Abundance and community structure of ammonia-oxidizing bacteria and archaea in a temperate forest ecosystem under ten-years elevated CO₂

Xien Long^{a,c}, Chengrong Chen^{b,**}, Zhihong Xu^c, Ram Oren^{d,e}, Ji-Zheng He^{a,*}

^aState Key Laboratory of Urban and Regional Ecology, Research Centre for Eco-Environmental Sciences, Chinese Academy of Sciences, 18 Shuangqing Road, Beijing 100085, China

^bEnvironmental Futures Centre and Griffith School of Environment, Griffith University, Nathan, Queensland 4111, Australia

^cEnvironmental Futures Centre and School of Biomolecular and Physical Sciences, Griffith University, Nathan, Queensland 4111, Australia

^dNicholas School of the Environment and Earth Science, Duke University, Durham, NC 27708, USA

^eDepartment of Forest Ecology and Management, Swedish University of Agricultural Sciences (SLU), SE-901 83 Umeå, Sweden

ARTICLE INFO

Article history:

Received 7 August 2011

Received in revised form

12 December 2011

Accepted 13 December 2011

Available online 26 December 2011

Keywords:

Ammonia-oxidizing bacteria and archaea

Elevated CO₂

Free air CO₂ enrichment (FACE) experiment

N fertilization

Nitrification

Temperate forest ecosystem

ABSTRACT

Ammonia-oxidizing bacteria (AOB) and archaea (AOA) are considered as the key drivers of global nitrogen (N) biogeochemical cycling. Responses of the associated microorganisms to global changes remain unclear. This study was to determine if there was a shift in soil AOB and AOA abundances and community structures under free-air carbon dioxide (CO₂) enrichment (FACE) and N fertilization in Duke Forest of North Carolina, by using DNA-based molecular techniques, i.e., quantitative PCR, restriction fragment length polymorphism (RFLP) and clone library. The N fertilization alone increased the abundance of bacterial *amoA* gene, but this effect was not observed under elevated CO₂ condition. There was no significant effect of the N fertilization on the thaumarchaeal *amoA* gene abundance in the ambient CO₂ treatments, while such effect increased significantly under elevated CO₂. A total of 690 positive clones for AOA and 607 for AOB were selected for RFLP analysis. Analysis of molecular variance (AMOVA) indicated that effects of CO₂ enrichment and N fertilization on the community structure of AOA and AOB were not significant. Canonical correspondence analysis also showed that soil pH rather than elevated CO₂ or N fertilization shaped the distribution of AOB and AOA genotypes. A negative linear relationship between the δ¹³C and archaeal *amoA* gene abundance indicated a positive effect of elevated CO₂ on the growth ammonia oxidizing archaea. On the other hand, the community structures of AOB and AOA are determined by the soil niche properties rather than elevated CO₂ and N fertilization.

© 2011 Elsevier Ltd. All rights reserved.

1. Introduction

Human activities such as the combustion of fossil fuels and deforestation have increased the atmospheric carbon dioxide concentration (CO₂) by ca. 35% mostly since the beginning of industrialization. Atmospheric CO₂ enrichment has been shown to significantly influence the structure and function of terrestrial ecosystems (Rosenzweig et al., 2007), adding to or interacting with the spatial and temporal effects of environmental variables (Ge et al., 2010). Soil microorganisms, encompassing the greatest diversity in terrestrial ecosystems, are the key drivers in almost all global biogeochemical cycles (Fuhrman, 2009). However, their responses to elevated CO₂ are poorly understood (Heimann and Reichstein, 2008) and even controversial (Horz et al., 2004; Schortemeyer et al., 1996).

Lithoautotrophic ammonia-oxidizing bacteria (AOB), which catalyze the first step of nitrification process (NH₃ → NO₂⁻) – a rate-limiting step in the global nitrogen (N) cycle – have been designated as the model for molecular microbial ecology. Evidence is building supporting the presence of ammonia-oxidizing archaea (AOA) in various soil environments (He et al., 2007; Leininger et al., 2006; Nicol and Schleper, 2006). The abundance, distribution and community structure of ammonia-oxidizing organisms can be significantly affected by environmental factors, including levels of ammonium, temperature (Tournier et al., 2008), moisture (Horz et al., 2004), salinity (Bernhard et al., 2010), and pH (He et al., 2007; Nicol et al., 2008). It has been shown that N fertilization significantly increased the abundance of soil AOB, but not AOA (Di et al., 2009). The effects of elevated CO₂ on the abundance of AOB are less consistent, ranging from no effect in forest ecosystems (O'Neill et al., 1987) or a white clover and perennial ryegrass dominated grassland ecosystem (Schortemeyer et al., 1996) to a decrease in an annual grass and forb dominated grassland ecosystem (Horz et al., 2004). On the other hand, little is known

* Corresponding author. Tel.: +86 10 6284 9788; fax: +86 10 6292 3563.

** Corresponding author. Tel.: +61 7 3735 7494; fax: +61 7 3735 7773.

E-mail addresses: c.chen@griffith.edu.au (C. Chen), jzhe@rcees.ac.cn (J.-Z. He).

regarding effects of elevated CO₂ on community structure of AOB and AOA. Moreover, the interactive effects of multiple factors (i.e., elevated CO₂, soil N, temperature and precipitation) on the abundance and community structure of both AOB and AOA have been scarcely studied. It has been suggested that global changes (e.g., elevated atmospheric CO₂ and N deposition) may change the resource availability (e.g., C or N) and quality, leading to shifts in the abundance and community structure of ammonia oxidizers (Horz et al., 2004).

Previous studies at the Duke free-air CO₂ enrichment (FACE) site indicated that both elevated CO₂ and N fertilization altered soil microbial community composition based on phospholipid fatty acid (PLFA) profiling (Feng et al., 2010), whereas another study suggested that the bacterial community composition was more influenced by spatial variations than the CO₂ enrichment (Ge et al., 2010). Increased allocation of C (carbohydrates) into the soil under elevated CO₂ (Palmroth et al., 2006) and enhanced soil N availability with N fertilization have led to the shifts in the abundance and composition of soil microbial communities (Finzi et al., 2006). Reports on the responses of AOB to elevated CO₂ are controversial in the literature. Some studies found that elevated CO₂ did not affect AOB abundance or nitrifying enzyme activity (Barnard et al., 2004; O'Neill et al., 1987; Schortemeyer et al., 1996), whereas others revealed that elevated CO₂ decreased the abundance of AOB in the grassland ecosystem, and this effect was enhanced under elevated precipitation (Horz et al., 2004). In this study, we examined the separate and combined effects of CO₂ enrichment (for ten-years) and N fertilization (for two years) on the abundance and community structure of soil AOB and AOA in a temperate forest, North Carolina, USA. It was hypothesized that: (1) whether both the abundance of AOA and AOB will increase under elevated CO₂ or N fertilization? And (2) whether the community structures of AOB and AOA will shift under long-term elevated CO₂?

2. Materials and methods

2.1. Site description and soil sampling

The FACE experiment was established in the Duke Forest (Orange County, North Carolina, USA) (35°58'41"N, 79°05'39"W). The site has a temperate climate with a mean annual temperature of 15.5 °C and average annual precipitation of 1140 mm (Oren et al., 2001). Duke Forest is dominated by loblolly pine (*Pinus taeda* L.) stand established in 1983. Six of the eight 30 m diameter circular plots were utilized in this study. Three experimental plots were fumigated with CO₂ to maintain the atmospheric CO₂ concentration of 200 μL L⁻¹ above the ambient (i.e., ~565 μL CO₂ L⁻¹ at the year of soil sampling), while three control plots were fumigated with ambient air only (365 μL CO₂ L⁻¹). The plots ($n = 6$) were arranged in a complete block design to lessen topographic variation and potential fertility gradients. The CO₂ enrichment experiment began on 27th August 1996 (Oren et al., 2001). For the N amendment study, each plot was split into two halves inserting an impermeable layer through the root zone (0.7 m) with one-half of each plot fertilized at a rate of 11.2 g N m⁻² yr⁻¹ and the other half unfertilized (Oren et al., 2001). For N fertilization, ammonium-nitrate pellets were applied in two half-doses in March and April of 2005, and applied in a single full-dose annually in March in the following years. Thus, four treatments were designated as A and E for ambient and elevated CO₂, and U and N for unfertilized and N-fertilized, respectively, resulting in 4 treatments: AU, EU, AN and EN.

Soils at the site are classified as the Enon series, a low-fertility, slightly acidic, Ultic Alfisol (pH 5.75, 0.1 mol L⁻¹ CaCl₂). For each treatment, 5 sub-samples of the topsoil (0–10 cm) were collected

and mixed to form one composite sample in August 2006. The sampling was conducted at ca. 1.5 years after inserting the impermeable layers and fertilization. Samples were taken along the central section of the half plot to avoid the disturbance of impermeable layers. Each composite sample was placed in a sterile plastic bag, sealed and kept on ice several hours before transporting to Australia by dry ice. All samples were passed through a 2.0-mm sieve and subsamples were stored at –80 °C for DNA analysis. Further details regarding FACE technology and site characteristics can be found in the previous publication (Oren et al., 2001).

2.2. Soil DNA extraction and PCR

Triplicate soil DNA of each treatment was extracted from 0.3 g of defrosted soil sample using the MoBio Powersoil DNA Isolation Kit (Carlsbad, USA) according to the manufacturer's instruction. The process of soil DNA extraction was under the uniform conditions like the same temperature, chemical reagents and the handler. The extracted DNA was high quality with ratios of A260/A230 and A260/A280 above 1.5 and 1.8, respectively (He et al., 2005). The extracted DNA was stored at –20 °C. The effect of soil inhibitors on PCR was tested in a dilution series of 10, 20 and 50-fold of the extracted DNA in the presence and absence of bovine serum albumin (BSA). It was found that a 10-fold dilution was enough to remove the effect of soil inhibitors on qPCR. The *amoA* genes of AOA and AOB were amplified using primers *amoA1F* (GGG GTT TCT ACT GGT GGT)/*amoA2R* (CCC CTC KGS AAA GCC TTC TTC) and *CrenamoA23F* (ATG GTC TGG CTW AGA CG)/*CrenamoA616R* (GCC ATC CAT CTG TAT GTC CA), respectively, as described previously (He et al., 2007; Tournia et al., 2008). The 50 μL reaction mixture contained: 10 μL 5× GoTaq Flexi Green Buffer (Promega), 2.5 mM MgCl₂, 750 μM each dNTP, 100 μM each primer, 1.25 units GoTaq DNA polymerase (Promega), 0.5 μL template DNA and ultraclean water to volume.

2.3. Establishment of AOB and AOA *amoA* gene fragment libraries and identification of associated genotypes

The obtained PCR products were purified using a QIAquick Gel Extraction Kit (Qiagen, Valencia, CA). Clone libraries were constructed using the pGEM®-T Easy Vector System and JM109 Competent Cells (Promega, Madison, WI) following manufacturer's instruction. Twelve bacterial *amoA* gene clone libraries and the same number of thaumarchaeal *amoA* gene clone libraries were established. Approximately 60 white clones were randomly picked up for checking with T7/Sp6 primers. The *amoA* genotypes were identified using the restriction fragment length polymorphism (RFLP). The positive clones of AOA were digested with MboI (NEB) and HaeIII (NEB) and AOB with MboI (NEB), and 5 μL of restriction digest fragments were loaded on a 3% agarose gel, running at 100 V in 1× TAE buffer for 60 min and digitally photographed using ChemiDoc™ XRS imaging system (Bio-Rad). The *amoA* gene patterns were checked using the Quantity-One software (Bio-Rad). Triplicate of each representative genotypes from the clone libraries were selected for sequencing (3130x1 Genetic Analyzer, Applied Biosystems). The obtained representative sequences were submitted to GenBank under accession numbers HM131547–HM131578.

2.4. Quantitative PCR analyses of total bacterial and archaeal rRNA and *amoA* genes

Quantitative PCR was performed on a Mastercycler thermocycler (Eppendorf). The plasmid was extracted using QIAprep Spin Miniprep Kit (QIAGEN) and DNA concentration determined using a NanoDrop 2000 UV–Vis Spectrophotometer (Thermo Scientific).

Standardization templates were performed with a dilution series (10^{-1} – 10^{-8} times) of the environmental sample plasmids with EASY Dilution (for Real Time PCR) (Takara). The reaction was performed in a 20 μ L volume containing 10 μ L SYBR[®] Premix Ex Taq[™] (Perfect Real Time) (Takara Bio), 200 μ M of each primer and 2 μ L of template DNA solution. Universal primer sets 338f (ACT CCT ACG GGA GGC AG)/518r (ATT ACC GCG GCT GCT GG) and A109f (ACK GCT CAG TAA CAC GT)/A334r (TCG CGC CTG CTG CTC CCC GT) were used to determine population size of total bacteria and archaea, respectively (Sun et al., 2009). The thermal cycling conditions for the bacterial 16S rRNA gene was as follows: an initial denaturation step at 95 °C for 2 min; 40 cycles of 95 °C for 20 s, 55 °C for 15 s, 72 °C for 30 s, 80 °C for 20 s; final extension at 72 °C for 60 s, followed by a melting curve cycle. For archaeal 16S rRNA gene, only annealing temperature was changed to 57 °C. The primer pairs for quantitative analysis of *amoA* genes were described as above. The thermal cycling conditions for the thaumarchaeal *amoA* (AOA) gene were as follows: an initial denaturation step at 95 °C for 2 min; 40 cycles of 95 °C for 15 s, 55 °C for 30 s, 72 °C for 45 s, 80 °C for 20 s; final extension at 72 °C for 60 s, followed by a melting curve analysis. The fluorescence intensity was detected at 80 °C. The thermal protocol for AOB was the same with AOA except the annealing temperature of 53 °C. Quantitative PCR was performed on purified template plasmid DNA to make a standard curve with a log–linear effect of target concentration ($r^2 = 0.999$) and an amplification efficiency of 0.950.

2.5. Statistical and phylogenetic analysis

Univariate statistical analyses were performed using Sigmaplot 11 (Systat Software, Inc., San Jose, CA). The abundance data were analyzed by analysis of variance (ANOVA) using split-plot model and generalized linear model (GLM). The RFLP genotypes of different treatments were analyzed using an AMOVA executed in Arlequin 3.5 (Excoffier and Lischer, 2010; He et al., 2006). Canonical correspondence analysis (CCA) was performed using the community ecology package “vegan” (Oksanen et al., 2010). Three important explanatory variables (pH, NH_4^+ -N and dissolved organic C (DOC)/dissolved organic N (DON) ratio) were selected for the CCA ordination diagrams by 1000 times permutation test. P -values ≤ 0.05 were considered significant. The Neighbor-joining method was followed to construct the phylogenetic tree using MEGA5 (Kumar et al., 2008) and based on the maximum composite likelihood model for calculating the evolutionary distance.

3. Results

3.1. Abundances of soil bacteria and archaea

Soil bacterial 16S rRNA gene abundance ranged from 0.49×10^9 to 2.45×10^9 copies g^{-1} dry soil weight (Fig. 1a). These values were significantly higher than those of archaeal 16S rRNA genes (1.32×10^6 – 6.61×10^6 copies g^{-1} dry soil weight) (Fig. 1a) ($p < 0.01$). Bacterial abundance reflected an interactive effect of CO_2 enrichment and N fertilization ($p = 0.024$), indicating that the N effect was restricted to ambient CO_2 conditions only (AU > AN; no difference between ambient and elevated CO_2 conditions). In contrast, no significant differences or interaction effect ($p > 0.05$) were detected in the archaeal 16S rRNA genes abundance among the treatments (Fig. 1a). There were a negative relationship between the abundance of the bacterial or archaeal 16S rRNA gene and the ratio of DOC to DON and a negative relationship between the archaeal 16S rRNA gene abundance and $\delta^{13}\text{C}$ value of the soil organic carbon ($p < 0.05$) (Table 2).

Fig. 1. Bacterial and archaeal 16S rRNA gene copy numbers under different treatments (a) and ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA) *amoA* gene copy numbers under different treatments (b). Abbreviations: AU, ambient CO_2 and unfertilized; AN, ambient CO_2 and fertilized; EU, elevated CO_2 and unfertilized; and EN, elevated CO_2 and fertilized.

3.2. Abundances of soil AOB and AOA

Bacterial *amoA* genes abundance ranged between 0.05×10^6 and 3.72×10^6 copies g^{-1} dry soil weight across all the treatments, similar to the number of thaumarchaeal *amoA* genes (0.09×10^6 – 1.86×10^6 copies g^{-1} dry soil weight; Fig. 1b). There was a significant interactive effect between N fertilization and CO_2 enrichment on the AOB abundance ($p = 0.022$). This effect was manifested in a similar number of the bacterial *amoA* gene copies under elevated CO_2 at both N levels (i.e., EU = EN) but higher number under ambient CO_2 conditions with N fertilization than under native soil N condition (i.e., AU < AN, with the elevated treatments not different from either of the ambient treatments; Fig. 1b). There was also a significant $\text{CO}_2 \times \text{N}$ effect on the number of thaumarchaeal *amoA* gene copies ($p = 0.031$), but the effect was limited to a CO_2 -induced increase in the number under native soil N only (i.e., EU > EN = AU = AN; Fig. 1b). In the unfertilized treatments with either ambient or elevated CO_2 (i.e., AU and EU), AOA have more copy numbers of *amoA* gene than AOB with the mean ratios of 2.3 and 4.1 respectively. In contrast, there was more AOB of the *amoA* gene than AOA under N fertilization in both ambient and elevated CO_2 (i.e., AN and EN), with mean ratios of 2 and 10, respectively. Linear regression analysis indicated a negative relationship between the archaeal *amoA* gene abundance and the $\delta^{13}\text{C}$ ($p < 0.05$).

3.3. Community and phylogenetic analysis of AOB and AOA

The community structure of AOB and AOA was analyzed via clone library followed by RFLP (Table 3). Three duplicates of unique genotypes were selected from each library for sequencing (in total 99 and 59 sequences of AOA and AOB, respectively). In this study, *amoA* gene sequences that shared $\geq 97\%$ similarity were defined as one genotype or operational taxonomic unit (OTU). Phylogenetic trees of the bacterial and thaumarchaeal *amoA* gene sequences and related GenBank sequences are shown in Figs. 2 and 3. For bacterial *amoA* genes, clusters 1, 2 and 9 were detected across all treatments with cluster 2 dominated. However, cluster 3a and cluster 11 were only observed under elevated CO₂ conditions (i.e., EU, EN, Fig. 2, Table 3). On the other hand, all thaumarchaeal *amoA* genes of different treatments fell within two clusters, designated as soil cluster 2 and soil cluster 5. Thaumarchaeal *amoA* sequences affiliated with soil cluster 2 were retrieved from various soils (He et al., 2007; Leininger et al., 2006), while most sequences of soil cluster 5 came from water column or marine. There were no significant shifts of *amoA* genotypes determined by RFLP in the AU, AN, EU and EN treatments and between soil cluster 2 and soil cluster 5, although soil cluster 5 predominated in all the treatments (Table 3).

3.4. Communities of AOB and AOA in response to environmental factors

Three important environmental variables (NH₄⁺-N, pH and DOC/DON ratio) and dominant *amoA* gene RFLP genotypes were included in the canonical correspondence analysis (Table 1). The first two axes of the CCA of the AOB communities versus

environmental factors explained 47.8% of the total variance in the *amoA* genotypes composition and 97.7% of the cumulative variance of the genotype–environment relationship (Fig. 4a). The DOC/DON ratio ($F = 2.573$, $p = 0.045$, 1000 permutations) was the most influential environmental gradient on the distribution of *amoA* genotype of AOB among the samples, followed by pH value ($F = 2.167$, $p = 0.084$) and NH₄⁺-N concentration ($F = 1.949$, $p = 0.118$). However, only 31.0% of the total variance and 83.2% of the cumulative variance of the AOA communities versus environmental variables were explained by the first two axes of the CCA (Fig. 4b). The CCA showed that only soil pH significantly affected the distribution of the AOA *amoA* genotypes ($F = 2.036$, $p = 0.028$, 1000 permutations).

3.5. Analyses of molecular variance of *amoA* gene sequences

The sequences of unique RFLP patterns and their statistical numbers of selected positive clones were used to analyse the molecular variance of *amoA* gene among different treatments. The AMOVA results showed that the variance (3.1%) of AOA *amoA* gene within the replicates was higher than the variance (0.59%) among the treatments. A small value for the overall fixation index (F_{SC}) was identical to the F_{ST} measure of the genetic distance among the samples. Those parameters were not significantly different from zero and, hence, revealed genetic homogeneity in the AU, AN, EU and EN treatments (Table 4). In this study, we obtained negative variance components of AOB *amoA* gene among the treatments. The variance components and F_{ST} evaluation of AMOVA are based on a multi-allelic model (Excoffier and Lischer, 2010), which relies on estimates of relationships between alleles in the same populations

Fig. 2. Neighbor joining distance tree based on an alignment of 413 bp *amoA* gene sequences. The sequences obtained in this study are indicated in bold including the following information: clone name, accession number in GenBank and treatments in the parentheses. *Nitrosomonas nitrosa* was used as the outgroup sequence. Bootstrap values ($>60\%$) are indicated at branch points. The scale bar represents 5% sequence divergence.

Fig. 3. Neighbor joining distance tree based on an alignment of 559 bp *amoA* gene sequences. The sequences obtained in this study are in bold and include the following information: clone name, accession number in GenBank and treatments in parentheses. *Nitrosopumilus maritimus* SCM1 was used as the outgroup sequence. Bootstrap values (>60%) are indicated at branch points. The scale bar represents 5% sequence divergence.

relative to the alleles of different populations. Usually, negative variance components are produced only if the estimated parameter value is close to zero, in another word, without a genetic structure among the treatments.

4. Discussion

4.1. Responses of soil bacterial and archaeal abundance to elevated CO₂ and N fertilization

Our results indicated that a 10-year elevated CO₂ exposure negated the N-induced reduction of soil bacterial abundance, which suggests that the negative effect of N fertilization (and perhaps N deposition) on the bacterial abundance decreases with increasing atmospheric CO₂ concentration. However, elevated CO₂ or N fertilization did not significantly affect the abundance of soil archaea. Previous studies also found that in comparison with fallow or without fertilizers' treatment, a range from 1.48 to 2.44-fold decrease of bacterial abundance was detected under long-term N fertilization in a wheat–maize rotation system and a wheat

continuous cropping system (He et al., 2007; Shen et al., 2010). Moreover, effects of long-term fertilization on specific bacterial and archaeal taxa revealed that ammonia sulphate significantly decreased the abundance of bacteria, archaea and most of the specific taxa, except for Acidobacteria (Wessén et al., 2010). In this study, we observed a 2.03-fold decrease of bacterial abundance with 2 years of N fertilization. Due to only two years' N fertilization addition and the possible bias of DNA PCR-based approaches, the changes in bacterial abundance among the treatments were not great enough to be explained by the fertilizer effect. Wallenstein et al. (2006) speculated that shifts of soil C/N ratio and pH value induced by N saturation decreased bacterial biomass under long-term fertilization in three forest ecosystems. Our results indicated that ratio of DOC to DON negatively influenced the abundance of bacteria and archaea (Table 2). However, effects of elevated CO₂ on soil bacterial and archaeal abundance were not widely covered. Natural CO₂ vent has been reported to decrease the abundance of soil bacteria and archaea under grass species (Oppermann et al., 2010). Yet, a four-year exposure to CO₂ in a FACE experiment site decreased the abundance of archaea but did not affect the

Table 1

pH, ammonium N (NH₄⁺–N), dissolved organic C (DOC), dissolved organic N (DON), ratio of DOC to DON, total carbon (TC), total nitrogen (TN), ratio of total carbon and nitrogen (C/N), δ¹³C and δ¹⁵N of the soil samples from the Duke forest plots.

Treatment	pH (H ₂ O)	NH ₄ ⁺ –N (mg kg ^{−1})	DOC (mg kg ^{−1})	DON (mg kg ^{−1})	DOC/DON	TC (%)	TN (%)	C/N	δ ¹³ C (‰)	δ ¹⁵ N (‰)
AU (plot_1)	4.94	189.37	447.72	75.45	5.93	6.16	0.23	26.78	−28.72	31.12
AU (plot_5)	5.00	178.84	857.90	98.80	8.68	3.87	0.19	20.37	−28.51	14.79
AU (plot_6)	4.61	187.90	912.88	93.67	9.75	7.64	0.30	25.47	−27.79	20.98
AN (plot_1)	5.20	216.33	445.73	90.61	4.92	4.31	0.18	23.94	−28.33	20.74
AN (plot_5)	4.69	266.11	1233.65	95.14	12.97	6.27	0.30	20.90	−27.80	15.35
AN (plot_6)	4.12	191.93	880.96	78.74	11.19	5.84	0.24	24.33	−27.54	13.26
EU (plot_2)	4.62	111.13	459.08	57.59	7.97	5.90	0.18	32.78	−34.56	21.68
EU (plot_3)	4.14	207.56	664.23	86.98	7.64	7.42	0.31	23.94	−33.84	13.59
EU (plot_4)	5.06	255.26	661.67	91.62	7.22	7.15	0.32	22.34	−33.99	20.22
EN (plot_2)	4.72	139.01	723.50	85.62	8.45	4.80	0.19	25.26	−35.35	17.76
EN (plot_3)	4.69	194.39	628.85	72.79	8.64	5.23	0.26	20.12	−34.76	8.72
EN (plot_4)	4.44	148.22	845.94	88.92	9.51	7.13	0.26	27.42	−32.67	16.02

A: Ambient CO₂; E: Elevated CO₂; U: Unfertilized; and N: Fertilized.

Table 2

Pearson's correlations (*r*) between soil properties and community size (qAOB, qAOA, qBacteria and qArchaea) measured at each individual sampling point (*n* = 12).

	qAOB	qAOA	qBacteria	qArchaea
pH	0.19	−0.26	0.39	0.05
NH ₄ ⁺ –N	0.45	−0.34	−0.16	−0.34
DOC	0.19	−0.54*	−0.39	−0.52*
DON	0.14	−0.56*	0.21	−0.04
DOC/DON	0.16	−0.37	−0.59**	−0.60**
TC	0.31	0.13	−0.10	−0.08
TN	0.39	−0.09	−0.14	−0.13
C/N	−0.04	0.43	−0.04	0.03
δ ¹³ C	−0.26	−0.60**	0.08	−0.58**
δ ¹⁵ N	−0.03	−0.01	0.47	−0.03

Abbreviations: DOC, dissolved organic carbon; DON, dissolved organic nitrogen; TC, total carbon; TN, total nitrogen; AOB, ammonia-oxidizing bacteria; AOA, ammonia-oxidizing archaea. Values labeled with ** and * are statistically significant at *p* < 0.05 and *p* < 0.10, respectively.

population size of soil bacteria under trembling aspen (Lesaulnier et al., 2008). It was believed that elevated CO₂ increased plant photosynthetic rate and productivity, and the increase in the above-ground photosynthesis will result in greater below-ground C allocation via root exudation and rhizodeposition (Hungate et al., 1997; Jackson et al., 2009; Palmroth et al., 2006; Phillips et al., 2006), which can directly stimulate changes in the size and community structure of soil microorganisms (Drigo et al., 2009). However, no significant difference in DOC between ambient CO₂ and elevated CO₂ was observed in this study which was partly in consistent with the study of Jackson et al. (2009), who found that no significant change of DOC between ambient and elevated CO₂ condition in the O horizon of soil. Moreover, a significant difference of δ¹³C between ambient CO₂ and elevated CO₂ indicated an increase in plant photosynthetic rate, which could supply greater below-ground labile carbon (Table 1). No differences of DOC among treatments can be explained by the soil respiration increase under elevated CO₂ (Luo and Zhou, 2006).

4.2. Responses of AOB and AOA abundance to elevated CO₂ and N fertilization

Quantitative PCR data show a positive effect of the N addition on AOB but no significant effect on AOA under ambient CO₂. Studies on the effect of N addition have shown an increasing in *amoA* gene abundance of soil AOB but not of AOA, with AOB being predominant over AOA in the N-rich environment (Di et al., 2009; Hallin et al., 2009). Progressive nutrient limitation (particularly N) induced by inputs of C resulting from enhanced photosynthesis under elevated CO₂ was the main cause of shifts in the abundance and community structure of soil microorganisms (Hungate et al., 1999; Oren et al., 2001; Schaeffer et al., 2007). However, a study on the Duke FACE site revealed that the carbon fixation faster than nitrogen in tree biomass under elevated CO₂ would delay the

progressive N limitation (Finzi et al., 2006). In the present study, the effect of N fertilization was different under elevated CO₂; this phenomenon had been discussed by Zak et al. (2000), who thought that the effect of N fertilization on microbial communities under elevated CO₂ depended on the plant species presented. Elevated CO₂ negated the N-induced increase of AOB observed under ambient CO₂, yet caused an increase of AOA under native soil N conditions, a response not observed under ambient CO₂ condition. Though a positive effect of elevated CO₂ on the net nitrification and potential denitrification was observed under the open top chambers fumigation system at a Scot pine seedlings stand forest (Carnol et al., 2002), in comparison with ambient CO₂ condition, carbon dioxide enrichment did not significantly increase the abundance of AOB in this study, perhaps due to the relative high level of NH₄⁺–N determined in the soils (185 and 191 μg g^{−1} for AU and EU, respectively, Table 1). However, elevated CO₂ did offset the effects of N fertilization on the abundance of AOB, suggesting a resource competition. The N fertilization increased the abundance of AOB under ambient CO₂ due to the increased availability of NH₄⁺–N, which is consistent with previous findings that higher ammonium concentration promoted the growth of AOB (Okano et al., 2004). Quantitative PCR data of archaeal *amoA* genes under elevated CO₂ condition (i.e., EU, Fig. 1b) are qualitatively consistent with the findings of Lesaulnier et al. (2008). They reported the abundance of two specific dominant OTUs of Crenarchaeota increased significantly under elevated CO₂. As in the above description, elevated CO₂ increased root exudation and rhizodeposition, which would increase the availability of labile organic carbon. Evidence had demonstrated that the labile organic carbon rather than ammonia availability presented a positive effect on the growth of AOA (Stopnisek et al., 2010). However, a negative linear relationship between the δ¹³C and archaeal *amoA* gene indicated a positive effect of elevated CO₂ on the growth of AOA (Table 2). Under elevated CO₂ and N fertilization, the competition for resources (nutrients and carbohydrates) is greater among different groups of soil microorganisms. It has been reported that fast-growing (r-selected) microorganisms grew better and dominated in response to elevated atmospheric CO₂ than the slow-growing (k-selected) microbes such as *Nitrosospira* or *Nitrosospira*-like bacteria (Dorodnikov et al., 2009). Therefore, it is likely that AOB could not compete well with other microbes in soil under elevated CO₂ and N fertilization, and thus showed no significant growth response to these conditions. Increased abundance of AOA under elevated CO₂ may be related to the potential ability of AOA to utilize both inorganic and organic material as C sources (Hallam et al., 2006; Zhang et al., 2010). However, the effect of elevated CO₂ on the abundance of AOA was mitigated by N fertilization. This, again, may relate to intensified resource competition under both elevated CO₂ and N fertilization as discussed above. Previous studies have demonstrated that different AOB species possess multiple *amoA* gene copy numbers but

Table 3

Fragment patterns of *amoA* genes and distribution of sequence types affiliated with *amoA* clusters in the clone libraries (%).

Treatment	AOA			AOB						
	Pattern Number ^a	Soil cluster 2 ^b	Soil cluster 5	Pattern Number	Cluster 1	Cluster 2	Cluster 4	Cluster 9	Cluster 11	Cluster 3a
AU	9	22.0%	78.0%	4	4.5%	70.8%	0	24.7%	0	0
AN	7	13.3%	86.7%	4	18.7%	55.8%	13.3%	12.2%	0	0
EU	9	25.7%	74.3%	5	13.9%	33.8%	0	31.2%	19.8%	1.3%
EN	8	21.2%	78.8%	4	19.2%	55.9%	0	19.8%	4.3%	0.9%

^a Pattern numbers are numbers of molecular fragments following digestion with HaeIII and MboI.

^b Proportion of sequences is calculated as proportion of analyzed clones affiliated with the fragment patterns belonging to one of the clusters among of the clone numbers used in the digestion analysis in one clone library.

Fig. 4. Canonical correspondence analysis triplots of AOB (a) and AOA (b) with the *amoA* genotypes determined by RFLP and the following environmental factors: soil pH, ammonia (NH_4^+), and DOC/DON ratio.

only one 16S rRNA gene per cell (Klotz and Norton, 1998; Norton et al., 1996). However, AOA species are considered to hold one copy of the 16S rRNA gene and equal number of the *amoA* gene (Leininger et al., 2006; Norton et al., 1996). Since *amoA* gene copy

number was not directly linked with the activity of the ammonia oxidizers, it did not directly reflect nitrification activity of the ammonia oxidizers.

4.3. Responses of AOB and AOA community structure to elevated CO_2 and N fertilization

CCA results revealed that soil niche properties significantly influenced the *amoA* genotype distribution of AOB and AOA rather than elevated CO_2 ($p > 0.1$) or N fertilization ($p > 0.1$). Previous study of this site already showed that the spatial difference rather than elevated CO_2 shaped the distribution of bacterial community (Ge et al., 2010). A study on the response of AOB and AOA in the rhizosphere of maize and soybean to elevated CO_2 showed no significant difference among treatments based on unweighted PCA of the *amoA* gene sequences (Nelson et al., 2010). In this study,

Table 4
Arlequin AMOVA analyses of *amoA* gene sequences of Duke FACE soils by RFLP genotype frequencies.

	Source of variation	Variance components	% of variation	F-statistics	p
AOA	Among treatments	0.025 Va	0.59	$F_{CT} < 0.01$	0.31 ± 0.02
	Replicates/treatment	0.130 Vb	3.10	$F_{SC} = 0.03$	0.00 ± 0.00
	Among all samples	4.033 Vc	96.31	$F_{ST} = 0.04$	0.00 ± 0.00
AOB	Among treatments	-0.013 Va	-4.10	$F_{CT} = -0.04$	0.72 ± 0.01
	Replicates/treatment	0.140 Vb	42.94	$F_{SC} = 0.41$	0.00 ± 0.00
	Among all samples	0.199 Vc	61.17	$F_{ST} = 0.39$	0.00 ± 0.00

phylogenetic analyses of bacterial *amoA* genes showed that *Nitrosospira* or *Nitrosospora*-like species dominated in all the treatments. *Nitrosospira* cluster 2 was predominant among the treatments (Fig. 2), which is in agreement with previous studies in which *Nitrosospira* cluster 2 sequences were found as the most common in acidic soils (Nugroho et al., 2005). The CCA plot indicated that a positive relationship between three bacterial *amoA* genotypes (HM131547, HM131550, HM131552) and pH value, showing that these genotypes may be related to a niche of acidic environment. The *amoA* genotype HM131549 (*Nitrosospira* cluster 2) and HM131553 (*Nitrosospira* cluster 4) appeared to link with a niche of high levels of DOC/DON ratio and NH_4^+-N . The *amoA* genotype HM131547 (*Nitrosospira* cluster 9) was negatively related to the level of NH_4^+-N , which is consistent with the previous findings that the *Nitrosospira* cluster 9 is present at the niche of low level of NH_4^+-N (Avrahami et al., 2003). Moreover, AMOVA of the bacterial *amoA* genotypes indicated that 42.9% (Table 4.) of the total variation occurred within the replicates, suggesting that the soil physical–chemical properties dominate the AOB distribution.

In contrast, no remarkable changes in community composition of AOA have been observed in the results of CCA and AMOVA. No significant phylogenetic changes among the treatments confirmed the above CCA and AMOVA results of archaeal *amoA* genotypes' composition. However, results of CCA in this study showed that only soil pH significantly influenced the composition of archaeal *amoA* genotype, which is consistent with the prior studies (He et al., 2007; Nicol et al., 2008).

Elevated CO_2 and N fertilization did not affect the community structure of AOB and AOA, indicating that both ammonia oxidizers had great ecophysiological diversity and as a marker, *amoA* genes were less significant than 16S rRNA genes in specific niche (Nelson et al., 2010). In this study, community structures of AOB and AOA were not influenced by N fertilization for only two-year N addition before the soils sampling.

5. Conclusion

This study partly demonstrated that AOB and AOA responded differently to elevated CO_2 and N fertilization, based on the abundance of *amoA* genes, and that there were significant interactions of multiple factors (e.g., elevated CO_2 and N fertilization) on both ammonia oxidizers, though their community structure did not significant shift with elevated CO_2 or N fertilization. These different responses may be attributed to the shifts in resource availability induced by the elevated CO_2 and N fertilization and to different characteristics of growth and resource competition of different ammonia oxidizer groups. The interactive effects of multiple environmental factors on these organisms frustrate the understanding of the mechanisms responsible for the responses of soil ammonia-oxidizing communities to the shifts in environmental factors. As ammonia oxidizers regulate the rate-limiting step in the N cycling of highly complex terrestrial ecosystems, the shifts in the abundance and community structure of soil AOA and AOB may have large effect on the N cycling in forest ecosystems, particularly in a world with continuously rising atmospheric CO_2 and N deposition. Our results imply that AOA may continue to dominate the soil nitrifying processes in this forest ecosystem because the atmospheric CO_2 could continue rising in the future. If, however, atmospheric N deposition would increase, or in stands receiving N fertilization, AOB may become similarly or of greater importance to ecosystem N cycling, possibly leading to the acceleration of soil nitrification. Further studies on the potential nitrification rate and *amoA* gene expression could offer more detailed information of the response of ammonia oxidizers to global change, like elevated CO_2 , soil warming and N deposition.

Acknowledgements

This research was jointly supported by the Australian Research Council, the Chinese Academy of Sciences and the Natural Science Foundation of China. The Duke FACE research was supported by the Office of Science (BER), U. S. Department of Energy, through its FACE facilities program. The authors thank Jeffrey S Phippen and other staffs at DukeUniversity for their assistances in soil sampling.

References

- Avrahami, S., Liesack, W., Conrad, R., 2003. Effects of temperature and fertilizer on activity and community structure of soil ammonia oxidizers. *Environmental Microbiology* 5, 691–705.
- Barnard, R., Barthes, L., Le Roux, X., Harmens, H., Raschi, A., Soussana, J.F., Winkler, B., Leadley, P.W., 2004. Atmospheric CO_2 elevation has little effect on nitrifying and denitrifying enzyme activity in four European grasslands. *Global Change Biology* 10, 488–497.
- Bernhard, A.E., Landry, Z.C., Blevins, A., de la Torre, J.R., Giblin, A.E., Stahl, D.A., 2010. Abundance of ammonia-oxidizing archaea and bacteria along an estuarine salinity gradient in relation to potential nitrification rates. *Applied and Environmental Microbiology* 76, 1285–1289.
- Carnol, M., Hogenboom, L., Jach, M.E., Remacle, J., Ceulemans, R., 2002. Elevated atmospheric CO_2 in open top chambers increases net nitrification and potential denitrification. *Global Change Biology* 8, 590–598.
- Di, H.J., Cameron, K.C., Shen, J.P., Winefield, C.S., O'Callaghan, M., Bowatte, S., He, J.Z., 2009. Nitrification driven by bacteria and not archaea in nitrogen-rich grassland soils. *Nature Geoscience* 2, 621–624.
- Dorodnikov, M., Blagodatskaya, E., Blagodatsky, S., Fangmeier, A., Kuzyakov, Y., 2009. Stimulation of r- vs. K-selected microorganisms by elevated atmospheric CO_2 depends on soil aggregate size. *FEMS Microbiology Ecology* 69, 43–52.
- Drigo, B., van Veen, J.A., Kowalchuk, G.A., 2009. Specific rhizosphere bacterial and fungal groups respond differently to elevated atmospheric CO_2 . *The ISME Journal* 3, 1204–1217.
- Excoffier, L., Lischer, H.E.L., 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources* 10, 564–567.
- Feng, X., Simpson, A.J., Schlesinger, W.H., Simpson, M.J., 2010. Altered microbial community structure and organic matter composition under elevated CO_2 and N fertilization in the Duke forest. *Global Change Biology* 16, 2104–2116.
- Finzi, A., Moore, D., DeLucia, E., Lichter, J., Hofmockel, K., Jackson, R., Kim, H., Matamala, R., McCarthy, H., Oren, R., 2006. Progressive nitrogen limitation of ecosystem processes under elevated CO_2 in a warm-temperate forest. *Ecology* 87, 15–25.
- Fuhrman, J.A., 2009. Microbial community structure and its functional implications. *Nature* 459, 193–199.
- Ge, Y., Chen, C.R., Xu, Z.H., Oren, R., He, J.Z., 2010. Spatial factor rather than elevated CO_2 controls soil bacterial community in a temperate forest ecosystem. *Applied and Environmental Microbiology* 76, 7429–7436.
- Hallam, S.J., Mincer, T.J., Schleper, C., Preston, C.M., Roberts, K., Richardson, P.M., DeLong, E.F., 2006. Pathways of carbon assimilation and ammonia oxidation suggested by environmental genomic analyses of marine Crenarchaeota. *PLoS Biology* 4, 520–536.
- Hallin, S., Jones, C.M., Schlöter, M., Philippot, L., 2009. Relationship between N-cycling communities and ecosystem functioning in a 50-year-old fertilization experiment. *The ISME Journal* 3, 597–605.
- He, J.Z., Xu, Z.H., Hughes, J., 2005. Pre-lysis washing improves DNA extraction from a forest soil. *Soil Biology and Biochemistry* 37, 2337–2341.
- He, J.Z., Xu, Z.H., Hughes, J., 2006. Molecular bacterial diversity of a forest soil under residue management regimes in subtropical Australia. *FEMS Microbiology Ecology* 55, 38–47.
- He, J.Z., Shen, J.P., Zhang, L.M., Zhu, Y.G., Zheng, Y.M., Xu, M.G., Di, H.J., 2007. Quantitative analyses of the abundance and composition of ammonia-oxidizing bacteria and ammonia-oxidizing archaea of a Chinese upland red soil under long-term fertilization practices. *Environmental Microbiology* 9, 2364–2374.
- Heimann, M., Reichstein, M., 2008. Terrestrial ecosystem carbon dynamics and climate feedbacks. *Nature* 451, 289–292.
- Horz, H.P., Barbrook, A., Field, C.B., Bohannan, B.J., 2004. Ammonia-oxidizing bacteria respond to multifactorial global change. *Proceedings of the National Academy of Sciences, USA* 101, 15136–15141.
- Hungate, B.A., Holland, E.A., Jackson, R.B., Chapin III, F.S., Mooney, H.A., Field, C.B., 1997. The fate of carbon in grasslands under carbon dioxide enrichment. *Nature* 388, 576–579.
- Hungate, B.A., Dijkstra, P.L., Johnson, D.E.W., Hinkle, C.R.S., Drake, B.T.G., 1999. Elevated CO_2 increases nitrogen fixation and decreases soil nitrogen mineralization in Florida scrub oak. *Global Change Biology* 5, 781–789.
- Jackson, R.B., Cook, C.W., Phippen, J.S., Palmer, S.M., 2009. Increased belowground biomass and soil CO_2 fluxes after a decade of carbon dioxide enrichment in a warm-temperate forest. *Ecology* 90, 3352–3366.

- Klotz, M.G., Norton, J.M., 1998. Multiple copies of ammonia monooxygenase (amo) operons have evolved under biased AT/GC mutational pressure in ammonia oxidizing autotrophic bacteria. *FEMS Microbiology Letters* 168, 303–311.
- Kumar, S., Nei, M., Dudley, J., Tamura, K., 2008. MEGA: a biologist-centric software for evolutionary analysis of DNA and protein sequences. *Briefings in Bioinformatics* 9, 299–306.
- Leininger, S., Urich, T., Schlöter, M., Schwark, L., Qi, J., Nicol, G.W., Prosser, J.I., Schuster, S.C., Schleper, C., 2006. Archaea predominate among ammonia-oxidizing prokaryotes in soils. *Nature* 442, 806–809.
- Lesaulnier, C., Papamichail, D., McCorkle, S., Ollivier, B., Skiena, S., Taghavi, S., Zak, D., van der Lelie, D., 2008. Elevated atmospheric CO₂ affects soil microbial diversity associated with trembling aspen. *Environmental Microbiology* 10, 926–941.
- Luo, Y., Zhou, X., 2006. Separation of source components of soil respiration. In: *Soil Respiration and the Environment*. Elsevier Inc., pp. 187–214.
- Nelson, D.M., Cann, I.K.O., Mackie, R.I., 2010. Response of archaeal communities in the rhizosphere of maize and soybean to elevated atmospheric CO₂ concentrations. *PLoS ONE* 5, e15897.
- Nicol, G.W., Schleper, C., 2006. Ammonia-oxidising Crenarchaeota: important players in the nitrogen cycle? *Trends in Microbiology* 14, 207–212.
- Nicol, G.W., Leininger, S., Schleper, C., Prosser, J.I., 2008. The influence of soil pH on the diversity, abundance and transcriptional activity of ammonia oxidizing archaea and bacteria. *Environmental Microbiology* 10, 2966–2978.
- Norton, J.M., Low, J.M., Klotz, M.G., 1996. The gene encoding ammonia monooxygenase subunit A exists in three nearly identical copies in *Nitrosospora* sp. NpAV. *FEMS Microbiology Letters* 139, 181–188.
- Nugroho, R.A., R. ling, W.F.M., Laverman, A.M., Zoomer, H.R., Verhoef, H.A., 2005. Presence of *Nitrosospora* cluster 2 bacteria corresponds to N transformation rates in nine acid Scots pine forest soils. *FEMS Microbiology Ecology* 53, 473–481.
- O'Neill, E.G., Luxmoore, R.J., Norby, R.J., 1987. Elevated atmospheric CO₂ effects on seedling growth, nutrient uptake, and rhizosphere bacterial populations of *Liriodendron tulipifera* L. *Plant and Soil* 104, 3–11.
- Okano, Y., Hristova, K.R., Leutenegger, C.M., Jackson, L.E., Denison, R.F., Gebreyesus, B., Lebauer, D., Scow, K.M., 2004. Application of real-time PCR to study effects of ammonium on population size of ammonia-oxidizing bacteria in soil. *Applied and Environmental Microbiology* 70, 1008–1016.
- Oksanen, J., Blanchet, G., Kindt, R., Legendre, P., O'Hara, R.G., Simpson, G.L., Solymos, P., Stevens, M.H.H., Wagner, H., 2010. *Vegan: Community Ecology Package*. R package version 1.17-1.
- Oppermann, B.I., Michaelis, W., Blumenberg, M., Frerichs, J., Schulz, H.M., Schippers, A., Beaubien, S.E., Krüger, M., 2010. Soil microbial community changes as a result of long-term exposure to a natural CO₂ vent. *Geochimica et Cosmochimica Acta* 74, 2697–2716.
- Oren, R., Ellsworth, D.S., Johnsen, K.H., Phillips, N., Ewers, B.E., Maier, C., Schafer, K.V.R., McCarthy, H., Hendrey, G., McNulty, S.G., Katul, G.G., 2001. Soil fertility limits carbon sequestration by forest ecosystems in a CO₂-enriched atmosphere. *Nature* 411, 469–472.
- Palmroth, S., Oren, R., McCarthy, H., Johnsen, K., Finzi, A., Butnor, J., Ryan, M., Schlesinger, W., 2006. Aboveground sink strength in forests controls the allocation of carbon below ground and its [CO₂]-induced enhancement. *Proceedings of the National Academy of Sciences, USA* 103, 19362–19367.
- Phillips, D.A., Fox, T.C., Six, J., 2006. Root exudation (net efflux of amino acids) may increase rhizodeposition under elevated CO₂. *Global Change Biology* 12, 561–567.
- Rosenzweig, C., Casassa, G., Karoly, D., Imeson, A., Liu, C., Menzel, A., Rawlins, S., Root, T., Seguin, B., Tryjanowski, P., 2007. Assessment of observed changes and responses in natural and managed systems. *Climate Change 2007: Impacts, Adaptation and Vulnerability*. Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change, pp. 79–131.
- Schaeffer, S.M., Billings, S.A., Dave, E., 2007. Laboratory incubations reveal potential responses of soil nitrogen cycling to changes in soil C and N availability in Mojave Desert soils exposed to elevated atmospheric CO₂. *Global Change Biology* 13, 854–865.
- Schortemeyer, M., Hartwig, U.A., Hendrey, G.R., Sadowsky, M.J., 1996. Microbial community changes in the rhizospheres of white clover and perennial ryegrass exposed to free air carbon dioxide enrichment (FACE). *Soil Biology and Biochemistry* 28, 1717–1724.
- Shen, J.P., Zhang, L.M., Guo, J.F., Ray, J.L., He, J.Z., 2010. Impact of long-term fertilization practices on the abundance and composition of soil bacterial communities in Northeast China. *Applied Soil Ecology* 46, 119–124.
- Stopnisek, N., Gubry-Rangin, C., Hofferle, S., Nicol, G.W., Mandic-Mulec, I., Prosser, J.I., 2010. Thaumarchaeal ammonia oxidation in an acidic forest peat soil is not influenced by ammonium amendment. *Applied and Environmental Microbiology* 76, 7626–7633.
- Sun, Y., Xing, W., Li, J., Lu, Y., Zuo, J., 2009. Microbial community in granules from a high-rate EGSB reactor. *Applied Biochemistry and Microbiology* 45, 593–598.
- Tourna, M., Freitag, T., Nicol, G., Prosser, J., 2008. Growth, activity and temperature responses of ammonia-oxidizing archaea and bacteria in soil microcosms. *Environmental Microbiology* 10, 1357–1364.
- Wallenstein, M.D., McNulty, S., Fernandez, I.J., Boggs, J., Schlesinger, W.H., 2006. Nitrogen fertilization decreases forest soil fungal and bacterial biomass in three long-term experiments. *Forest Ecology and Management* 222, 459–468.
- Wessén, E., Hallin, S., Philippot, L., 2010. Differential responses of bacterial and archaeal groups at high taxonomical ranks to soil management. *Soil Biology and Biochemistry*.
- Zak, D.R., Pregitzer, K.S., King, J.S., Holmes, W.E., 2000. Elevated atmospheric CO₂, fine roots and the response of soil microorganisms: a review and hypothesis. *New Phytologist* 147, 201–222.
- Zhang, L.M., Offre, P.R., He, J.Z., Verhamme, D.T., Nicol, G.W., Prosser, J.I., 2010. Autotrophic ammonia oxidation by soil thaumarchaea. *Proceedings of the National Academy of Sciences, USA* 107, 17240–17245.