to 2.5 g soil aliquots ($n = 6$) in 400 μl aqueous solution. This volume corresponded well to the amount of water evaporating during 24 h of incubation (data not shown). Of the six treatment replicates, two samples, serving as blanks, were immediately frozen at −20 °C and four replicates were incubated at 20 °C for 24 h. Subsequently, nitrite in samples and blanks was extracted with 2 M KCl, analysed photometrically and nitrification activities were calculated (Schinner et al. 1996).

2.4. DNA extraction and quantification by real-time PCR

From three replicate microcosms per treatment DNA was extracted from 0.5 g bulk soil using the UltraClean soil DNA Extraction Kit (MoBio Laboratories, Inc.). Three replicate DNA extractions per soil core were pooled and subjected to PCR amplification.

Functional marker genes encoding archaeal and bacterial ammonia monooxygenase (archaeal *amoA*, bacterial *amoA*) were quantified by real-time PCR using an iCycler IQ (Biorad). The 25 μl PCR reaction mix contained 15 μg BSA, 0.675 μl DMSO, 12.5 μl of Q Mix (Biorad, 100 mM KCl, 40 mM Tris–HCl, 6 mM $MgCl_2$, 0.4 mM each of dNTP, 1.25 U iTaq DNA Polymerase, SybrGreen I, 20 mM fluorescein) and approximately 25 ng template DNA. The reaction mix contained 0.5 μM of the primers amoA-1F (GGGGTTTCTACTGGTGGT) and amoA-2R (CCCCTCKGSAAAGCCTTCTTC) (Rotthauwe et al. 1997) amplifying the bacterial *amoA* gene (491 bp). The cycling conditions were 95 °C for 3 min, followed by 45 cycles of 95 °C, 57 °C, 72 °C and 81 °C for 60 s each. The real-time PCR reaction of the archaeal *amoA* gene (635 bp) contained 0.3 μM of the primers Arch amoA1F (STAATGGTCTGGCTTAGACG) and Arch amoA2R (GCGGCCATCCATCTGTATGT) (Francis et al. 2005). The cycling conditions were 95 °C for 5 min, followed by 45 cycles of 45 s at 95 °C, 60 s at 53 °C, 60 s at 72 °C and 60 s at 78 °C. Fluorescence acquisition was performed at a temperature at which all primer dimers had melted but specific products had not. Fluorescence emission was detected at 81 °C and 78 °C for bacterial *amoA* and archaeal *amoA*, respectively.

Abundances of bacterial and archaeal *amoA* genes refer to copy numbers per gram dry soil. Standards for qPCR were generated by serial dilution of stocks containing a known number of plasmids carrying the respective functional gene as an insert. Reaction efficiencies of qPCRs were 90% (±2) for archaeal *amoA* and 85% (±4.1) for bacterial *amoA*. R^2 values were 0.99 for all runs. Inhibition tests with serially diluted soil DNA revealed high concentrations of template reduced PCR efficiency. To eliminate these effects, reduced amounts of template DNA were used in PCR reactions (25 ng).

2.5. Community profiling by T-RFLP analysis

For T-RFLP analysis fluorescently end-labelled forward primers (carboxyfluorescein phosphoramidite marked – FAM) were used

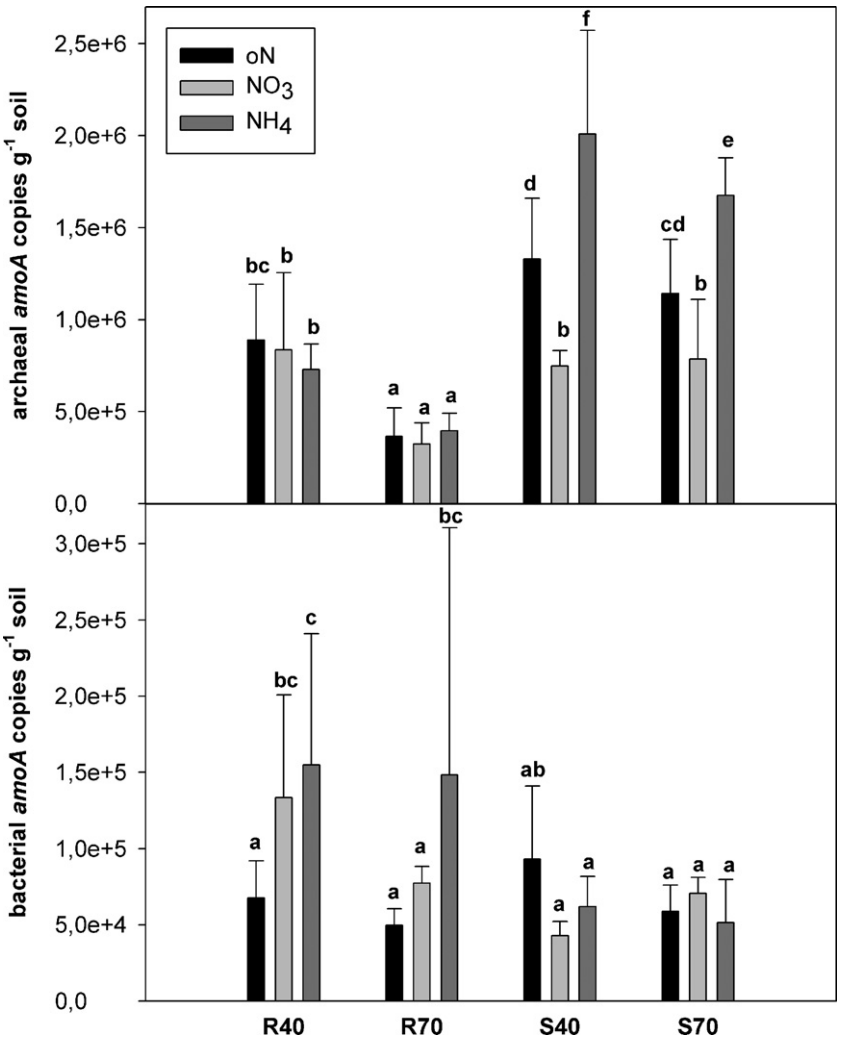

Fig. 1. Mean abundances of archaeal *amoA* genes (copies g^{-1} soil) and bacterial *amoA* genes (copies g^{-1} soil) in R and S soil at 40% WFPS (40) and 70% WFPS (70), treated with NO_3^- and NH_4^+ and without treatment (oN). Error bars represent the standard deviation. Different lower case characters represent significant differences at the 95% confidence level, $p < 0.05$.

for the amplification of archaeal and bacterial *amoA* genes. PCR amplifications were performed in T1 thermocyclers (Biometra). The amplification of bacterial (491 bp) and archaeal *amoA* fragments (635 bp) was performed in 25 μl reactions containing 2.5 μl (2 μM each) of the primers Arch-amoAF and Arch-amoAR, or amoA-1F and amoA-2R, 2.5 μl dNTPs (2 mM), 3 μl $MgCl_2$ (25 mM), 2.5 μg BSA (Sigma, 10 mg ml^{-1}), 1 μl DMSO, ~20 ng DNA, 1 U Firepol Polymerase (Solis Biodyne) in 10× reaction buffer provided with the enzyme. The PCR amplification of archaeal *amoA* genes involved an initial denaturation step of 95 °C for 5 min, followed by 30 cycles of 95 °C for 45 s, 53 °C for 60 s, 72 °C for 60 s and a final elongation step of 10 min at 72 °C. The PCR amplification of bacterial *amoA* genes involved an initial denaturation step of 95 °C for 5 min, followed by 38 cycles of 95 °C for 30 s, 54 °C for 40 s, 72 °C for 60 s and a final elongation step of 10 min at 72 °C.

For generating T-RFLP profiles at least 3 replicate PCR reactions from each sample were pooled and purified with Invisorb Spin PCRapid Kit (Invitek) before digest as recommended previously (Hartmann et al. 2007). A 10 μl reaction volume containing 150 ng FAM marked bacterial or archaeal *amoA* PCR-product, 1 μl reaction buffer and 2U *RsaI* restriction enzyme (Invitrogen) was incubated for 3 h at 37 °C. Digests were purified using Sephadex columns. HIDI loading buffer (Formamide) and GeneScan-500 ROX length standard (Applied Biosystems) were added to the digests and denatured at 92 °C for 2 min prior to analysis on an ABI Prism 3100 Genetic Analyzer (Applied Biosystems). Lengths of T-RFs were determined by comparing with internal standards using GeneScan 3.1 software (2001, Applied Biosystems). Background noise was corrected by eliminating peaks which constituted less than 1% of the total signal and only peaks which were present in more than 1 sample were included in the analysis. Electropherograms were analysed using the Molecular Fingerprint Analysis Software by SequentiX (Digital DNA Processing, Klein Raden, Germany). Prediction of sequences corresponding to specific T-RFs was performed in silico using a database of all publicly available sequences (from the NCBI database) using the ARB (Ludwig et al. 2004) and TRiFLe packages (Junier et al. 2008).

2.6. Statistical analysis

Analysis of variance of real-time PCR data (one-way ANOVA, multiple range test) was performed using the Statgraphics Plus 5.0 package. A LSD test was used for comparing nitrification activities and gene abundances between treatments. Multivariate statistical analysis of T-RFLP profiles was performed on standardised, square-root transformed data using Primer 6 software (Primer and Permanova+ PRIMER-E, Version 1.1.0, Plymouth, UK). Similarity between treatments on the basis of T-RFLP profiles was calculated

Table 2
Mean values of nitrification activity (ng N g^{-1} 24 h^{-1}) in the in R and S soil at 40% WFPS (40) and 70% WFPS (70), treated with NO_3^-, NH_4^+ and without treatment (oN). Different lower case characters represent significant differences at the 95% confidence level, $p < 0.05$, n.a., no detectable activity.

WFPS (%)	Rothwald				Schottenwald		
	40		70		40	70	
NO_3^-	142.3	*bc*	254.7	*cd*	n.a.	n.a.	
NH_4^+	205.5	*cd*	369.6	*d*	n.a.	n.a.	
oN	243.6	*cd*	335.7	*d*	n.a.	25.4	*ab*

using the one-way analysis of similarity (ANOSIM) including 10,000 permutations with significant differences considered as $p < 0.01$ (Clarke 1993). Permutational multivariate ANOVA (PERMANOVA) including a Monte Carlo test, was used to test for effects of factors and interactions. Non-metric multidimensional scaling (nmMDS), running 10,000 permutations and using Bray–Curtis similarity calculation was used for demonstrating the relatedness of individual samples under different treatments (Kenkel and Orloci 1986; Minchin 1987), including stress values in the plots. To identify differences in the T-RF occurrence and abundance between specific treatments ANOVA (Scheffé test, 95% confidence level) was used (Statgraphics Plus 5.0) (Buckley and Schmidt 2001).

3. Results

3.1. Nitrification activities

Nitrification activities ranged from 142.3 to 369.6 ng N g^{-1} 24 h^{-1} in R soils (Table 2), whereas no significant nitrification activity was detected in S soil. In R soil the variability between the replicates was high, indicating high sample heterogeneity. Neither nitrogen amendment, nor increased soil moisture significantly affected nitrification activities in R and S soils.

3.2. Abundance of archaeal and bacterial amoA genes

Changes in the soil water content affected the number of archaeal *amoA* genes in soils of the Rothwald forest (Fig. 1). At 40% WFPS R soils contained 1.8–2.6 times more archaeal *amoA* gene copies than at 70% WFPS. In S soils this effect was not found. In S soil NH_4^+ addition increased archaeal *amoA* copy numbers, whereas upon NO_3^- treatment archaeal *amoA* abundance was lowest. However, mineral nitrogen amendment did not affect the number of archaeal *amoA* genes in R soils (Fig. 1).

The relative numerical contribution of archaeal *amoA* genes to the total *amoAs* of putative ammonium oxidizers ranged between 76% (±24%) and 93% (±0.9%) in R soil and between 90% (±5%) and 97% (±2%) in S soil. The highest numerical contribution (97%) of archaea to the putative ammonium oxidising community was detected in NH_4^+ treated S soils at both water contents.

The abundance of bacterial *amoA* genes was affected by N treatment in R soils (Fig. 1). In R soil at 40% WFPS, 1.8 and 2.6 times more *amoA* copies were detected after NO_3^- and NH_4^+ treatment compared to oN soil ($p < 0.05$). At 70% WFPS *amoA* copies increased in NH_4^+ amended R soil however numbers did not differ significantly from NO_3^- or oN treatments ($p = 0.09$). In S soil neither water content nor nitrogen application had an effect on bacterial *amoA* gene copy numbers.

3.3. Community profiles

Archaeal *amoA* community profiles recorded three major fragments with abundances between 6% and 16% of the total abundance (130 bp, 169 bp, 299 bp) in both soils. One dominant fragment (136 bp) comprised an abundance of 37% and 49% of the total abundance in R and S soil, respectively.

The T-RF of 59 bp in length dominated in R (21% of the total abundance) and the T-RF of 387 bp in length dominated in S soil (13% of the total abundance), but comprised ≤1% of the total abundance in the other soil, respectively.

ANOSIM and PERMANOVA analysis demonstrated a significant difference between the archaeal *amoA* T-RFLP profiles from R and S soils (Global $R = 0.82$, $p = 0.01\%$). In R soils moisture significantly affected the archaeal *amoA* community structure ($R = 0.28$, $p = 0.2\%$). Archaeal *amoA* T-RFLP profiles demonstrated distinct clusters for R soils at 40% WFPS and 70% WFPS (p(MC) = 0.004), (Fig. 2A) however, no such effect was seen in S soils (p(MC) = 0.70) (Fig. 2B). Across all N treatments the T-RF of 59 bp in length was significantly more abundant in R soils at 40% than at 70% WFPS ($p = 0.0002$). The fragment of 59 bp length corresponded to a group within the soil/sediment cluster described previously (Francis et al. 2005). The T-RFs of 102 bp, 169 bp, 196 bp and 299 bp in length significantly differed in abundance between the two water contents in R soil ($p < 0.005$).

Control (oN) and NO_3^- treated S soil differed in community structure ($R = 0.27$, $p < 0.02$), whereas nitrogen treatment effects on the archaeal *amoA* community structure were not significant in R soil.

In bacterial *amoA* community profiles six and three T-RFs, respectively, showed abundances of more than 5% in R (46 bp, 271 bp, 376 bp, 379 bp, 485 bp, 459 bp) and S (46 bp, 247 bp, 485 bp) soils. Both soils shared the two most abundant fragments of 46 bp and 485 bp length, however with differing abundance. Overall, bacterial *amoA* communities of R and S soils differed significantly (Global $R = 0.44$, $p = 0.01\%$). PERMANOVA analysis showed an interacting effect of site and treatment (p(MC) = 0.008), indicating a distinct response to N treatment in R and S soils. R soils amended with NH_4^+ and R control soils (oN) formed separable clusters (p(MC) = 0.008), whereas no such effect was observed for S soils (Fig. 3). The major difference between control (oN) and NH_4^+ treated R soils was due to two T-RFs of 288 bp and 485 bp length, which were 23- and 2.6 times more abundant in oN soils than in NH_4^+ treated soils, respectively ($p < 0.01$). The fragment of 288 bp length was of low total abundance, comprising 0.3% of the total abundance in R soil. Sequences from the database did not match the fragment of 485 bp length, which may indicate a novel sequence, previously undetected. Increased soil moisture did not induce a statistically significant change in bacterial *amoA* community profiles.

4. Discussion

4.1. Treatment effect: water amendment

The divergent changes in functional gene abundances and community composition observed upon nitrogen and water amendment in Rothwald and Schottenwald soils indicate specific effects on AOA and AOB in the two contrasting soils. The AOA community composition was affected by the different water contents in the Rothwald soil and archaeal *amoA* genes were more abundant at 40% than at 70% WFPS, indicating sensitivity to anaerobic conditions. In Schottenwald soil on the other hand, bacterial as well as archaeal *amoA* gene abundances remained unaffected by the soil water content. The Rothwald soil, being well aerated, rich in organic carbon and of higher pH is likely to provide more favourable conditions for bacterial nitrifiers than the more acidic clay soil of the Schottenwald. The pH value has been identified as the primary variable influencing bacterial *amoA* abundance, with greater abundance observed at a higher soil pH (Hayden et al. 2010). While AOB may be more abundant and active at higher pH values (Nicol

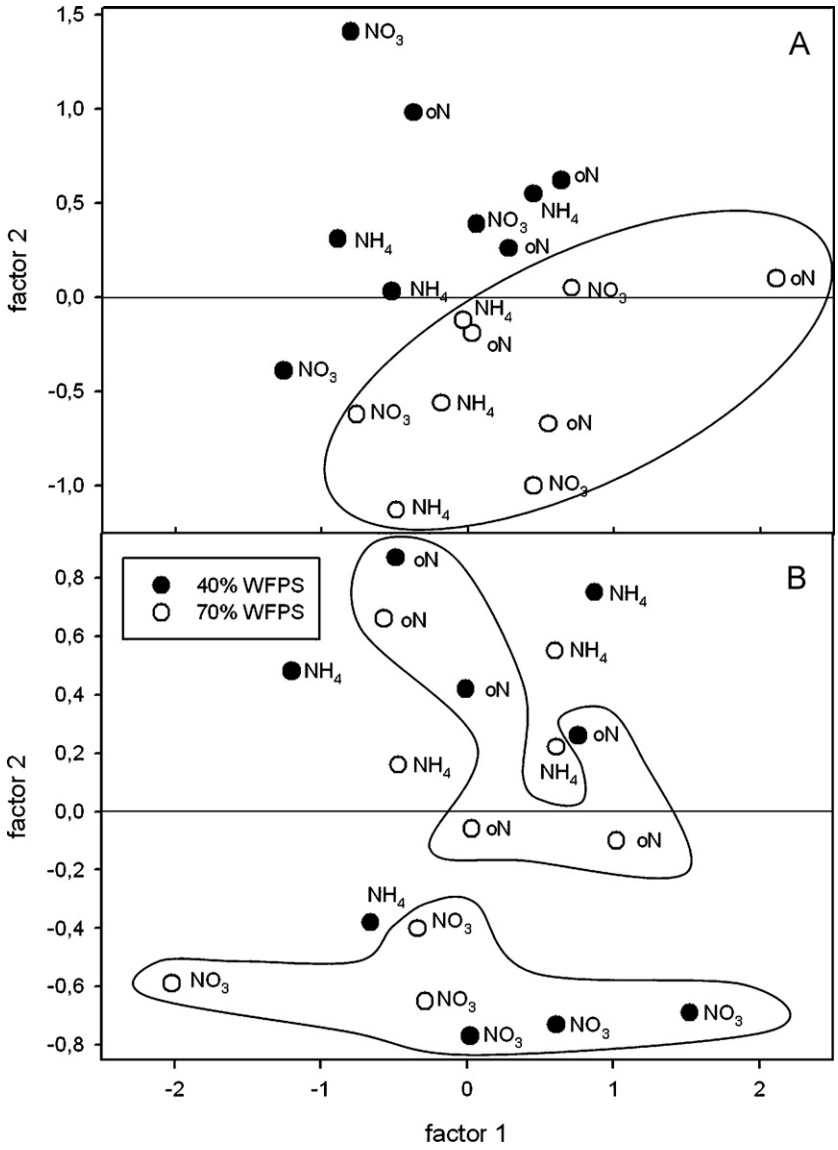

Fig. 2. Plots (nmMDS) based on Bray–Curtis similarities showing the relative similarities of the archaeal *amoA* (AOA) community structure based on T-RFLP profiles of (A) Rothwald and (B) Schottenwald soil at 40% WFPS (40) and 70% WFPS (70), treated with NO_3^- and NH_4^+ and without treatment (oN).

et al. 2008; Ceccherini et al. 2008), archaeal *amoA* abundance and expression levels are likely to decrease with increasing soil pH. The one log higher soil pH of the Rothwald than the Schottenwald forest may have been more favourable to AOB in the Rothwald soil. Despite the observed changes in bacterial and archaeal *amoA* abundance, nitrification activities remained widely unaffected by the various treatments. Mendum et al. (1999) using ^{15}N pool dilution techniques reported an increase in nitrification rate three days after NH_4NO_3 application to arable soil. While multiple nitrogen turnover processes occur simultaneously in soil, turnover rates measured in Rothwald and Schottenwald soil provide information on net nitrification rates only. The lack of fertilisation effects on net nitrification in our study may be due to the reduction of NO_2^- by denitrifiers in moist soils at 40% and 70% WFPS. Compared to the C_{org}-rich Rothwald soil, in the silty loam Schottenwald soil O_2 may have become limiting at lower soil moisture contents, resulting in gaseous N loss via denitrification (Szukics et al. 2009) and undetectable nitrification activities. Long-term field observations have shown 2–10-fold higher NO_3^- concentrations in Rothwald than in Schottenwald soils, indicating higher in situ nitrification activities in Rothwald versus Schottenwald soils (Hackl et al. 2004; Kitzler et al. 2006). Low nitrification activities in the Schottenwald soil may also be ascribed to the continuous nitrogen input through atmospheric deposition. A recent study described reduced potential nitrification activities in agricultural soils after long-term $(NH_4)_2SO_4$ fertilisation associated with a pH-decrease, affecting the availability of ammonia (Enwall et al. 2007).

4.2. Treatment effect: N amendment

In both forest soils ammonia oxidizers responded to N amendment within 4 days. Previous studies have reported a rapid increase in AOB abundance following $(NH_4)_2SO_4$ amendment, resulting in the doubling of the population after 1.5 days (Cavagnaro et al. 2008) and a peak abundance after 7 days of incubation (Okano et al. 2004) and archaeal *amoA* transcripts increased in number one day after the addition of NH_4Cl (Treusch et al. 2005). In the present study, the addition of NH_4^+ stimulated either bacterial or archaeal ammonia oxidizers in the two different forest soils. Upon NH_4^+ addition the AOB population size increased in the Rothwald soil, whereas in the Schottenwald soil the AOA population size increased. These changes in *amoA* gene abundances

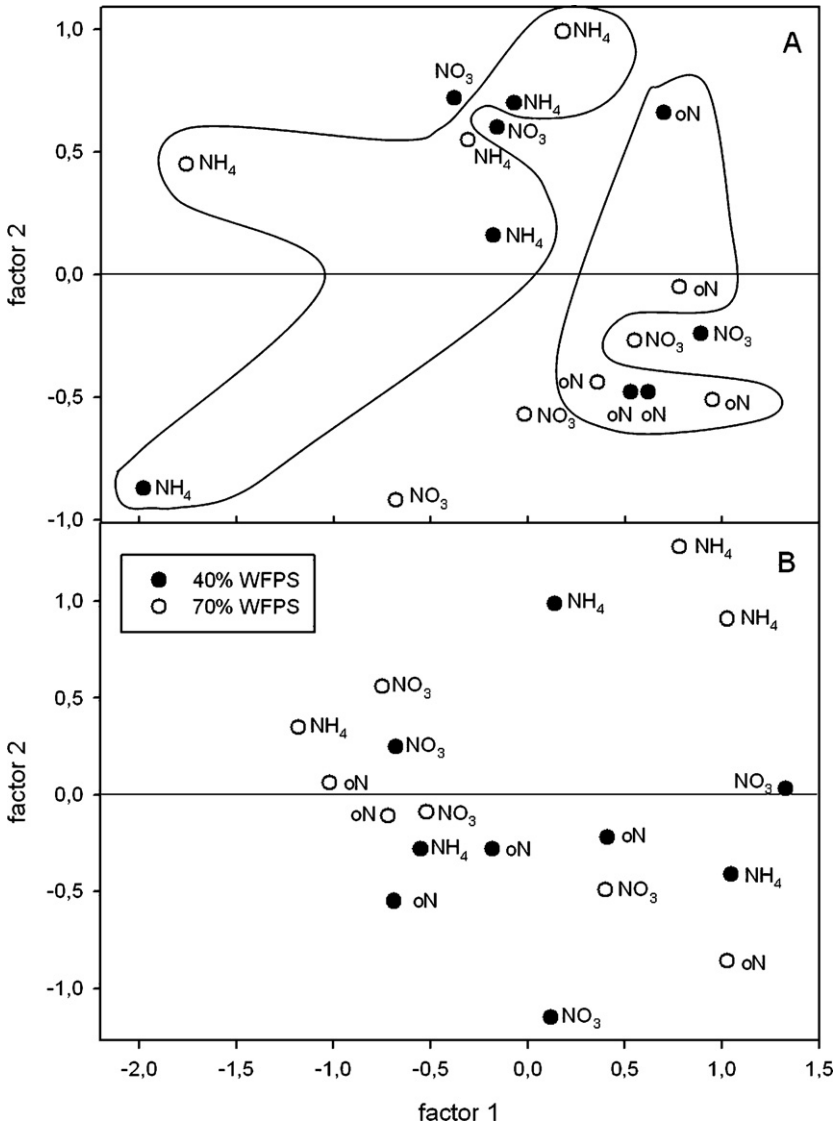

Fig. 3. Plots (nmMDS) based on Bray–Curtis similarities showing the relative similarities of the bacterial *amoA* (AOB) community structure based on T-RFLP profiles of A) Rothwald and B) Schottenwald soil at 40% WFPS (40) and 70% WFPS (70), treated with NO_3^- and NH_4^+ and without treatment (oN).

were also reflected in the community composition, as in the Rothwald soil the AOB community structure shifted in response to N application, whereas the AOA community remained unaffected. Similarly, in agricultural soil bacterial *amoA* community profiles changed upon NH_4^+ treatment but patterns of archaeal *amoA* genes remained unchanged over a period of 28 days (Jia and Conrad 2009). Shifts in the AOB community structure, but no significant changes within the AOA community were observed after long-term fertilisation (17 years) of alkaline soil (Shen et al. 2008). By contrast, in the more acidic Schottenwald soil AOA abundance and community composition changed upon N application, whereas AOB remained unaffected. These results indicate differences in the eco-physiology of AOA and AOB, suggesting that they may grow under different environmental conditions. Increased expression of archaeal *amoA* genes after spiking soils with NH_4^+ has been reported previously (Treusch et al. 2005). Assuming that AOA, like AOB, grow chemolithoautotrophically through aerobic oxidation of NH_4^+ to NO_2^- (Könneke et al. 2005), an increased abundance of *amoA* genes after NH_4^+ amendment also suggests enhanced nitrification potential. Accordingly, archaea may be considered the predominant ammonia oxidizers in the Schottenwald soil, as their population size increased upon NH_4^+ application, whereas bacteria may represent the predominant ammonia oxidizers in the Rothwald forest soil. Since archaeal nitrification may not deliver the same intermediates, the commonly used assay for assessing nitrification activity (Schinner et al. 1996) does not necessarily comprise archaeal nitrification. The assay is based on measuring the increase in NO_2^- concentration as a consequence of NH_4^+ oxidation to NH_2OH and its subsequent oxidation to NO_2^- catalysed by the enzyme hydroxylamine oxidoreductase (HOA). However, HOA gene homologues encoding hydroxylamine oxidoreductase have not yet been detected in Crenarchaeota (Nicol and Schleper 2006). Since it is still unclear if NO_2^- truly represents an intermediate in archaeal nitrification, potential archaeal nitrification activity in the Schottenwald soil may be unaccounted for. Likewise, potential nitrification rates correlated with AOB but a lack of correlation with archaeal *amoA* genes was reported recently for agricultural soils (Ying et al. 2010).

Moreover, a different but chemolithoautotrophic lifestyle of soil AOA is possible. For instance, in agricultural soil NH_4^+ fertilisation stimulated bacterial, but not archaeal *amoA* abundance and $^{13}CO_2$ was assimilated by bacteria rather than by archaea (Jia and Conrad 2009). Besides chemolithoautotrophy (Könneke et al. 2005), mixotrophy (Hallam et al. 2006) and even heterotrophy

(Herndl et al. 2005; Ouverney and Fuhrman 2000) have previously been reported for marine archaeal isolates. Assuming an alternative lifestyle of soil AOA to chemolithotrophy, archaeal *amoA* gene abundance would change independently from ammonia oxidation. High abundances of AOA, which were found in many soils, have been interpreted as indicators for the significance of archaeal ammonium oxidation for nitrification (Leininger et al. 2006). Based on qPCR of *amoA* genes, several studies have reported a numerical dominance of AOA over AOB in various ecosystems, including agricultural soils and grasslands (He et al. 2007; Shen et al. 2008), semiarid soils (Adair and Schwartz 2008), the marine water column (Wuchter et al. 2006), marine sediments (Park et al. 2008) and the temperate forest soils Rothwald and Schottenwald. Our results suggested that, notwithstanding the numerical dominance of AOA in two different forest soils, AOA as well as AOB may play significant roles in nitrification under specific environmental conditions. Divergent responses of AOA and AOB to increased N availability and soil moisture further imply that both groups are characterized by different physiologies and/or enzyme characteristics. As a consequence, it can be assumed that AOA and AOB accomplish soil nitrification under distinct conditions and that they are specifically affected by soil and management factors.

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