RESEARCH ARTICLE

Dynamics of ammonia-oxidizing communities in barley-planted bulk soil and rhizosphere following nitrate and ammonium fertilizer amendment

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Abstract

Oxidation of ammonia by nitrifying microorganisms is a major pathway that fertilizer nitrogen (N) may take upon application to agricultural soils, but the relative roles of bacterial (AOB) vs. archaeal (AOA) ammonia oxidizers are controversial. We explored the effects of various forms of mineral N fertilizer on the AOB and AOA community dynamics in two different soils planted with barley. Ammonia oxidizers were monitored via real-time PCR and terminal restriction fragment length polymorphism analysis of bacterial and archaeal *amoA* genes following the addition of either $[NH_4]_2SO_4$, NH_4NO_3 or KNO_3. AOB and AOA communities were also studied specifically in the rhizospheres of two different barley varieties upon $[NH_4]_2SO_4$ vs. KNO_3 addition. AOB changed in community composition and increased in abundance upon ammonium amendment in bulk soil and rhizosphere, with changes in bacterial *amoA* copy numbers lagging behind relative to changes in soil ammonium. In both soils, only T-RFs corresponding to phylotypes related to *Nitrosospira* clade 3a underwent significant community changes. Increases in AOB abundance were generally stronger in the bulk soil than in the rhizosphere, implying significant ammonia uptake by plant roots. AOA underwent shifts in the community composition over time and fluctuated in abundance in all treatments irrespective of ammonia availability. AOB were thus considered as the main agents responsible for fertilizer ammonium oxidation, while the functions of AOA in soil N cycling remain unresolved.

Introduction

It is well acknowledged that up to 50% of the fertilizer nitrogen (N) applied to agricultural soils is not taken up by the crop plants (FAO, 1995, 2009), but the pathways that the various fertilizer forms actually take upon field application are not fully understood. Fertilizer N entering the soil ammonium pool is subject both to assimilation by plants and microorganisms and to microbial nitrification. Chemoautotrophic bacteria affiliated with the β-subgroup of proteobacteria, which use ammonia-N as their sole energy source, were long thought to play an exclusive role in soil ammonia oxidation (Kowalchuk & Stephen, 2001; Prosser & Nicol, 2008). However, archaea assigned to the mesophilic *Crenarchaeota* group also possess ammonia monooxygenase-encoding *amoA* genes, enabling them to catalyze the first and rate-limiting step of nitrification (Leininger *et al.*, 2006). The functional redundancy of ammonia-oxidizing bacteria (AOB) and archaea (AOA) in soil environments has been postulated (Schauss *et al.*, 2009). Nevertheless, archaeal nitrification has recently been attributed a minor role in agricultural soils, based on the putative oligotrophic lifestyle of AOA (Erguder *et al.*, 2009; Martens-Habbena *et al.*, 2009) and on the observation that archaeal *amoA* gene patterns were uncoupled from ammonia oxidation activity (Di *et al.*, 2009; Jia & Conrad, 2009).

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In common fertilization practice, different chemical N forms are introduced into agricultural soils, which exert specific controls on the nitrifying microbial groups: ammonium availability is the most important factor determining the nitrification rate in soil, while nitrite and nitrate are known to inhibit nitrification (Azam *et al.*, 1995). This has major agronomic and environmental implications, because the nitrate set free by nitrifiers as a mobile N form may easily leach out of soils, causing N losses and polluting groundwater. In addition to emission of NO_x during nitrification, the nitrate produced serves as a substrate in denitrification, and may hence be reduced to radiatively active NO_x and N_2O gases (Conrad, 1996).

Our understanding is limited, however, on how AOB vs. AOA communities respond to the amendment of various forms of mineral N fertilizer to agricultural soils. AOB communities have previously been found to be affected by ammonium fertilization (e.g. Chu *et al.*, 2007; Enwall *et al.*, 2007), but fertilizer effects on AOA communities are less well studied and seem to be incongruent (He *et al.*, 2007; Shen *et al.*, 2008; Di *et al.*, 2009; Schauss *et al.*, 2009; Wang *et al.*, 2009). Furthermore, information is lacking on how ammonia oxidation by AOB and AOA interacts with plant N uptake and with the activities of other rhizosphere and endosphere microbial community members. The competitiveness of AOB and AOA in scavenging fertilizer N may be differentially influenced in the rhizosphere vs. the bulk soil, and may also vary during plant growth and in interaction with different plant genotypes.

The present study aims to explore the dynamics in AOB and AOA abundance and community composition in two agricultural soils planted with barley as affected by KNO_3, NH_4NO_3 and $[NH_4]_2SO_4$ amendment, respectively. In addition to studying bulk soils, we analyzed AOB and AOA community dynamics in the rhizospheres of barley plants following amendment of KNO_3 vs. $[NH_4]_2SO_4$. The effects of the plant genotype on soil ammonia oxidizers were examined using two different varieties of barley. Part of the plants within one variety were inoculated with the plant growth-promoting bacterium *Burkholderia phytofirmans* strain PsJN (Sessitsch *et al.*, 2005) to assess the potential effects of plant inoculation on rhizosphere N processes.

Materials and methods

Soils

Soil samples were collected from the top layer of two arable fields situated in Purkersdorf (48°15′0″N, 16°0′0″E) and Niederschleinz (48°35′59″N) in Lower Austria. Purkersdorf soil was classified as a cambisol from sandy loamy flysch material with sandy clay loam texture and had a pH of 5.7, while Niederschleinz soil was a chernozem from loess with a pH of 7.2. The soils were held at 4 °C during transportation to the lab, where they were sieved to 2 mm and then stored at 4 °C until analysis. The site characteristics of the sampling locations are given in Table 1 and have been described in detail in Inselsbacher *et al.* (2009).

Experimental design

Two experiments were performed, which focused on N fertilizer effects on AOB and AOA communities (1) in two different top soils planted with barley and (2) in the rhizospheres of two different barley varieties. These experiments are, in the following, referred to as experiments 1 and 2, respectively.

In experiment 1, 90 1-L pots were filled with 300 g of sieved and homogenized soil from the Purkersdorf or

Table 1. Site characteristics and physical and chemical properties of the soils studied (Inselsbacher *et al.*, 2009)

	Purkersdorf	Niederschleinz
Soil type	Gleyic Cambisol from sandy loamy flysch	Chernozem from loess
Geographic site	48°12′25″N	48°35′59″N
	16°10′37″E	15°10′24″E
Altitude (m.a.s.l.)	248	244
Management	Winter barley	Arable field
Water condition	Moist	Moderately dry
Clay (%)	1.7	17.7
Silt (%)	64.9	74.2
Sand (%)	33.4	8
pH (H_2O)	5.67	7.15
$CaCO_3$ (%)	0.06	8.5
Exchange capacity (mval%)	11.2	15.4
Total C (mg C g^{-1} DW)	16.2 (0.5)	26.4 (0.2)
Total N (mg C g^{-1} DW)	1.63 (0.05)	1.86 (0.02)
Base saturation (%EC)	81.4	98.1
Bulk density (g DW mL^{-1})	1.06	0.96

Niederschleinz agricultural sites, respectively. One barley var. Morex (BCC906) plant was grown from seed in each pot. Ten days after the sowing, 40 mg N in solution was applied per pot to the soils in the form of either KNO_3, NH_4NO_3 or $[NH_4]_2SO_4$ using a syringe that was inserted at multiple spots to allow for a uniform distribution (Inselsbacher *et al.*, 2009). Soils were additionally amended with 10 mL of a 37 mM K_2HPO_4 solution to prevent nutrient insufficiencies. Soils from three replicate pots per treatment and soil type were destructively sampled at five different time points: directly after fertilization (0 day), and after 2 days (2 days), 1 (7 days), 2 (14 days) and 3 weeks (21 days) following fertilization. At the first sampling event (0 day), unamended and unplanted soils from three replicate pots were sampled as controls in addition to the samples given above. Before soil sampling, the barley plants were removed carefully. The soil contained in the individual pots was mixed thoroughly, comprising both bulk and rhizosphere soil, but strongly dominated by bulk soil. Therefore, soils in experiment 1 are, in the following, referred to as bulk soils. Aliquots of 9 and 0.5 g of homogenized soil were used for chemical and molecular microbiological analyses, respectively.

Experiment 2 was designed for the analysis of the nitrifying communities in the barley rhizosphere and for studying plant genotype effects. As seeds of the variety Morex used in experiment 1 were no longer available, seeds of barley var. MR 3/51 (HOR 11371) and barley var. Arupo (BCC812) were sown in small (4 cm diameter) turf pots containing 20 g of Purkersdorf soil. An additional set of barley var. MR 3/51 seeds was used that had been inoculated with a green fluorescent protein (GFP)-labeled, kanamycin-resistant culture of *B. phytofirmans* PsJN (PsJN::gfp2x). This was done by surface sterilizing the seeds (by washing for 2 min each with 5% NaOCl and 70% ethanol, followed by two washings with sterile water) and then leaving them for 5 min in a two times diluted overnight culture of the inoculant at a final concentration of 6.75×10^7 CFU mL^{-1}. Five days after the sowing, 30 plantlets of each variety were transferred together with the turf pots into 1 L pots containing 300 g of Purkersdorf soil. After another 5 days, liquid N fertilizer in the form of either $[NH_4]_2SO_4$ or KNO_3 at a final concentration of 40 mg N per pot was inserted into the soil with a syringe as done in experiment 1. Thirty-five milligrams of K_2O was added to each pot to prevent growth deficiencies. Three replicate pots each were destructively sampled at four time points within 4 weeks: immediately after N amendment (0 day), and after 2 weeks (14 days), 3 weeks (21 days) and 4 weeks (28 days), resulting in 72 samples in total from four sampling occasions. The barley plants were removed by carefully detaching the roots from the soil contained in the turf pots, and rhizosphere soil adhering to the barley roots was collected using a fine spatula. Approximately 15 g of rhizosphere soil was collected per plant for subsequent use in chemical and molecular microbiological analyses. In addition, approximately 0.5 g of soil adhering to the seed surface (spermosphere) of the PsJN-inoculated plants was collected and incubated in 1 mL 0.9% NaCl at room temperature for 2 h with agitation. Hundred microliters of serial dilutions (10^{-1}–10^{-3}) of the suspension were then plated on Luria–Bertani plates amended with kanamycin (50 mg mL^{-1}), which, after 3 days of incubation at 28 °C, were screened for GFP-labeled PsJN colonies under UV light. In addition, the fresh and dry weights (DW) of the shoots and roots of all barley plants were determined.

Soil chemical analyses

The soil water content was determined after drying at 105 °C, and pH was measured using a glass electrode in 0.0125 M $CaCl_2$. NH_4-N was measured in extracts prepared from 2 g aliquots of homogenized soil in 15 mL KCl (1 M) by a modified indophenol reaction (Kandeler & Gerber, 1988). NO_3-N was measured in soil extracts prepared from 5 g soil in 0.0125 M $CaCl_2$ by ion chromatography (Inselsbacher *et al.*, 2009).

Analyses of AOB and AOA and total bacterial communities

DNA was extracted from 0.5 g of soil taken from three replicate pots per treatment and sampling occasion using the FastDNA® Spin Kit for Soil (MP Biomedicals, Solon, OH) as described by the manufacturer, and was quantified photometrically (Nanodrop ND-1000; Nanodrop Technologies, Wilmington, DE).

SYBR-green assays were performed in an iCycler iQ Multicolor Real-Time PCR Detection System (Bio-Rad Laboratories). Genes encoding the ammonia monooxygenase catalytic subunit A (*amoA*) were amplified using the primers *amoA*1F/*amoA*2R (5′-GGGGTTTCTACTGGTGGT-3′/5′-CCCCTCKGSAAAGCCTTCTTC-3′, Rotthauwe *et al.*, 1997) for bacteria and Arch-*amoA*F/Arch-*amoA*R (5′-STAATGGTCTGGCTTAGACG-3′/5′-GCGGCCATCCATCTGTATGT-3′, Francis *et al.*, 2005) for archaea. Twenty-five microliters reactions were composed of 12.5 μL 2× IQ™ SYBR®-Green Supermix (Bio-Rad Laboratories), 0.5 μM of each primer for *amoA*/Arch-*amoA*, 2.5% (v/v) dimethyl sulfoxide (DMSO) and 2.4% (w/v) bovine serum albumine (BSA). Standards and samples were processed in triplicate. The thermocycler program for *amoA*/Arch-*amoA* PCR was carried out using the following protocol: 95 °C for 3 min, 45 cycles of 95 °C for 1 min, 57 °C/53 °C for 1 min, 72 °C for 1 min and data collection at 78 °C for 1 min. Melting curve analysis was performed in order to confirm the specificity of the PCR product. To check for inhibition, a two times dilution series of one representative DNA sample for each

soil was carried out. No inhibiting effects could be seen for any of the samples.

PCR products of each targeted gene were cloned (Strataclone PCR cloning Kit; Stratagene) and sequenced to confirm specificity. Plasmids were isolated using the Quantum Prep Plasmid Miniprep Kit (Bio-Rad Laboratories) and DNA concentrations were determined using spectroscopy to calculate copy numbers. Standard curves from serial dilutions of known amounts of the target genes were generated for each run, showing correlation coefficients (R^2) of 0.994–1.000 and PCR efficiencies of 88–102%.

Terminal restriction fragment length polymorphism (T-RFLP) analysis was performed on PCR-amplified 16S rRNA gene and bacterial and archaeal *amoA* gene fragments. *AmoA* genes from bacteria and archaea were amplified using the same primers as for Real Time PCR amplification, except that the forward primers were 6-carboxyfluorescein (FAM) labeled at the 5́-end. For amplification of bacterial 16S rRNA genes, a 6-carboxyfluorescein (FAM)-labeled primer 8F (5′-AGAGTTTGATCCTGGCTCAG-3′, and the primer 1520R (5′-AAGGAGGTGATCCAGCCGCA-3′, Massol-Deya *et al.*, 1995) were used. Amplifications were performed in 25 μL reactions containing 1 × buffer, 3 mM $MgCl_2$, 0.2 mM dNTPs, 4%(v/v) DMSO, 1% (w/v) BSA, 1 U of FIREpol polymerase (Solis Biodyne, Estonia), and 0.2 μM of each primer for the amplification of archaeal *amoA* and 16S rRNA genes, but 0.24 μM of each primer for bacterial *amoA* amplification. Ten to 100 ng DNA was added to the reactions. Amplifications were performed in a Whatman T1 thermocycler using the following programs for bacterial/archaeal *amoA* reactions: an initial denaturing step at 95 °C for 5 min, followed by 35/30 cycles of 1 min at 95 °C, 1 min at 60 °C/53 °C, 1 min at 72 °C and a final extension step at 72 °C for 10 min. The program for 16S rRNA gene amplification was set as follows: 95 °C for 5 min, 30 cycles of 95 °C 1 min, 53 °C 1 min and 72 °C 2 min, with a final extension step at 72 °C for 10 min.

16S rRNA and bacterial and archaeal *amoA* genes were cleaved using the AluI restriction enzyme. The choice of restriction enzyme was based on *in silico* testing of 20 cloned sequences for theoretical T-RF lengths following cleavage with either RsaI, AluI or HaeIII and evaluation of the resulting profiles. To reduce bias, two PCR products were pooled for each digestion assay. Ten-microliter mixtures for bacterial and archaeal *amoA* analysis contained 7 μL PCR product, 1 × buffer and 0.5 μL AluI (10 U μL^{-1}; Promega), and were incubated for 3 h at 37 °C. For 16S rRNA gene analysis, the amount of restriction enzyme was doubled.

Digestion assays were purified by passage through a DNA grade Sephadex G50 (GE Healthcare) columns. Ten microliters of the purified product was mixed with 10 μL HiDi-formamide (Applied Biosystems) and 0.3 μL 500 ROX™ Size Standard (Applied Biosystems) and denatured at 95 °C for 2 min. Detection of FAM-labeled terminal restriction fragments was carried out by capillary electrophoresis using an ABI 3100 automatic DNA sequencer.

Sequence analysis of bacterial and archaeal *amoA* genes from Niederschleinz and Purkersdorf soils was performed by constructing in total four clone libraries from pooled samples taken at all time points from each of the two soils. PCR products were generated as described above for the T-RFLP analysis using bacterial and archaeal *amoA*-specific (but unlabeled) primers, which were then purified using a QIAquick gel 20 extraction kit (Qiagen, Chatsworth, CA) and cloned into the PCR vector pSC-A-amp/kan and transformed into competent StrataClone SoloPack cells (Strataclone 21 PCR cloning Kit; Stratagene) according to the manufacturers' protocol. Forty-seven to 70 clones per library were sequenced by AGOWA (http://www.agowa.de/; Berlin, Germany) using the M13F primer (5′-GTAAAACGA CGGCCAG-3′). Sequences were edited and terminal restriction fragments were determined *in silico* using the software CLC Sequence viewer 5.1 (http://www.clcbio.com/; CLC Bio, Denmark). To check for similarities, sequences were compared with entries in the NCBI database using the Basic Local Alignment Search Tool (BLAST) (http://blast.ncbi.nlm.nih.gov/).

Bacterial and archaeal *amoA* sequences, respectively, were aligned with a selection of sequences deposited in the NCBI database using CLUSTALW. Neighbor joining and PhyML algorithms in the ARB software package (Ludwig *et al.*, 2004) were used for calculating trees.

Measurement of the plant N content

The plant N content was determined using an elemental analyzer as described in ÖNORM G 1073 (ÖNORM, 1988).

Statistical analyses

T-RFLPs were transformed into numerical data using GENESCAN 3.7 software (Applied Biosystems). To reduce background noise, peaks with intensities higher than three times the SD were binned and normalized as described in Abdo *et al.* (2006) using the statistical program R, together with the filtering and binning macro provided at the IBEST homepage (http://www.ibest.uidaho.edu/tools/trflp_stats/instructions.php). Peaks that occurred in at least two replicates and with a percentage ≥ 2% were considered as major ones. Discriminant analysis using the statistical program SPSS (11.0 for Windows) was performed for each T-RFLP data matrix. Functional gene abundance data were subjected to ANOVA using the Duncan *post hoc* test to test for significant differences between sampling times. ANOSIM was performed using the statistical program PRIMER 5 (PRIMER-E, Plymouth) as described in Clarke (1993) to test for differences between treatments and time points.

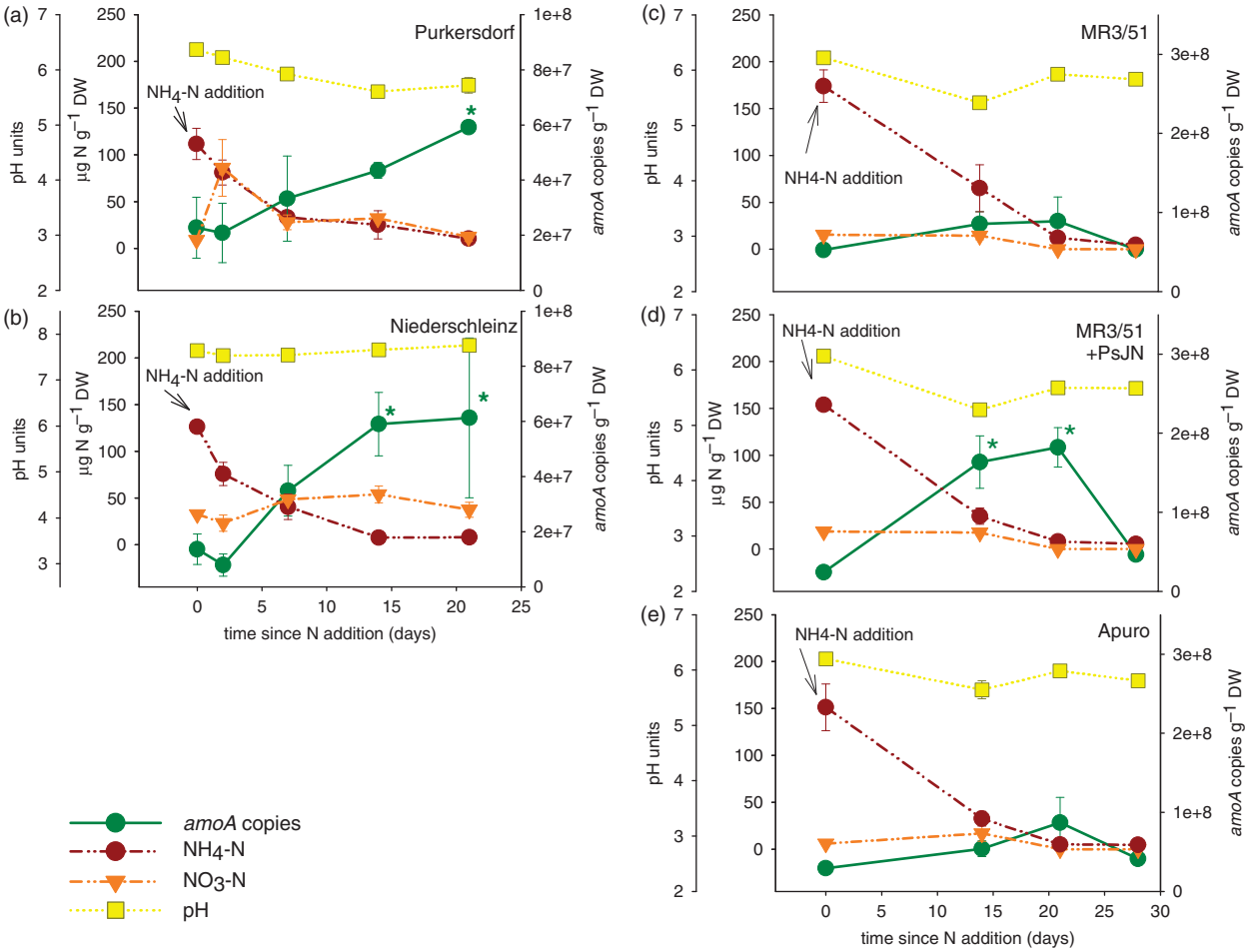

Fig. 1. Dynamics in AOB *amoA* copy numbers following $[NH_4]_2SO_4$ amendment in relation to soil NH_4-N and NO_3-N concentrations, and soil pH values. Barley-planted bulk soils from (a) Purkersdorf and (b) Niederschleinz and Purkersdorf rhizospheres beneath (c) barley var. MR3/51, (d) barley var. MR3/51 inoculated with *Burkholderia* strain PsJN and (e) barley var. Apuro were repeatedly analyzed immediately after N amendment (day 0) and at intervals of about 7 days. Symbols represent means ($n = 3$) ± SEs. Significant differences in AOB abundance between time points are indicated by $^*P < 0.05$.

Nucleotide sequence accession numbers

The environmental *amoA* sequences generated in this study have been deposited in the GenBank nucleotide sequence database under accession numbers HM803691 through HM803924.

Results

Soil pH and mineral N

Soil pH and mineral N contents were monitored during 3 and 4 weeks following N amendment in experiments 1 and 2, respectively. In experiment 1, pH in Purkersdorf soil decreased from 6.5 (0 day) to 5.6 and 6.3 (21 days) upon $[NH_4]_2SO_4$ and NH_4NO_3 addition, respectively, while Niederschleinz soil showed only minor variations in pH over time (Fig. 1a and b, Supporting Information, Fig. S1a and b). Following KNO_3 addition, pH changed less than with ammonia fertilization; however, the pH again varied more in Purkersdorf than Niederschleinz soil, increasing up to 6.8 in the former (Fig. 2a and b). In experiment 2, the initial pH of 6.4 in Purkersdorf rhizosphere decreased upon $[NH_4]_2SO_4$ addition to minimum 5.2 after 2 weeks and was increased to 5.9 after 4 weeks (Fig. 1c–e). Following KNO_3 addition, the pH in the Purkersdorf rhizosphere was continuously decreased from 6.4 to a minimum 5.8 (Fig. 2c–e).

The ammonium concentrations in Purkersdorf and Niederschleinz soils were 7.61 (± 0.34) and 7.32 (± 0.57) µg NH_4-N g^{-1} DW at the beginning of experiment 1. After $[NH_4]_2SO_4$ and NH_4NO_3 amendment, the added ammonium became continuously depleted within 21 days in Purkersdorf soil. In Niederschleinz soil, the ammonium decreased to the initial concentrations on days 14 and 21 in

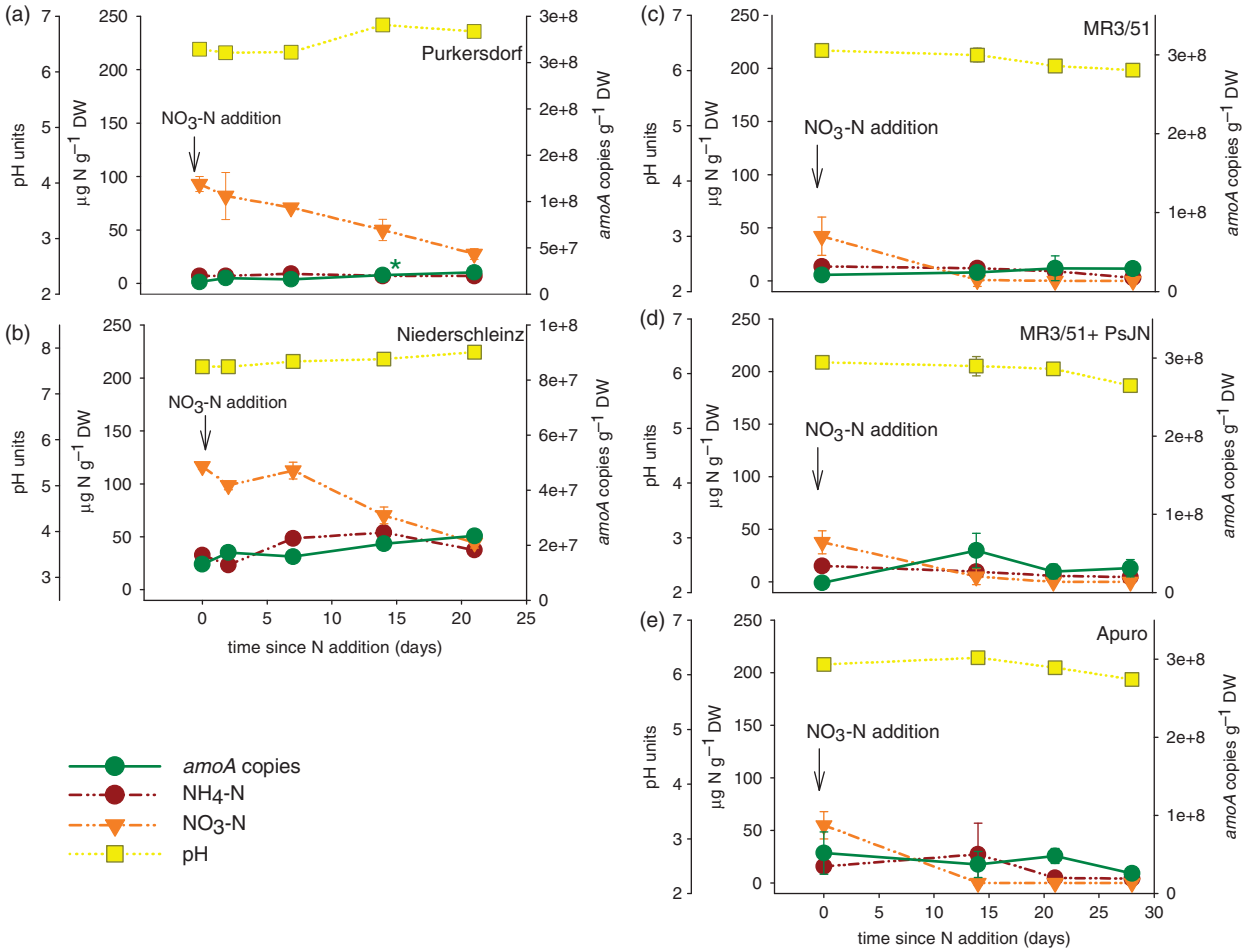

Fig. 2. Dynamics in AOB *amoA* copy numbers following KNO_3 amendment in relation to soil NH_4-N and NO_3-N concentrations, and soil pH values. Barley-planted bulk soils from (a) Purkersdorf and (b) Niederschleinz, and Purkersdorf rhizospheres beneath (c) barley var. MR3/51, (d) barley var. MR3/51 inoculated with *Burkholderia* strain PsJN and (e) barley var. Apuro were repeatedly analyzed immediately after N amendment (day 0) and at intervals of about 7 days. Symbols represent means ($n = 3$) ± SEs. Significant differences in AOB abundance between time points are indicated by $^*P < 0.05$.

$[NH_4]_2SO_4$- and NH_4NO_3-amended soils, respectively (Fig. 1a and b, Fig. S1a and b). KNO_3 amendment did not have any effect on the soil ammonium pool sizes (Fig. 2a and b).

The initial nitrate concentration in Purkersdorf soil was 9.64 (± 2.82) µg NO_3-N g^{-1} DW, which, following NH_4NO_3 and $[NH_4]_2SO_4$ amendment, increased to 63.90 (± 5.83) and 86.08 (± 30.37) µg NO_3-N g^{-1} DW. Nitrate in Niederschleinz soil was initially 22.07 (± 7.51) µg NO_3-N g^{-1} DW, and following NH_4NO_3 and $[NH_4]_2SO_4$ addition, increased to maximum 101.20 (± 21.65) and 53.93 (± 8.84) µg NO_3-N g^{-1} DW, respectively (Fig. 1a and b, Fig. S1a and b).

The ammonium concentration in the barley rhizosphere was 15.19 (± 1.06) µg NH_4-N g^{-1} DW at the beginning of experiment 2. Upon $[NH_4]_2SO_4$ amendment, the added ammonium decreased continuously to 4.58 (± 0.11) µg NH_4-N g^{-1} DW on day 28 (Fig. 1c–e). In the KNO_3 amendments, the initial NH_4-N concentration decreased continuously to 2.89 (± 0.64) µg NH_4-N g^{-1} DW on day 28 (Fig. 2c–e). Nitrate concentrations were generally low in the rhizosphere. The initial nitrate concentration of 13.42 (± 1.83) µg NO_3-N g^{-1} DW soil remained constant during the first 2 weeks following $[NH_4]_2SO_4$ amendment and then decreased below the detection limit (Fig. 1c–e).

AOB and AOA *amoA* gene abundances

In experiment 1, $[NH_4]_2SO_4$ amendment to Purkersdorf and Niederschleinz soils resulted in a six- and 4.5-fold increase in AOB *amoA* copy numbers at least from day 7 following N addition, with the maximum copy numbers occurring on day 21 (Fig. 1a and b). In the NH_4NO_3-fertilized soils, a corresponding, but a less pronounced increase in the AOB *amoA* copy numbers occurred (Fig. S1a and b). Following KNO_3 addition, the AOB *amoA* copy

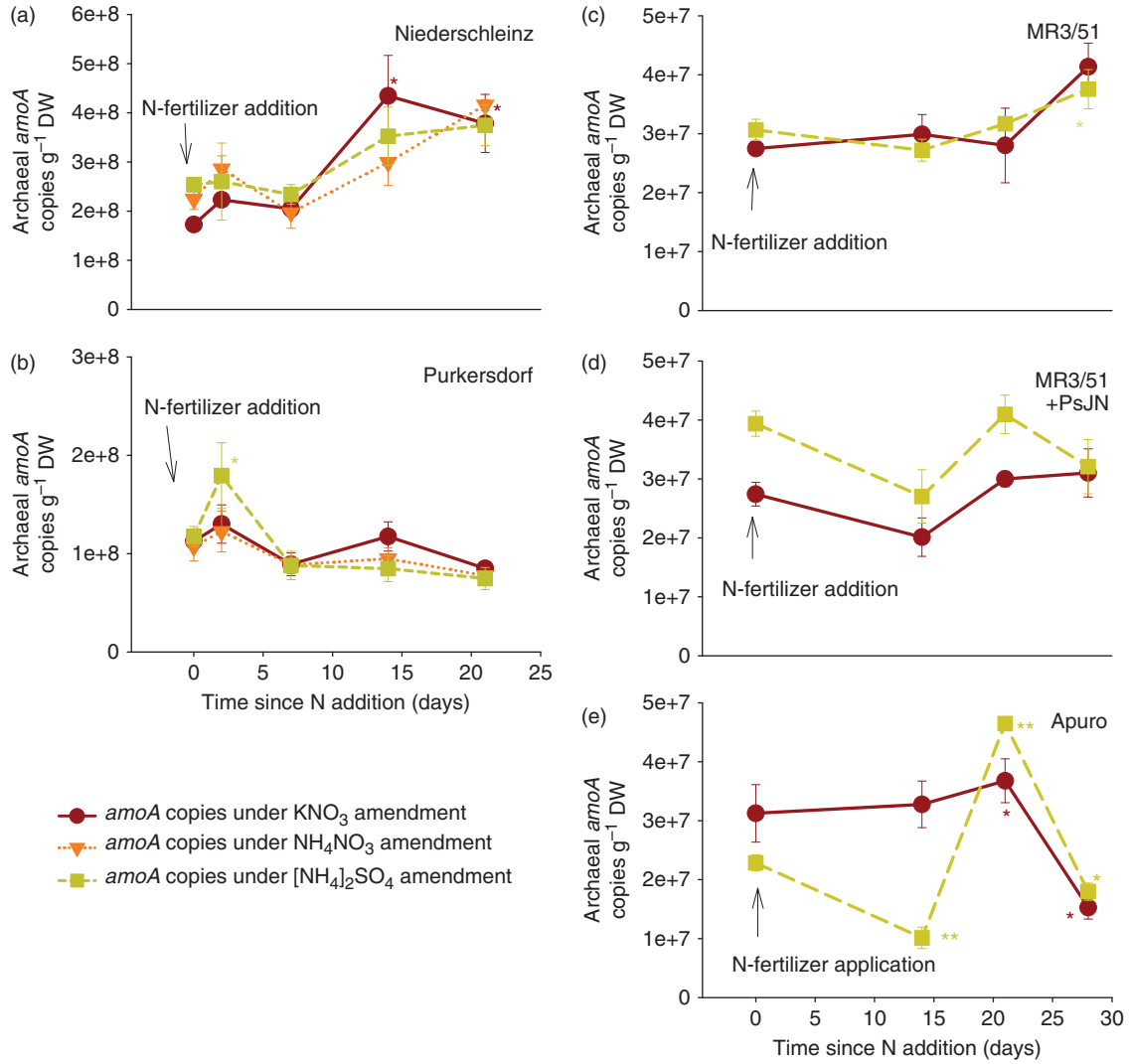

Fig. 3. Dynamics in AOA *amoA* copy numbers following various N amendments. Barley-planted (a) Purkersdorf and (b) Niederschleinz bulk soils received $[NH_4]_2SO_4$, NH_4NO_3 and KNO_3 amendments; and Purkersdorf rhizospheres beneath (c) barley var. MR3/51, (d) barley var. MR3/51 inoculated with *Burkholderia* strain PsJN and (e) barley var. Apuro were amended with either $[NH_4]_2SO_4$ or KNO_3. Soils were repeatedly analyzed immediately after N addition (day 0) and at intervals of about 7 days. Symbols represent means ($n = 3$) ± SEs. Significant differences in AOA abundance between time points are indicated by $^{*}P < 0.05$ and $^{**}P < 0.01$.

numbers were higher in Purkersdorf soil on day 14 vs. day 0, but otherwise remained unchanged. In Niederschleinz soil, the AOB *amoA* copies remained constant in number after KNO_3 addition (Fig. 2a and b).

AOB *amoA* copies also increased in the barley rhizosphere following $[NH_4]_2SO_4$ amendment, although to a lesser extent than in the bulk soils. This increase was the strongest and significant in the rhizosphere of PsJN-inoculated MR3/51 plants. In the rhizospheres of uninoculated barley var. MR3/51 and var. Arupo plants, the AOB *amoA* copy numbers were similar and the copy number increases were not significant at $P < 0.05$. AOB *amoA* copies decreased rapidly after the depletion of the added ammonium, so that the initial *amoA* copy numbers were reached on day 28 in all soils (Fig. 1c–e). Following KNO_3 amendment, the AOB *amoA* copy numbers in the rhizospheres of the different plants remained unchanged (Fig. 2c–e).

Throughout the experiment, AOA *amoA* copies fluctuated in abundance in both soils, but other than with AOB, changes in the AOA *amoA* copy numbers were similar in all N treatments (Fig. 3). Only in Purkersdorf soil, 2 days after $[NH_4]_2SO_4$ addition, did the AOA *amoA* numbers exceed those in the other treatments (Fig. 3a).

AOA *amoA* genes were 10.2 (Purkersdorf) and 21.0 (Niederschleinz) times more abundant than AOB *amoA* genes in the initial (untreated) soils (Table 2). Following

$[NH_4]_2SO_4$ amendment, however, archaeal over bacterial *amoA* dominance decreased to 1.3 (Purkersdorf) and 6.1 (Niederschleinz). In Purkersdorf soil, a similar, but less pronounced decrease in archaeal to bacterial *amoA* copies was seen after NH_4NO_3 and KNO_3 amendment, resulting in ratios of 2.5 and 3.6, respectively (Table 2). However, in Niederschleinz soil AOA *amoA* genes finally dominated over AOB ones by 26.6 following NH_4NO_3 amendment, and following KNO_3 amendment, the ratio of archaeal to bacterial *amoA* copies increased to 248.3 on day 14 and amounted to 196.3 on day 21 (Table 2).

As in the bulk soils, AOA *amoA* copies showed similar fluctuations following $[NH_4]_2SO_4$ and KNO_3 amendment also in the barley rhizospheres (Fig. 3c–e). AOA *amoA* gene copies were initially equally abundant as the AOB ones in the barley rhizosphere, but were outnumbered up to 8fold by AOB *amoA* genes following $[NH_4]_2SO_4$ addition (Table 3). Amendment with KNO_3 resulted in no or a less pronounced decrease in AOA to AOB *amoA* copy numbers.

16rRNA gene and AOB and AOA *amoA* community structures and AOB and AOA *amoA* sequence analysis

In experiment 1, the T-RFLP community profiles of 16S rRNA and AOB and AOA *amoA* genes from soils receiving three different N amendments sampled on day 0 and day 21 ($n = 3$ per treatment) were compared among each other and with unfertilized, unplanted control soils ($n = 3$) (Fig. 4). Peaks accounting for significant differences among treatments and sampling times ($P < 0.05$) using ANOVA based on Duncan's *post hoc* test are presented in Table 4. In addition, key statistical data from the canonical discriminant analysis are shown to indicate the resolution of discrimination among the T-RFLP data obtained in the plots. Profiles from Purkersdorf vs. Niederschleinz soil contained 32 vs. 21 T-RFs for 16S rRNA gene, 9 vs. 4 T-RFs for AOB *amoA* and 11 vs. 9 T-RFs for AOA *amoA* communities. Twelve 16S rRNA gene T-RFs and 2 AOB *amoA* T-RFs were common to both soils, while the remaining T-RFs were unique to either soil. The Purkersdorf AOA *amoA* T-RFLP profiles contained the same nine T-RFs that were also present in the Niederschleinz profiles, together with two additional unique T-RFs.

Table 2. Ratios of archaeal to bacterial *amoA* copies in Purkersdorf and Niederschleinz soils under the various N amendments

	Purkersdorf			Niederschleinz		
Time*	KNO_3	NH_4NO_3	$[NH_4]_2SO_4$	KNO_3	NH_4NO_3	$[NH_4]_2SO_4$
No	10.2	10.2	10.2	21.0	21.0	21.0
0 day	8.6	10.4	5.2	119.5	35.7	18.6
2 days	7.5	7.5	8.6	127.9	25.9	32.9
7 days	5.6	4.0	2.6	138.6	17.4	6.7
14 days	5.7	2.9	1.9	248.3	11.8	6.0
21 days	3.6	2.5	1.3	196.3	26.2	6.1

*Soils were analyzed immediately after N amendment (0 day) and after 2, 7, 14, and 21 days; no untreated control soils.

Table 3. Ratios of archaeal to bacterial *amoA* copies in the rhizospheres of barley MR3/51, barley MR3/51 inoculated with *Burkholderia* strain PsJN, and barley Apura grown on Purkersdorf soil

	Barley variety	Day 0*	Day 14*	Day 21*	Day 28*
$[NH_4]_2SO_4$	MR3/51	0.58	0.32	0.36	0.71
	MR3/51 PsJN	1.64	0.17	0.23	0.69
	Apuro	0.78	0.19	0.53	0.44
KNO_3	MR3/51	1.31	1.24	0.95	1.42
	MR3/51 PsJN	2.25	0.37	1.11	1.00
	Apuro	0.60	0.88	0.77	0.59

*Soils were analyzed immediately after N amendment (day 0) and after 14, 21 and 28 days.

In discriminant analyses of T-RFLP data, summed up eigenvalues for functions 1 and 2 explained at least 87% of the total variance in all cases (Table 4). Most of the functions had highly significant Wilk's λ values ($P < 0.005$) and high canonical correlation coefficients ($r > 0.934$), except for functions 1 and 2 in the analysis of AOB *amoA* in Niederschleinz soil, which did not fully explain data variability.

In the 16S rRNA gene discriminant plot from Purkersdorf soil, T-RFs from $[NH_4]_2SO_4$- and NH_4NO_3-treated samples from day 21 each formed most distinct clusters relative to the other samples. In the discriminant plot of 16S rRNA gene profiles from Niederschleinz soil, T-RFs from all samples from day 21 formed clusters according to the various N treatments, which were well separated along function 1 from day 0 and untreated control samples (Fig. 4a).

Similar to the 16S rRNA gene profiles, the AOB *amoA* T-RFs from $[NH_4]_2SO_4$- and NH_4NO_3-treated Purkersdorf soils from day 21 were also well distinguished against the remaining soils in the discriminant plot. Among Niederschleinz AOB *amoA* T-RFs, only those from soils amended with $[NH_4]_2SO_4$ and sampled on day 21 formed a separate cluster (Fig. 4b).

AOA *amoA* T-RFs from Purkersdorf soil formed separate clusters in the discriminant plot according to the various treatments and sampling times. In the discriminant plot of AOA *amoA* T-RFs from Niederschleinz soil, NH_4NO_3-amended samples from day 21 were most strongly separated from the other samples, which were also grouped according to treatment, but to a lesser extent than the Purkersdorf samples (Fig. 4c). AOA *amoA* profiles from differentially treated Purkersdorf and Niederschleinz samples, respectively, differed regarding the relative abundances of several peaks, but consistently contained the same major peaks (Table 4).

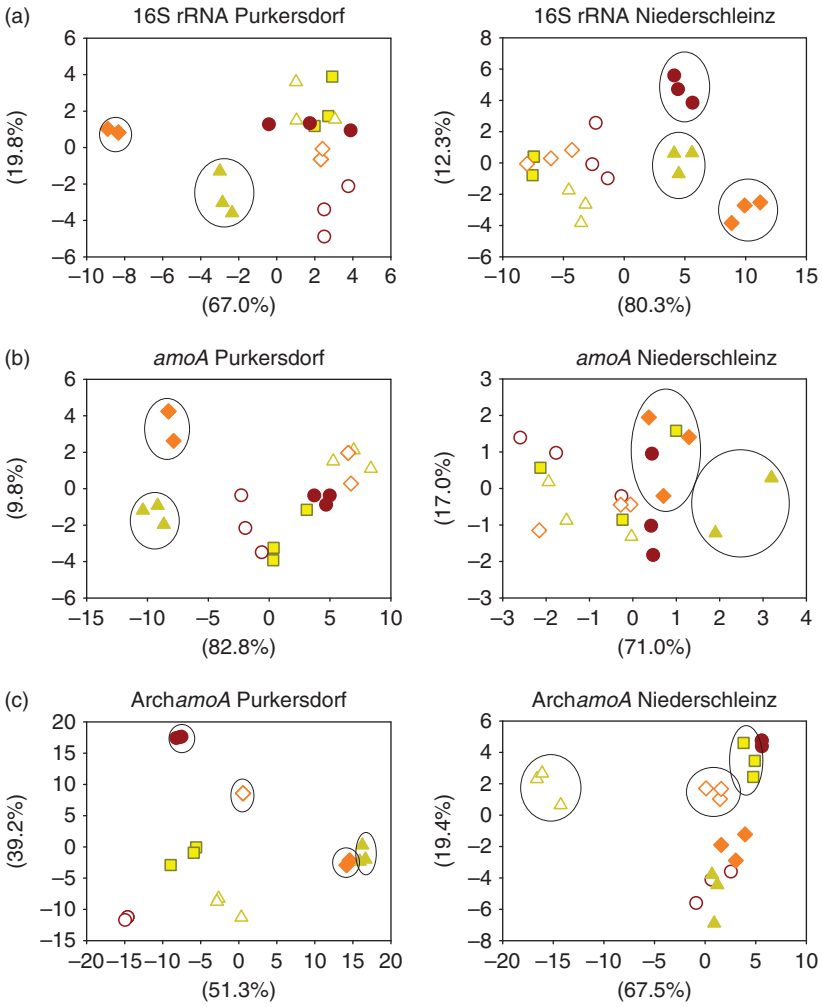

Fig. 4. Score plots of discriminant analysis performed on T-RFLP data of (a) 16S rRNA gene, (b) AOB *amoA* and (c) AOA *amoA* gene fragments from Purkersdorf and Niederschleinz soils. Besides the analysis of unplanted, unfertilized control soils ($n = 3$) (■), soils ($n = 3$) receiving $[NH_4]_2SO_4$ (Δ, ▲), NH_4NO_3 (◇, ◆) and KNO_3 (○, ●) were analyzed immediately after N amendment (open symbols) and after 21 days (filled symbols). Encircled symbol groups signify that T-RFs were discriminated from other samples in ANOSIM analysis.

AOB and AOA *amoA* clones from both soils were screened for those T-RFs that differed significantly among the fertilization treatments. Of those four T-RFs in the AOB community profiles from Purkersdorf soil, which differed significantly among the fertilization treatments, only one fragment corresponded to the respective clones in the library (Tables 4 and 5). The one T-RF accounting for significant differences in the Niederschleinz AOB profiles was represented by two clones in the library (Table 5). Bacterial *amoA* clones from Purkersdorf and Niederschleinz showed homologies of 91.1–99.8% and 95.7–99.8% to published bacterial *amoA* genes (data not shown). The majority of *amoA* sequences found belonged to *Nitrosospira* cluster 3 organisms. Of 60 clones from Purkersdorf soil, 36 and 19 were affiliated with cluster 3a and 3b organisms, respectively. The remaining five clones, however, did not group with either of the *Nitrosospira* clusters, but showed the highest homologies to a *Brevibacterium* sp. WJ *amoA*-like gene (data not shown). Thirty-eight and 27 *amoA* sequences from Niederschleinz soil, respectively, were most closely related to the *Nitrosospira* clusters 3a and 3b, while one clone again grouped with the *Brevibacterium* sp. WJ *amoA*-like gene (data not shown). In both soils, AOB phylotypes that changed in response to the fertilization treatments belonged exclusively to *Nitrosospira* cluster 3a (Fig. 5).

Four and two of the AOA T-RFs that differed among fertilization treatments in Purkersdorf and Niederschleinz soils corresponded to in total 43 and 25 clones, respectively (Table 5). Archaeal *amoA* clones from the two soils had sequence homologies to published sequences of 83.5–99.8% and 79.2–99.8%, respectively (data not shown). All AOA clone sequences were positioned in the soil cluster of uncultured *Crenarchaeota* and not in the marine or the sediment clusters (data not shown). AOA phylotypes from Purkersdorf soil that changed in response to fertilization were affiliated with agricultural soil (33 clones) as well as meadow soil (10 clones)-derived sequences. By contrast, all the responsive AOA phylotypes from Niederschleinz clustered with sequences from agricultural soils (Fig. 6).

Table 4. Statistical analysis of 16S rRNA gene and AOB and AOA *amoA* T-RFLP data

Soil	16S rRNA gene	AOB *amoA*	AOA *amoA*
Purkersdorf			
ΣT-RFs[†]	32	9	11
FC 1 (%)[‡]	67.0	82.0	51.3
(*R*/Wilk's λ)	(0.979/0.000)	(0.991/0.000)	(0.997/0.000)
FC 2 (%)[‡]	19.8	9.8	39.2
(*R*/Wilk's λ)	(0.933/0.010)	(0.934/0.007)	(0.996/0.000)
Cumulative (%)[‡]	86.8	92.2	90.5
Significant T-RFs[§]	209, 281, 241, 248, 274	64, 71, **201**, 211	137, 138, 139, **165**, 169, 170, **208**, **209**, 246, **260**
Niederschleinz			
ΣT-RFs[†]	21	4	9
FC 1 (%)[‡]	80.3	71.0	67.5
(*R*/Wilk's λ)	(0.991/0.000)	(0.859/0.106*)	(0.992/0.000)
FC 2 (%)[‡]	12.3	17.0	19.4
(*R*/Wilk's λ)	(0.945/0.005)	(0.635/0.404*)	(0.975/0.000)
Cumulative (%)[‡]	92.6	88.1	87.0
Significant T-RFs[§]	153, 196	**226**	136, 139, 165, 170, **246**, **261**

*No statistical significance ($P > 0.05$).

[†]Number of major peaks.

[‡]Eigenvalues of functions (FC) 1 and 2 derived from canonical discriminant analysis and the corresponding correlation coefficients (*R*) and Wilk's λ values.

[§]T-RFs that are significantly different among soils receiving various fertilizer treatments based on ANOVA analysis ($P < 0.05$), bold numbers indicate corresponding sequences in the clone library.

Plant biomass and N content and *B. phytofirmans* strain PsJN cell numbers

$[NH_4]_2SO_4$ fertilization resulted in higher above ground biomass of both barley var. MR 3/51 and var. Arupo plants as compared with the KNO_3 treatment (data not shown). In barley var. MR 3/51, the N content was also higher following $[NH_4]_2SO_4$ vs. KNO_3 amendment (data not shown). Inoculation with *B. phytofirmans* strain PSJN, however, had no consistent effect on the plant biomass. Only following $[NH_4]_2SO_4$ amendment the root biomass of PsJN-inoculated MR3/51 plants (37.03 ± 0.35 mg DW) was enhanced relative to noninoculated MR3/51 plants and to (also noninoculated) var. Arupo plants (22.92 ± 0.36 mg DW), while at the same time, the plant N content was lower. *Burkholderia phytofirmans* strain PsJN was recovered from the soils of all inoculated plants, with CFUs ranging from 3.83×10^4 to 3.74×10^5 g^{-1} soil FW.

Discussion

Soil type and physicochemical qualities have been found to be influential on microbial activities including N transformations (Cheneby *et al.*, 2000; Sessitsch *et al.*, 2001; Girvan *et al.*, 2003). Purkersdorf and Niederschleinz soils differed in the major soil characteristics (Table 1) and hosted distinct bacterial communities, including the AOB subsets, while the AOA communities in the two soils were more similar in composition, as evidenced in the community profiling by the numbers of shared and unique T-RFs, respectively. Nevertheless, the AOB nitrifiers in both soils were affected in abundance and community composition only by the $[NH_4]_2SO_4$ amendment (Figs 1, 2 and 4), and changes in the AOA community size and composition correspondingly occurred in $[NH_4]_2SO_4$-, NH_4NO_3- and KNO_3-amended soils (Figs 3 and 4). Although Niederschleinz soil contained two times more AOB and three times more AOA than Purkersdorf soil, the two soils had similar AOA to AOB ratios of 10 and 21. This contrasts with other soil habitats, where AOA have been found to dominate over AOB up to several hundred times (Leininger *et al.*, 2006). That AOB and AOA were similarly represented in the two soils may partly account for the concordant responses to the various N amendments.

AOB abundances in the Purkersdorf and Niederschleinz soils increased six and 4.5 times upon $[NH_4]_2SO_4$ addition, respectively (Fig. 1a and b). Fertilizer NH_4-N levels in the soils declined immediately following application, together with gradually increasing NO_3-N, but AOB numbers increased only with a delay of 2–7 days. Apparently, ammonia oxidation was brought about initially through enhanced activity of the resident AOB community. While *amoA*-based quantification of AOB reflects the dynamics in the AOB population size, it does not provide evidence of the actual ammonia-oxidizing activity.

As compared with the bulk soils, the same $[NH_4]_2SO_4$ treatment led to a minor, maximum fourfold increase in

Table 5. Sequence affiliations of cloned bacterial and archaeal *amoA* sequences that corresponded to TRFs differing significantly in the fertilization treatments (see Table 2) with entries in the NCBI database

Clone library	Number of clones	Actual TRF*	*In silico* TRF†	Accession no.‡	Isolation source	% identity§
(P2) Purkersdorf Archaeal *amoA*	1	165	163	GQ142486.1	Kobresia meadow soil from Mount Mila in Tibetan Plateau	98.42
	8	208/209	208	EU025151.1	Sediment of Changjiang Estuary and East China Sea	98.7–99.4
	2	208/209	208	GQ142714.1	Kobresia meadow soil from Mount Mila in Tibetan Plateau	98.9–99.1
	1	260	259	GQ142575.1	Kobresia meadow soil from Mount Mila in Tibetan Plateau	98.3
	4	260	259	GQ906636.1	Confluence of the Ohio and Mississippi River water	98.1–99.2
	6	260	259	GQ906638.1	Confluence of the Ohio and Mississippi River water	98.3–99.1
	4	260	259	GQ142340.1	Kobresia meadow soil from Mount Mila in Tibetan Plateau	98–98.3
	17	260	259	GQ142486.1	Kobresia meadow soil from Mount Mila in Tibetan Plateau	98.1–98.6
(P1) Purkersdorf Bacterial *amoA*	1	201	201	DQ480828.1	Soil aggregates after tillage	100
	2	201	201	HM113503.1	Soil under different land management	99.6–99.8
	4	201	201	DQ480844.1	Soil aggregates after tillage	99.4–99.6
(N2) Niederschleinz Archaeal *amoA*	1	246	250	FN423454.1	China, Gansu Province; maize and faba bean-intercropped soil	99.2
	2	261	259	FN423433.1	China, Gansu Province; maize and faba bean-intercropped soil	99.4–99.5
	2	261	259	FN423454.1	China, Gansu Province; maize and faba bean-intercropped soil	99.1–99.2
	1	261	259	FN423460.1	China, Gansu Province; maize and faba bean-intercropped soil	99.2
	10	261	259	FN691256.1	China, Gansu Province; arable soil	92.4–99.8
	3	261	259	FN691259.1	China, Gansu Province; arable soil	98–99.2
	3	261	259	FN691266. 1	China, Gansu Province; arable soil	98.9–99.7
	2	261	259	HM055560.1	Sludge sample from wastewater treatment plant	84.1–98.3
	1	261	260	GQ906643.1	Confluence of the Ohio and Mississippi River water	85.2
(N1) Niederschleinz Bacterial *amoA*	2	226	225	FJ940186.1	Vegetable soil	99.4–99.8

*Length of TRF derived from T-RFLP analysis using AluI restriction.
†TRF determined *in silico* for AluI restriction.
‡Accession number of closest match in the NCBI database.
§Percent sequence homology with closest NCBI match.

bacterial *amoA* genes in the barley rhizosphere (Fig. 1c–e). Plants together with N-assimilating microorganisms compete fiercely with nitrifiers for NH_4-N, which may have restricted the growth of AOB in the rhizosphere. Similarly, competition for NH_4-N was shown to affect the gross nitrification rates negatively in the rhizosphere of wild oat (Herman *et al.*, 2006). Organic carbon (C) or nitrification inhibitors released from plant roots may additionally inhibit autotrophic AOB (Verhagen & Laanbroek, 1991; Strauss & Lamberti, 2000; Subbarao *et al.*, 2007).

Besides scavenging ammonium, barley roots appeared to also compete efficiently for nitrate, as has been evidenced previously especially during early barley plant growth stages (Inselsbacher *et al.*, 2010). While in the bulk soils the nitrate concentrations increased transiently and then fluctuated in a similar way in the various fertilization regimes, the nitrate concentrations declined to low levels immediately after N addition in the barley root environment (Figs 1 and 2), suggesting rapid uptake by plants and nitrate-assimilating microorganisms. Nitrate probably was also subject to denitrification, which is a common process in anaerobic microsites within the rhizosphere (Cavigelli & Robertson, 2000; Philippot *et al.*, 2007; Philippot *et al.*, 2009).

The rhizospheres of the two different barley genotypes had similar numbers of AOB. Higher AOB numbers, however, were measured following $[NH_4]_2SO_4$ amendment in the rhizosphere of barley var. M3/51 inoculated with the plant growth-promoting *B. phytofirmans* strain PsJN

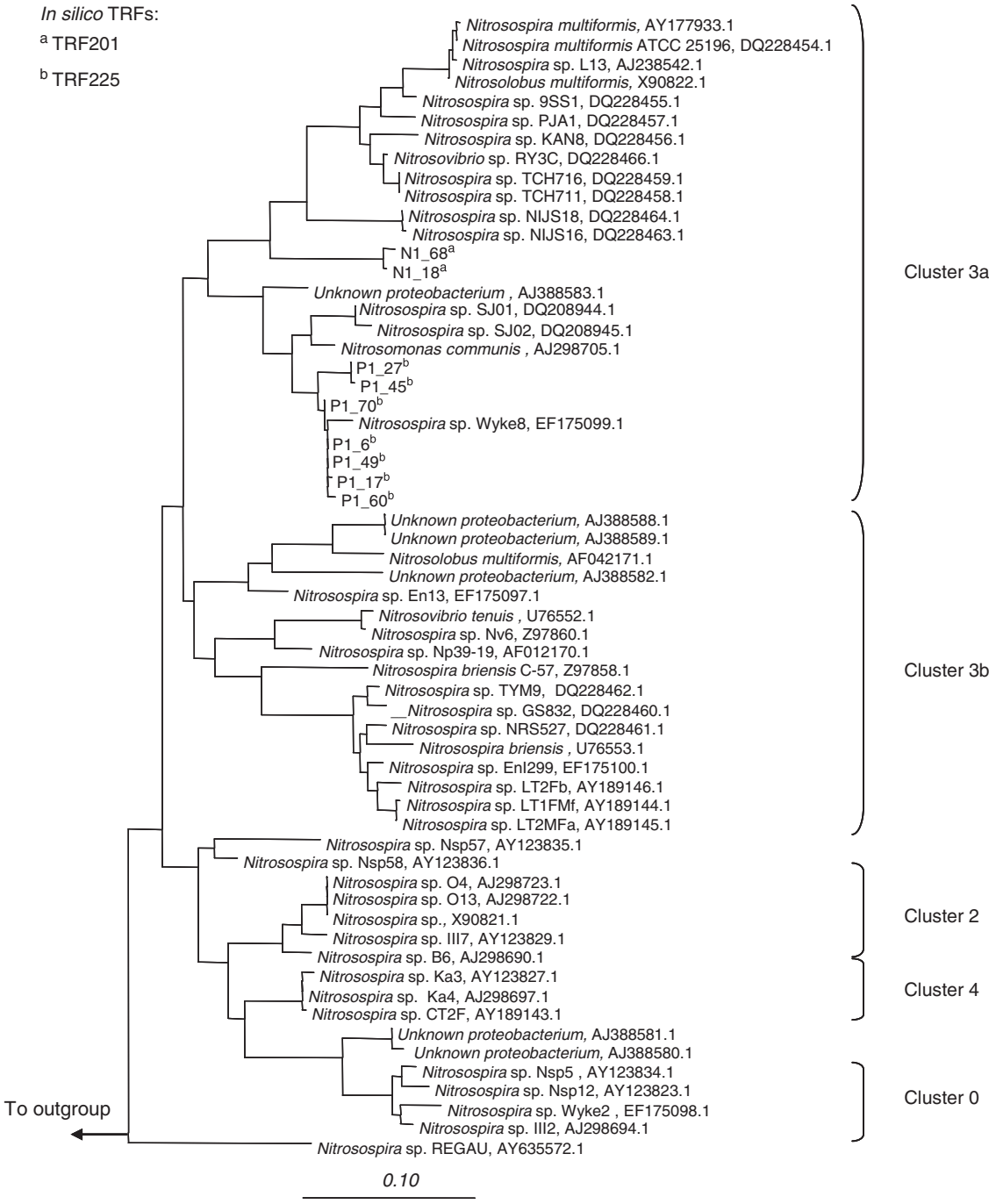

Fig. 5. Bacterial *amoA* tree constructed using PhyML algorithms in the ARB software package (Ludwig *et al*., 2004). Cluster assignments were made based on Avrahami *et al*. (2002) and Koops *et al*. (2003). Environmental clones (approximately 450 bp sequence length) from this study that correspond to significantly different T-RFs in N amendments appear with the site (P or N), and the respective *in silico* T-RF lengths of the various phylotypes are indicated. The bar represents 10% amino acid sequence divergence. The *amoA* sequences of the *Nitrosomonas europaea/Nitrococcus mobilis* and *Nitrosomonas marina/Nitrosomonas oligothropha* lineages were used as an outgroup reference.

(Fig. 1d). This suggests that soil N cycling in the rhizospheres of PsJN-inoculated vs. noninoculated plants was differentially influenced by ammonium fertilization, which, in addition, resulted in higher root biomass and lower N contents in inoculated plants. The PsJN strain efficiently colonizes the rhizospheres and endospheres of numerous plant species and has the potential for high 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity and IAA

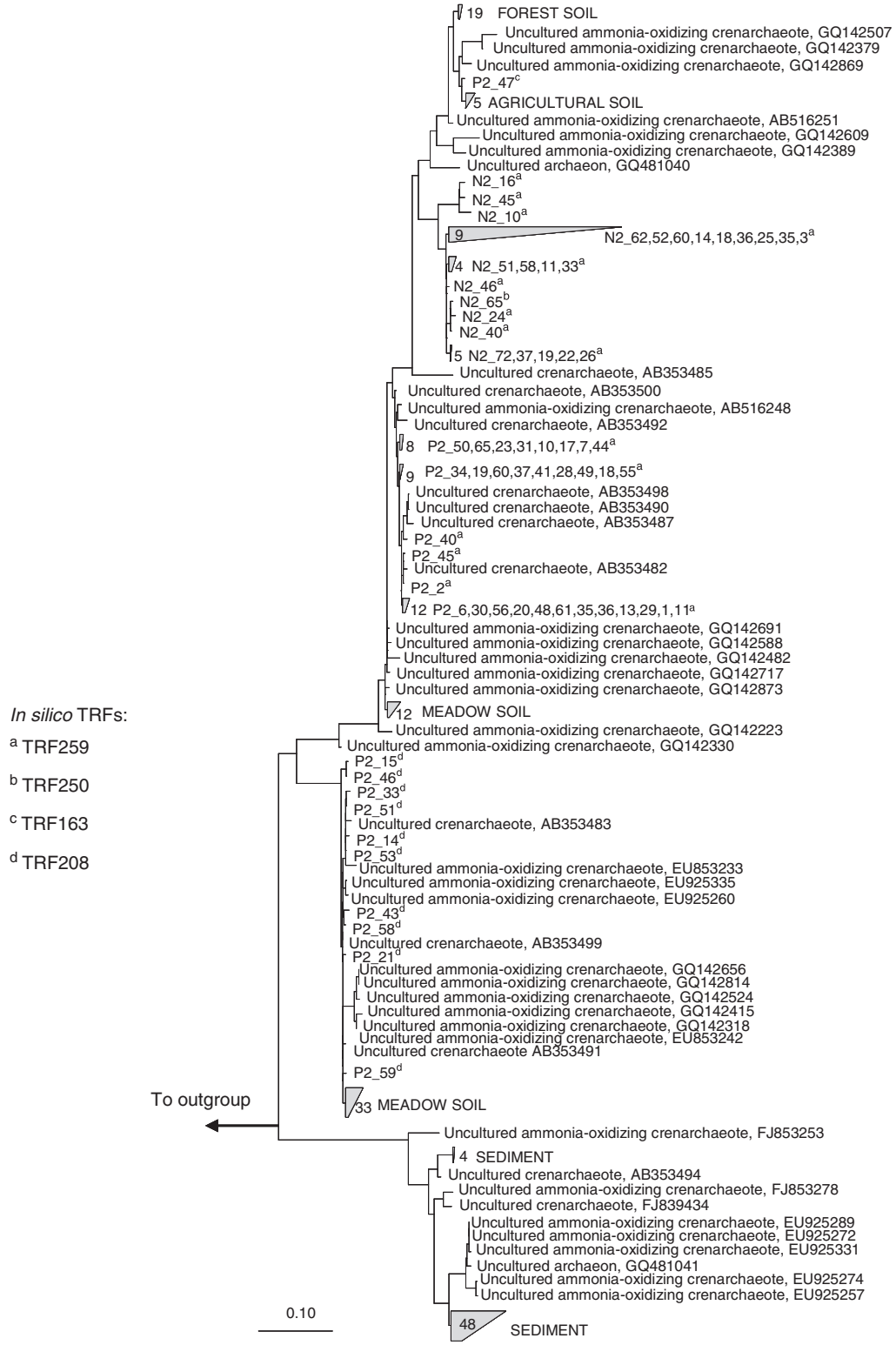

Fig. 6. Archaeal *amoA* tree constructed using PhyML algorithms in the ARB software package (Ludwig *et al.*, 2004). Clades are labeled according to the most frequently represented environment. Environmental clones (approximately 600 bp sequence length) from this study that correspond to significantly different T-RFs in N amendments appear with the site (P or N), and the respective *in silico* T-RF lengths of the various phylotypes are indicated. The bar represents 10% amino acid sequence divergence. The tree was rooted with 10 sequences of a deep marine water clade (DM).

production (Sessitsch *et al.*, 2005; unpublished data), but it does not fix atmospheric N. The effects of PsJN on soil N cycling have not been described earlier, and the mechanisms potentially involved herein are unclear. These might include effects of rhizosphere-colonizing PsJN on the root architecture or, via competition for nutrients with endophytic PsJN bacteria, effects on the plant N budget. Overall, inoculation with the PsJN strain did not promote the growth of the barley plants under conditions of high N fertilizer input, and the potential effects of the inoculant strain on rhizosphere N cycling need to be further tested for consistency.

Apart from AOB numbers, the AOB community composition was also affected by ammonium fertilizer amendment to Purkersdorf and Niederschleinz soils. While in both soils the AOB community structures changed specifically only upon ammonium amendment, they did so more distinctly in Purkersdorf than in Niederschleinz soil (Fig. 4b). In the former, AOB community changes may have been partly induced by the change in pH (Prosser & Embley, 2002; Koops *et al.*, 2003; Nicol *et al.*, 2008), brought about by the release of hydrogen ions during ammonia oxidation and also through exchange of NH_4^+ for H^+ during plant N uptake. In Niederschleinz soil, by contrast, the soil pH was more stable, presumably due to strong carbonate buffering (Table 1, Figs 1 and 2).

While the total AOB communities in both soils also contained *Nitrosospira* cluster 3b organisms and some affiliates to an *amoA*-like sequence from a novel actinobacterial nitrifying strain (data not shown), only AOB phylotypes related to *Nitrosospira* cluster 3a appeared to be responsive to ammonium amendment (Fig. 5). Cluster 3a organisms within the subgroup have previously exhibited community shifts in response to changes in temperature (Avrahami and Conrad, 2003), and *Nitrosospira* cluster 3 organisms in general have been found ubiquitously in soil environments (e.g. Fierer *et al.*, 2009), but seem to dominate especially in N-fertilized soils (Bruns *et al.*, 1999; Avrahami *et al.*, 2003; Chu *et al.*, 2007).

The changes in the AOB community size were similar in $[NH_4]_2SO_4$- and NH_4NO_3-amended soils, although they were more pronounced in the former (Fig. S1). That no distinct effects on AOB abundance were seen after KNO_3 amendment signifies that N addition in the form of ammonium was the critical factor determining the prevalence of AOB in the soils studied. Higher AOB abundances in ammonia-fertilized vs. -unfertilized soils have repeatedly been reported both in microcosms and on the field (Hermansson & Lindgren, 2001; Okano *et al.*, 2004; Enwall *et al.*, 2007). However, $(NH_4)_2SO_4$ amendment to an organic farm soil did not change AOB abundances (Cavagnaro *et al.*, 2008). Differential effects on both AOB and AOA abundances were also seen when various combinations of fertilizer N, phosphorus and potassium as well as organic manure were applied in long-term soil treatments (He *et al.*, 2007). Similarly, the effects on AOB community composition upon the addition of mineral N fertilizers varied, including pronounced (Mendum & Hirsch, 2002) or minor changes in AOB community profiles (He *et al.*, 2007) and increased or reduced AOB diversity (Chu *et al.*, 2007).

In contrast to the changes seen in AOB abundance, suggesting utilization specifically of the ammonia substrate, fluctuations in AOA numbers seemed to be basically unrelated to ammonium availability. Higher AOA numbers occurred in Purkersdorf soil 2 days after $[NH_4]_2SO_4$ addition (Fig. 3a), but then both soil ammonium and nitrate were high (Fig. 1a). Ammonium amendment to other soils including rice paddies resulted in increased AOB, but unchanged AOA abundances, suggesting the involvement of mainly AOB in ammonia oxidation (Shen *et al.*, 2008; Di *et al.*, 2009; Wang *et al.*, 2009). Recently, a greater importance of AOB than AOA for ammonia oxidation in an agricultural soil has been demonstrated through DNA stable isotope probing (Jia & Conrad, 2009). Considering the high ammonia conversion rates displayed by AOB isolates (Prosser, 1989; Okano *et al.*, 2004) and the distinct growth responses of AOB following ammonium fertilizer amendment to Purkersdorf and Niederschleinz soils, it seems most likely that AOB and not AOA were mainly responsible for oxidizing the added ammonium.

Other than in the bulk soils, AOA in the barley rhizospheres did not dominate over AOB, but were equally abundant before N fertilizer addition. Following $[NH_4]_2SO_4$ amendment, however, the ratios of AOA to AOB *amoA* copies changed in favor of AOB, reinforcing the notion that AOB and not AOA were the dominant nitrifiers in the fertilizer-amended soils. As ammonia-oxidizing rates were low in AOA isolates (Könneke *et al.*, 2005; de la Torre *et al.*, 2008), a backup function of AOA was suggested either under disturbed (Schauss *et al.*, 2009) or under low-nutrient conditions (Erguder *et al.*, 2009; Martens-Habbena *et al.*, 2009), which may be supported by our findings.

Fluctuating AOA numbers in the various fertilizer treatments may imply that the AOA communities responded to fluctuations in substrate availability, which may include soil nitrate that was supplied either as fertilizer or resulted from oxidation of the fertilizer ammonium. AOA abundance seemed to be influenced by nitrate especially in the Niederschleinz soil, where AOA dominated over AOB most strongly following KNO_3 amendment. Because nitrate is prone to rapid translocation with the soil water in addition to microbial and plant uptake, alteration in the net nitrate concentration was only transiently detectable in the soils. Notably, a potential role of AOA in nitrate reduction has been indicated previously by the presence of denitrification genes in AOA fosmid clones (Treusch *et al.*, 2005; Hallam *et al.*, 2006). While ammonia-based chemolithoautotrophic

energy metabolism by AOA has been supported in studies of the so far only free-living AOA isolate, '*Candidatus* Nitrosopumilus maritimus' strain SCM1 (Könneke *et al.*, 2005), nitrate reduction by AOA has not been evidenced. This stresses the need for retrieving AOA isolates from soils, which would enable to further explore AOA metabolism and lifestyle traits.

In both the Purkersdorf and the Niederschleinz soils, the relative abundances of an otherwise constant number of AOA phylotypes were shifted upon receiving various N amendments, resulting in the segregation of the AOA communities in discriminant plots (Fig. 4c). Structural changes in AOA communities were caused primarily or exclusively by phylotypes related to agricultural soil-derived sequences in Purkersdorf and Niederschleinz soil, respectively (Fig. 6), which, through their varying representation, might exhibit plasticity in function with respect to different fertilization regimes. Notably, AOA communities appeared to differentiate immediately after fertilizer addition, suggesting that they can rapidly metabolize various N forms. Based on their high prevalence in ecologically diverse habitats and the possession of genes encoding putative functions in C fixation and the oxidation of various reduced N compounds, a more versatile metabolism of AOA than AOB has been implicated (Leininger *et al.*, 2006). Furthermore, AOA metabolism may be particularly responsive to oxygen and carbon dioxide released by roots into the rhizosphere, as has been suggested in studies of a rice paddy soil (Chen *et al.*, 2008). Nevertheless, while information on gene functions relating to specific metabolic traits of AOA has been increasing, the lifestyles and ecological functions of AOA in their *in situ* environments are still unknown.

Concluding, elevated soil nitrate and ammonium concentrations and altered pH values were short-term effects of $[NH_4]_2SO_4$ and NH_4NO_3 amendments to barley-planted soils, because the initial levels were reestablished within 3–4 weeks. AOB community dynamics lagged behind approximately 1 week relative to changes in ammonium availability and were unaffected by KNO_3 addition, whereas AOA abundances fluctuated in a similar way in the various fertilizer treatments. These results imply that AOB were the main agents responsible for ammonium oxidation in two different fertilizer-amended agricultural soils, while the roles of AOA in soil N cycling need to be further elucidated.

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Supporting Information

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

Fig. S1. Dynamics in AOB *amoA* copy numbers in relation to soil NH_4-N and NO_3-N concentrations, and soil pH values, following NH_4NO_3 amendment.

Table S1. Copy numbers (g DW^{-1} soil) of AOB and AOA *amoA* gene fragments in Purkersdorf and Niederschleinz soils amended with KNO_3, $[NH_4]_2SO_4$, and NH_4NO_3 at five time points following fertilizer amendments (no = untreated control).

Table S2. Copy numbers (g DW^{-1} soil) of AOB and AOA *amoA* gene fragments in Purkersdorf rhizospheres beneath barley var. MR3/51 (G1), barley var. MR3/51 inoculated with *Burkholderia* strain PsJN (G1 PSJN) and barley var. Apuro (G2) in $[NH_4]_2SO_4$ and KNO_3 treatments.

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