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Change in gene abundance in the nitrogen biogeochemical cycle with temperature and nitrogen addition in Antarctic soils

Jaejoon Jung [a,1], Jinki Yeom [a,1], Jisun Kim [a], Jiwon Han [a], Hyoun Soo Lim [b], Hyun Park [b], Seunghun Hyun [a], Woojun Park [a,*]

[a] *Department of Environmental Science and Ecological Engineering, Korea University, Anam-Dong 5Ga, Seungbuk-Ku, Seoul 136-713, Republic of Korea*
[b] *Korea Polar Research Institute, Incheon 406-840, Republic of Korea*

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Abstract

The microbial community (bacterial, archaeal, and fungi) and eight genes involved in the nitrogen biogeochemical cycle (*nifH*, nitrogen fixation; bacterial and archaeal *amoA*, ammonia oxidation; *narG*, nitrate reduction; *nirS*, *nirK*, nitrite reduction; *norB*, nitric oxide reduction; and *nosZ,* nitrous oxide reduction) were quantitatively assessed in this study, via real-time PCR with DNA extracted from three Antarctic soils. Interestingly, AOB *amoA* was found to be more abundant than AOA *amoA* in Antarctic soils. The results of microcosm studies revealed that the fungal and archaeal communities were diminished in response to warming temperatures (10 °C) and that the archaeal community was less sensitive to nitrogen addition, which suggests that those two communities are well-adapted to colder temperatures. AOA *amoA* and *norB* genes were reduced with warming temperatures. The abundance of only the *nifH* and *nirK* genes increased with both warming and the addition of nitrogen. NirS-type denitrifying bacteria outnumbered NirK-type denitrifiers regardless of the treatment used. Interestingly, dramatic increases in both NirS and NirK-types denitrifiers were observed with nitrogen addition. NirK types increase with warming, but NirS-type denitrifiers tend to be less sensitive to warming. Our findings indicated that the Antarctic microbial nitrogen cycle could be dramatically altered by temperature and nitrogen, and that warming may be detrimental to the ammonia-oxidizing archaeal community. To the best of our knowledge, this is the first report to investigate genes associated with each process of the nitrogen biogeochemical cycle in an Antarctic terrestrial soil environment.

Keywords: Ammonia-oxidizing archaea; Ammonia-oxidizing bacteria; Climate change; Nitrification; Denitrification; Nitrous oxide

1. Introduction

The nitrogen biogeochemical cycle carried out by microorganisms has been investigated previously in diverse environments including soils, estuaries, sediments, coral ecosystems and freshwater and ocean ecosystems. The most intensively studied process thus far has been the initial nitrification step (ammonia oxidation), which can be carried out by both bacteria and archaea (Prosser and Nicol, 2008). Since the discovery of the archaeal *amoA* (ammonia monooxygenase subunit alpha) from a metagenomic studies conducted in open ocean (Venter et al., 2004) and soil (Treusch et al., 2005), the ammonia-oxidizing archaea (AOA) have been recognized as the predominant ammonia-oxidizing microbial community in various environments, including soils (Leininger et al., 2006) and marine ecosystems (Beman et al., 2007). The detection of archaeal *amoA* sequences in harsh conditions such as alkaline soil (Shen et al., 2008), hot spring sediments (Reigstad et al., 2008) and cold deep sea water (Nakagawa et al., 2007) supports the general supposition of the worldwide ubiquity of the AOA. The determinative environmental factors for the AOA community may be as follows: ammonium levels, organic carbon, temperature, salinity, dissolved oxygen level, pH, sulfide and phosphate levels (Erguder et al., 2009). A great deal

* Corresponding author. Tel.: +82 2 3290 3067; fax: +82 2 953 0737.
E-mail address: wpark@korea.ac.kr (W. Park).
[1] These authors contributed equally to this article.

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of detailed information about AOA ecology has already been compiled (Erguder et al., 2009).

Nitrate reductase encoded by the *narGHJI* operon is responsible for the reduction of nitrate to nitrite (Philippot and Hojberg, 1999). Two types of nitrite reductase catalyze the reduction of nitrite to nitric oxide: a cytochrome *cd*1 encoded for by *nirS*, or a Cu-containing enzyme encoded by *nirK*. Nitric oxide is subsequently reduced by the nitric oxide reductase encoded by *norB*, producing nitrous oxide, a powerful greenhouse gas. Nitrous oxide contributes to global warming and climate change much more profoundly than carbon dioxide or methane. The final step in denitrification is the reduction of nitrous oxide to gaseous dinitrogen by nitrous oxide reductase encoded by *nosZ*. However, some denitrifiers do not harbor complete sets of genes required for complete denitrification (Zumft, 1997).

The Antarctic terrestrial ecosystem is also subject to climate change. The rate of surface temperature increase has averaged more than 0.1 °C per decade over the past 50 years (Steig et al., 2009). The majority of climate change models predicts increases in overall annual precipitation and temperature, and also that the impact of these changes will be greater at the polar region (Maxwell, 1992). Changes in soil moisture will influence microbial activity, organic matter turnover rates and ammonium availability via mineralization. The nitrogen cycle in Antarctic soils will therefore be influenced by climate change. Nitrogen fixation could be inhibited by increased nitrogen availability, which functions as a negative feedback in this process. Increased mineralization could accelerate nitrification and may, in turn, promote denitrification and nitrous oxide production (Paul and Clark, 1996). However, studies of the nitrogen cycle in the polar region have primarily been carried out in ocean ecosystems, and only *amoA* has been of interest in many of these cases, aside from nitrogen fixation or denitrification genes. The nitrogen cycle and the impacts of climate change have yet to be adequately evaluated in Antarctic soils. Therefore, the principal objective of this study was to evaluate nitrogen cycle potential by assessing the abundance of genes involved in every step of the nitrogen pathway. Microbial responses to warming effects and nutrient availability were assessed at the microcosm scale.

2. Materials and methods

2.1. Soil sampling and treatment

The Antarctic soils were sampled from Antarctic King Sejong Station (62° 13′ 25.1S, 58° 47′ 10.4W) and the Cape Burk area Cape Burk 1: 74° 45.326S, 136° 48.743W; Cape Burk 2: 74° 45.999S, 136° 47.974W) (Fig. 1). All soil samples were collected from topsoil (<10 cm) and subjected to storage at below −80 °C until analysis. The Korea Polar Research Institute (KOPRI) provided soil samples. In an effort to determine the effects of warming on the microbial community and the nitrogen biogeochemical cycle, soil microcosms were incubated at 4 °C and 10 °C (50 g of soils in 500 ml bottle) for 30 days. Incubation temperatures mimicking warming climate (10 °C) were selected based on the following: i) the number of days with temperature above the freezing point is expected to increase at Antarctic King Sejong Station in King George Island, which has been affected by increased temperature for the past few decades; ii) ten Celsius degrees is sufficiently high to expect a suppression response from the microbial community in the extremely cold Antarctic climate. Soil samples were supplied with 100 mg of urea kg^{-1} soil, since nitrogen input in Antarctic soil derives largely from animal feces and the area surrounding the station is a penguin protection area. The responses of genes involved in the nitrogen cycle to nitrogen amendment were evaluated after 30 days of incubation.

2.2. Quantification of soil nitrogen

Analysis of total nitrogen was conducted via the Kjeldahl method, as described by Bremner (1996). Soil samples were dried and ground to pass through a 0.5 mm sieve for total nitrogen analysis. A mixture of soils (1 g), K_2SO_4, and concentrated H_2SO_4 were placed in a Kjeldahl flask and digested with a Kjeldahl digester (BUCHI digest System K-437). Digested samples were distilled (BUCHI Distillation Unit B-324) in accordance with the manufacturer's recommendations and titrated using indicator solution and standard H_2SO_4 solution. Ammonium and nitrate concentrations were determined via a steam-distillation method as previously described (Mulvaney, 1996). Sieved soil samples were agitated with 2 M KCl solution and the filtered extract was distilled with MgO. The recipient solution was titrated to determine the ammonium concentration. Additional distillation was conducted with MgO and Devarda's alloy. The second recipient solution was titrated to determine the nitrate concentration. Nitrite concentrations were determined via a modified version of the Griess–Losvay method (Mulvaney, 1996). The filtrated extract was treated with a diazotizing reagent and a coupling reagent (*N*-(1-naphthyl)-ethylenediamine). Absorbance at 540 nm was proportional to the nitrite concentration.

2.3. Nitrification activity assay

Nitrification activity was determined via Kandeler's method (Schinner et al., 1995). Five grams of soil were incubated with 20 ml of 1 mM ammonium sulfate and 0.1 ml of 1.5 M sodium chlorate for 24 h at room temperature. Additional incubation was carried out for 30 min with 5 ml of 2 M potassium chloride, followed by filtration. For color development, 5 ml of filtered extract, 3 ml of 0.19 M ammonium chloride (pH 8.5) and 2 ml of color reagent were incubated for 15 min. Nitrification activity was determined by absorbance at 520 nm.

2.4. Quantitative real-time PCR analysis

The microbial community and the abundance of nitrogen cycle genes were assessed via quantitative real-time PCR (qPCR) using an iCycler iQ real-time PCR detection system (Bio- Rad, US). Soil DNA was extracted from 250 mg of soil

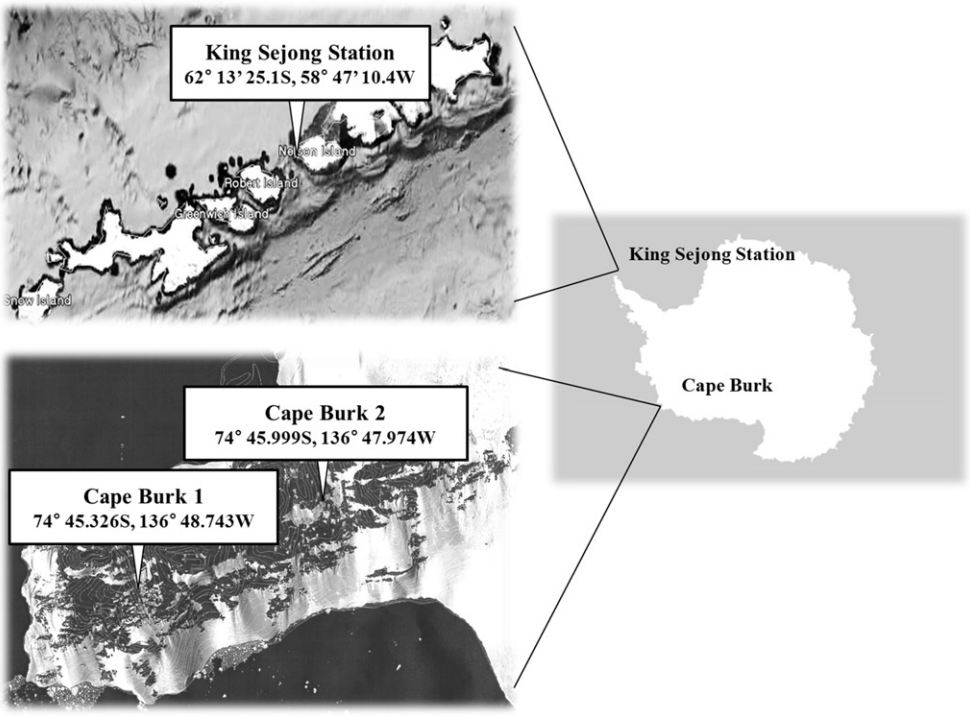

Fig. 1. A map of sampling sites. Images were captured from Google Earth and edited.

with a NucleoSpin soil kit (Macherey–Nagel, Germany), in accordance with the manufacturer's instructions. For qPCR, 100 ng of undiluted template soil DNA, 5 pmol of primers (Table 1) and 2× SYBR Green iCycler iQ mixture (Bio-Rad, US) were mixed in a total 25 μl reaction volume. Clear caps and 8 strip tubes were used (Axygen, USA). The PCR protocol was conducted as follows: 2 min of 95 °C, 40 cycles consisting of 45 s at 95 °C, 45 s at 60 °C, and 45 s at 72 °C. Fluorescence was measured at the end of each 72 °C incubation and analyzed with iCycler iQ software (version 3.0). Melting curve analyses (60–95 °C, 0.5 °C increments) were carried out to ensure PCR specificity. To assess the presence of PCR inhibitor in isolated soil DNA, qPCR was conducted in diluted soil DNA and a mixed DNA template containing soil DNA and PCR products from reference strains. For quantification, PCR products from strains were cloned with pGEM-T easy vector (Promega, US) and transformed into *Escherichia coli* Top10. Isolated cloned plasmids were restricted and used as a template. Standard curves for each gene are shown in Table S1. In the case of AOB *amoA*, AOA *amoA*, and *narG*, two primer sets were used for comparison. Gene abundances of AOB *amoA*, AOA *amoA*, and *narG* were evaluated using the primers designed by Okano et al. (2004), Francis et al. (2005), De la Torre et al. (2008) and Bru et al. (2007), respectively. Four independent experiments were carried out and averages with standard deviations are shown.

2.5. Statistical analyses

Gene copy numbers determined by qPCR were analyzed by *t*-tests. Copy numbers of genes from different samples were compared at a significance level of 5%. Data analysis was performed with SAS 9.1.3 (SAS Institute, US) software. Bars with different letters are statistically significantly different.

3. Results

3.1. Soil characteristics

Soil characteristics including soil texture, water content, pH, total organic carbon (TOC), nitrogen concentration and nitrification activity were analyzed and are summarized in Table 2. In brief, water contents of the Antarctic soils were low (0.79–9.74%). All tested soils were acidic (pH 4.6–5.5). Total nitrogen and organic carbon varied according to the sampling site. Inorganic nitrogen concentrations were low in Antarctic soils, and no nitrites were detected in any of the soils. Nitrification activity was highest in the Cape Burk 1 sample (55.2 ± 0.0 ng N g^{-1} d^{-1}).

3.2. Abundance of bacteria, fungi and archaea in Antarctic soils

Along with bacteria and archaea, fungi are an important player in the nitrogen biogeochemical cycle, which involves denitrification (Shoun et al., 1992). Therefore, the microbial community was quantified using domain-specific primers (Fig. 2). In all three Antarctic soils, bacteria were the predominant microbial domain (1.9×10^9–5.8×10^{12} copies/g). The archaea were the second most dominant domain in all samples, evidencing the lowest levels of variation by site (1.0×10^5–1.0×10^7 copies/g). Despite variations in soil

Table 1
PCR primers used for quantification of bacterial, archaeal and fungal community and nitrogen cycle genes.

Target gene (reference strain)	Primer	Primer sequence (5′ → 3′)	Product size (bp)	Reference
Bacterial 16S rRNA gene	341F	CCTACGGGAGGCAGCAG	193	Watanabe et al., 2001
(*Acinetobacter oleivorans* DR1)	534R	ATTACCGCGGCTGCTGGCA		
Archaeal 16S rRNA gene	Arch 349F	GYGCASCAGKCGMGAAW	457	Takai and Horikoshi, 2000
(*Haloterrigena jeotgali* A29)	Arch 806R	GGACTACVSGGGTATCTAAT		
Fungi ITS (*Postia placenta* ATCC 11538)	ITS1F	CTTGGTCATTTAGAGGAAGTAA	420–825[a]	Manter and Vivanco, 2007
	ITS4	TCCTCCGCTTATTGATATGC		
Bacterial ammonium monooxygenase;	amoA-1F	GGGGTTTCTACTGGTGGT	491	Rotthauwe et al., 1997
amoA (*Nitrosomonas europaea*)	amoA-2R	CCCCTCKGSAAAGCCTTCTTC		
	A189	GGHGACTGGGAYTTCTGG	669	Okano et al., 2004
	amoA-2R-TG	CCCCTCTGGAAAGCCTTCTTC		
Archaeal ammonium monooxygenase;	amo196F	GGWGTKCCRGGRACWGCMAC	81	Treusch et al., 2005
amoA (*Haloterrigena jeotgali* A29)	amo277R	CRATGAAGTCRTAHGGRTADCC		
	Arch_amoA_F	AATGGTCTGGSTTAGAMG	633	De la Torre et al., 2008;
	Arch_amoAR	GCGGCCATCCATCTGTATGT		Francis et al., 2005
Nitrogenase reductase;	nifHF	AAAGGYGGWATCGGYAARTCCACCAC	400	Rösch and Bothe, 2005
nifH (*Pseudomonas stutzeri* A1501)	nifHRb	TGSGCYTTGTCYTCRCGGATBGGCAT		
Cu-containing nitrite reductase;	nirK 1F	GGMATGGTKCCSTGGCA	514	Braker et al., 1998
nirK (*Agrobacterium tumefaciens* C58)	nirK 5R	GCCTCGATCAGRTTRTGGTT		
Nitrite reductase;	nirS cd3AF	GTSAACGTSAAGGARACSGG	425	Michotey et al., 2000;
nirS (*Ralstonia eutropha* H16)	nirS R3cd	GASTTCGGRTGSGTCTTGA		Throback et al., 2004
Nitrate reductase;	W9	MGNGGNTGYCCNMGNGGNGC	442	Gregory et al., 2000
narG (*Escherichia coli* APEC O1)	T38	ACRTCNGTYTGYTCNCCCCA		
(*Pseudomonas aeruginosa* PAO1)	narG-f	TCGCCSATYCCGGCSATGTC	173	Bru et al., 2007
	narG-r	GAGTTGTACCAGTCRGCSGAYTCSG		
Nitrate reductase;	cnorB2F	GACAAGNNNTACTGGTGGT	389	Braker and Tiedje, 2003
norB (*Pseudomonas aeruginosa* PAO1)	cnorB6R	GAANCCCCANACNCCNGC		
Nitrous oxide reductase;	nosZ-F	CGYTGTTCMTCGACAGCCAG	453	Kloos et al., 2001
nosZ (*Pseudomonas aeruginosa* PAO1)	nosZ-R	CGSACCTTSTTGCCSTYGCG		

[a] PCR product size varies by species.

characteristics, no significant differences in archaea abundance were detected in the Cape Burk 1 and 2 samples. Fungi were the smallest microbial community in the Antarctic soils. Similarly sized microbial communities were found in the Cape Burk soils.

3.3. Predominant AOB amoA in Antarctic soils

The abundance of ammonia-oxidizing bacteria (AOB) *amoA* did not vary significantly in the different soils (1.9×10^4–2.5×10^4 copies/g), even though the total number of copies of bacterial 16S rRNA genes did differ among different soils (Fig. 3a). Strikingly, AOB *amoA* predominated in all Antarctic soils other than AOA *amoA*. The ratios of AOB *amoA*: AOA *amoA* were 6.18, 435.16, and 484.13 in the Sejong, Cape Burk 1, and Cape Burk 2 samples, respectively. The ratios of AOB *amoA*: bacterial 16S rRNA genes were 3.8×10^{-7}, 8.3×10^{-6}, and 9.8×10^{-7} in the Sejong, Cape Burk 1, and Cape Burk 2 samples, respectively, indicating that only a small fraction of the total bacterial community was

Table 2
Characteristics of Antarctic soils.

Characteristics	Sejong	Cape Burk 1	Cape Burk 2
Soil texture	Loam sand	Sand	Sand
Water content (%)	9.74 ± 0.9	0.79 ± 0.0	3.32 ± 0.3
pH	4.8 ± 0.2	5.5 ± 0.1	4.6 ± 0.2
TOC (mg/kg)	5641.1 ± 232.9	416.2 ± 126.6	1454.1 ± 55.1
Nitrogen (mg/kg)			
Total	651.0 ± 116.8	1535.8 ± 85.1	1346.8 ± 223.6
NH^{4+}–N	1 ± 0.3	3 ± 0.3	ND
NO^{3-}–N	6.5 ± 0.7	ND	6.1 ± 2.7
NO^{2-}–N	ND	ND	ND
Nitrification activity (ngN g^{-1} d^{-1})	5.0 ± 0.0	55.2 ± 0.0	ND

ND: Not detected; TOC: Total organic carbon.

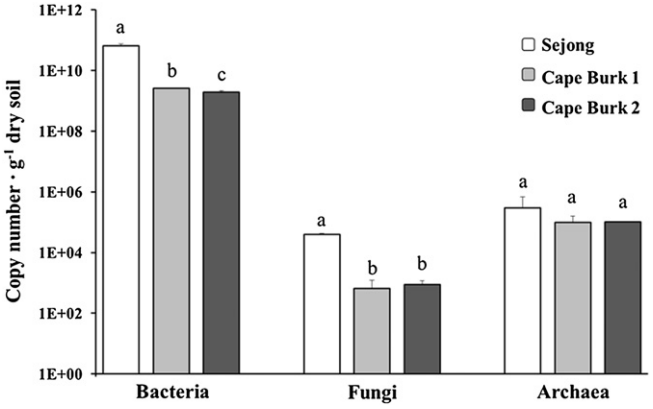

Fig. 2. The abundance of microorganisms in Antarctic soils. Average and standard deviations were calculated from triplicate microcosms. Bars with different letters are statistically significantly different. ($P < 0.05$).

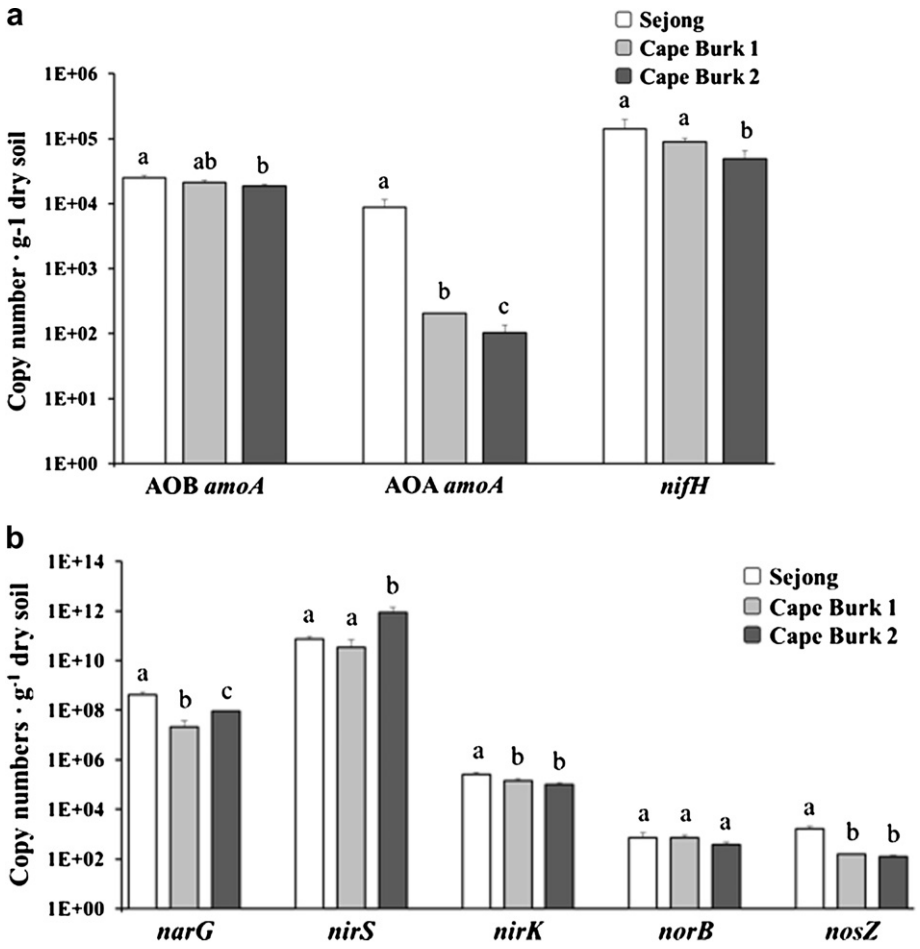

Fig. 3. The abundance of genes involved in (a) nitrification (AOB *amoA*, AOA *amoA*), nitrogen fixation (*nifH*), (b) denitrification (*narG*, *nirS*, *nirK*, *norB*, and *nosZ*) in Antarctic soils. Average and standard deviations were calculated from triplicate microcosms. Bars with different letters are statistically significantly different. ($P < 0.05$).

AOB *amoA*. A relatively larger portion of the total archaea was ammonia oxidizers, as shown by the ratios of AOA *amoA*: archaeal 16S rRNA genes (2.9×10^{-2}, 2.1×10^{-3}, and 1.0×10^{-4}, respectively). The abundance of *nifH* was 4.0×10^{4}–1.4×10^{5} copies/g and minimal variation was observed among sampling sites. The abundances of AOB *amoA* and AOA *amoA* were compared using two different primer sets (Figs. S1 and S2). The results of two AOB and AOA *amoA* primers were within 5-fold and 10-fold in most treatments. However, 60-fold underestimated results were noted in the nitrogen-added samples using the new AOB *amoA* primers (relatively recently developed primers, Okano et al., 2004). Large discrepancies (54-fold underestimated) were also noted in 4 °C-incubated Sejong soil with new AOA *amoA* primers (Francis et al., 2005; De la Torre et al., 2008).

3.4. Abundance of denitrification genes

The abundances of denitrification genes − *narG* (nitrate reductase), *nirS* (cytochrome cd_1 nitrite reductase), *nirK* (copper-containing nitrite reductase), *norB* (nitric oxide reductase), and *nosZ* (nitrous oxide reductase) − were assessed (Fig. 3b). Cape Burk soils harbored similar copy numbers of *nirK*, *norB* and *nosZ*. *nirS* predominated over *nirK* in all soils, even though their function is the same. Cape Burk soils have more *norB* than *nosZ*. In the absence of nitrous oxide reductase encoded for by *nosZ*, nitrous oxide gas could be generated by nitric oxide gas. It is difficult to assess the actual nitrous oxide gas emissions without carrying out further experiments. Primers designed by Gregory et al. (2000) were likely to fail to quantify the abundance of *narG* in 10 °C-incubated soils, when compared to the result from primers designed by Bru et al. (2007) (Fig. S3).

3.5. The effect of increased temperature and nitrogen addition

Antarctic soils were subjected to warming and nitrogen amendment in microcosms to evaluate the responses of microbial communities and the abundance of nitrogen cycle genes (Fig. 4). Bacterial communities in Antarctic soils were reduced by 66.4% (3.2×10^{9} copies/g) and 62.6% (5.1×10^{8} copies/g) in Sejong and Cape Burk 1, respectively. However, the bacterial community was recovered by 70.4-fold (6.4×10^{7} copies/g) in the Cape Burk 2 samples. The fungal and archaeal communities were reduced by warming in all tested soils. The fungal

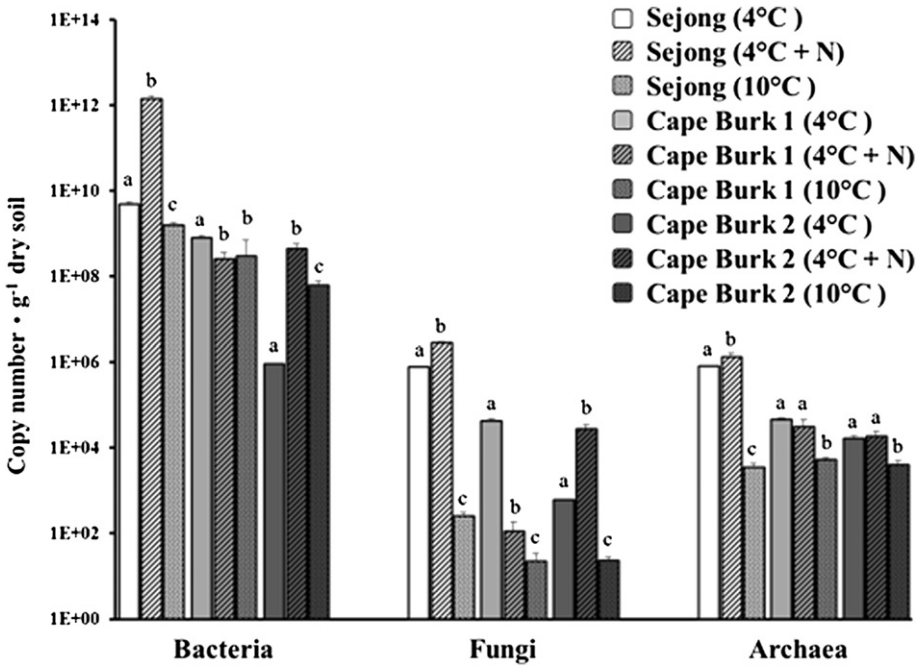

Fig. 4. The abundance of microorganisms in Antarctic soils incubated with a nitrogen source at 4 °C and without a nitrogen source at 10 °C for 30 days. Average and standard deviations were calculated from triplicate microcosms. Bars with different letters are statistically significantly different. ($P < 0.05$).

community was reduced by 3 orders of magnitude in Sejong and Cape Burk 1 (8.0×10^5 and 4.3×10^4 copies/g, respectively) and 1 order of magnitude in Cape Burk 2 (5.9×10^2 copies/g). The archaeal community was also reduced by 2 orders of magnitude (8.1×10^5 copies/g), 88.0% (4.0×10^4 copies/g), and 74.8% (1.2×10^4 copies/g) in the Sejong, Cape Burk 1, and Cape Burk 2 samples, respectively. Urea supplied as a nitrogen source exerted an inverse effect on those warmed microbial communities in many cases, thereby implying that nutritional factors could assist microbial communities in overcoming unfavorable environments, such as warming. Ammonia-oxidizing communities were also diminished by the warming effect in comparison with non-warming microcosms (Fig. 5a). However, their responses to warming differed according to site. AOB *amoA* decreased by 46.9% (3.1×10^4 copies/g) in the Sejong samples, whereas AOB *amoA* in the Cape Burk 1 and Cape Burk 2 samples were increased by 1 order of magnitude (3.7×10^4 and 1.7×10^4 copies/g, respectively). The warming effect on the AOA *amoA* community was also apparent, resulting in 1 order of magnitude reduction in the Sejong and Cape Burk 1 samples (9.0×10^3 and 1.3×10^4 copies/g, respectively), and 2 orders of magnitude reduction (2.0×10^4 copies/g) in the Cape Burk 2 sample. The nitrogen-fixing community benefited from warming, increasing the abundance of *nifH* by 1 order of magnitude (4.0×10^4–4.2×10^4 copies/g). The warming effect was detrimental to the abundance of *norB*, but was beneficial to *nirS* (Cape Burk 1, 2), *nirK*, and *nosZ*. The warming effect was more apparent in *nirK* than *nirS*; meanwhile, the *nirS* community was more sensitive to nitrogen addition (Fig. 4b). This result indicates that each step of denitrification in Antarctic soils has a unique set of optimal conditions for its activity. The impact of nitrogen addition was limited to the nitrite reduction step of denitrification, resulting in improved abundance of *nirS*, and *nirK*. The abundance of *norB* and *nosZ* did not evidence obvious changes.

4. Discussion

The nitrogen cycle in Antarctic soil is poorly understood, since the only currently available research data about the nitrogen cycle in Antarctic soils is the result of functional microarray analysis and qPCR, as conducted by Yergeau et al. (2007). In contrast with the limited number of studies conducted thus far with terrestrial samples, the nitrogen cycle in the polar aquatic environment has been studied for decades (Klingensmith and Alexander, 1983), and a variety of relevant aspects, including seasonal variation (Christman et al., 2011) and vertical variation (Pouliot et al., 2009) have been thoroughly evaluated. In addition to the rarity of research conducted in Antarctic soil, the partial processes of the nitrogen cycle, such as nitrogen fixation and denitrification, have been matters of great interest to researchers and a small number of genes have been analyzed (Yergeau et al., 2007).

The most significant result of this study is the finding that bacterial *amoA* predominates over archaeal *amoA* in Antarctic soil. Many previous studies have shown that archaeal *amoA* are more abundant than bacterial *amoA* in a variety of environments, including terrestrial (Leininger et al., 2006), estuarine (Caffrey et al., 2007), hot spring sediment (Hatzenpichler et al., 2008), coastal ecosystems (Wuchter et al., 2006) and marine ecosystems (Beman et al., 2007). The prevalence of bacterial *amoA* over archaeal *amoA* has generally been observed in estuarial environments, and high salinity is a major factor in the observed increases in the ammonia-oxidizing bacterial

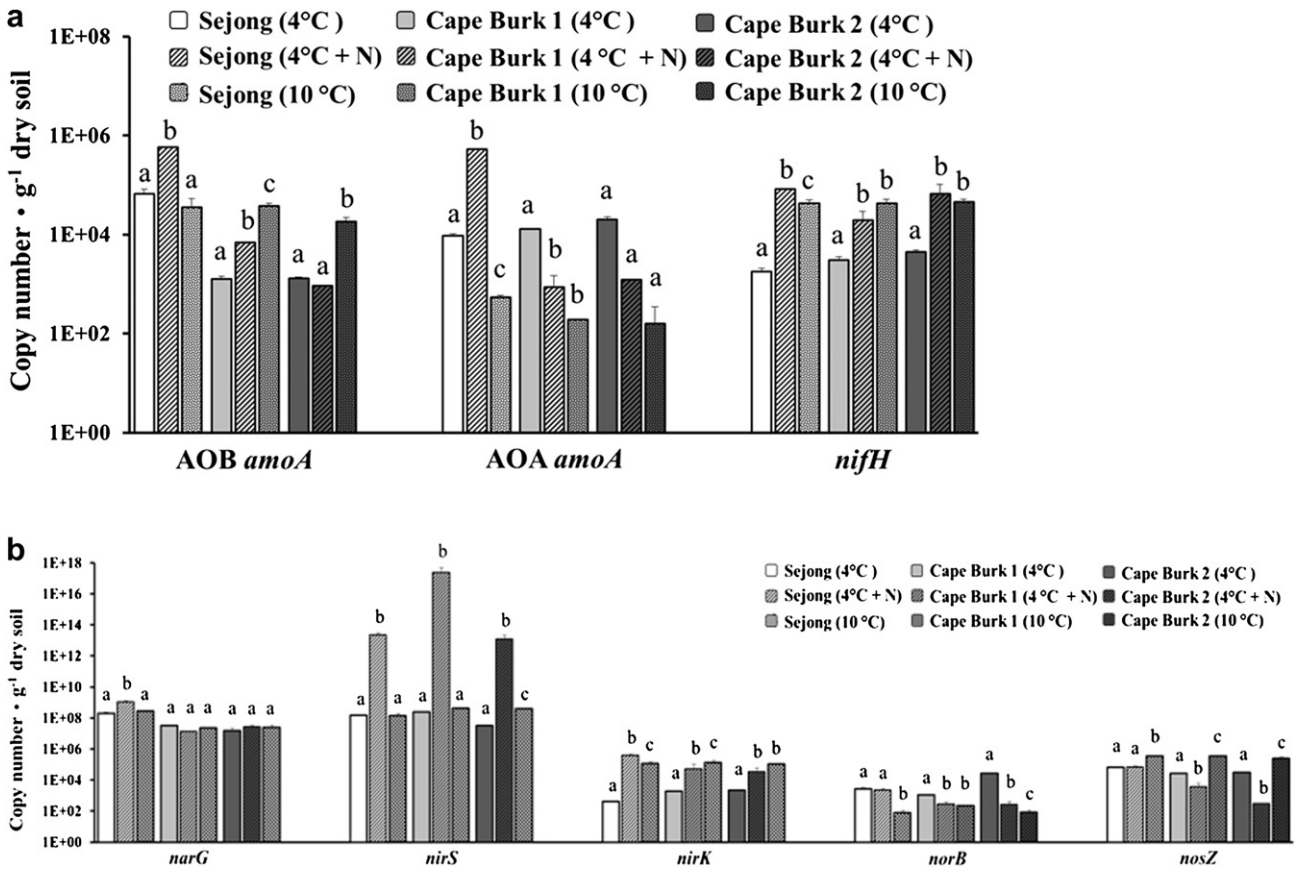

Fig. 5. The abundance of genes involved in (a) nitrification (AOB *amoA*, AOA *amoA*), nitrogen fixation (*nifH*), and (b) denitrification (*narG*, *nirS*, *nirK*, *norB*, and *nosZ*) in Antarctic soils incubated for 30 days with a nitrogen source at 4 °C and without a nitrogen source at 10 °C. Average and standard deviations were calculated from triplicate microcosms. Bars with different letters are statistically significantly different. ($P < 0.05$).

community with the C:N ratio (Bernhard et al., 2005). Although salinity was not assessed in this study, hypersalinity could be anticipated in Antarctic soil water, as saline and ionic compounds are concentrated in the thin film-like water available to microorganisms after freezing (Cowan and Tow, 2004). Denitrification activity is not restricted to the bacterial domain as shown in *Fusarium oxysporum* MT-811. In this study, the primers that amplify denitrification genes (*narG*, *nirS*, *nirK*, *norB*, and *nosZ*) were designed to detect bacterial denitrification genes. Therefore, assessments of denitrification thus far might have been underestimated. However, bacterial *narG*, *nirS*, and *nirK* involved in the first two denitrification steps were clearly more abundant than *norB* and *nosZ* responsible for the reduction of nitric oxide and nitrous oxide, thereby suggesting incomplete nitrogen pathways in terrestrial ecosystems. Truncated denitrification pathways can be attributed to nitrous oxide emissions (Singh et al., 2010). The detected differences in the abundance of *norB* and *nosZ* might constitute important evidence of nitrous oxide emissions from Antarctic soils along with environmental factors; more detailed analyses will be required to clarify this. We found that the NirS-type denitrifier community was more abundant than the NirK-type in all soil samples tested in this study. The NirS-type denitrifiers were also found to predominate in sediments of the macrotidal estuarial environment, and salinity was identified as an important factor affecting the NirS-type denitrifiers (Abell et al., 2010). We were unable to expressly identify the soil characteristics relevant to the denitrification community. The poor correlation between nitrogen concentration and the abundance of nitrogen cycle genes in Antarctic soil has been reported in previous studies (Yergeau et al., 2007). Significant increases in *nirK* abundance were observed in soils incubated at 10 °C. Temperature has been shown to exert an effect on the size and composition of soil denitrifiers (Braker et al., 2010; Yergeau et al., 2007). The temperature sensitive-AOA *amoA* community may have been compensated by the addition of a nitrogen source. These results imply that increased temperatures may prove harmful in low temperature-adapted communities, but favorable environmental factors such as nutrients could assist a certain microbial community in overcoming detrimental environments. To overcome the detection limit of PCR primers, we compared the results from different primer sets in cases of AOB, AOA *amoA*, and *narG* quantification (Figs. S1–S3). Most results varied within a 10-fold range and did not influence the overall comparison results. However, several cases, such as the AOB *amoA* in nitrogen-added Cape Burk 2, AOA *amoA* Sejong soil incubated in 4 °C and *narG* in soils incubated in 10 °C showed greater variations by primers. In particular, the abundance of *narG* might not be appropriately estimated with old primers. Even though many reasons for these large gaps in results could

be suspected, such as too much degeneracy in primers (7 in old forward primers), limited numbers of available sequences at the time of primer design, different lengths of PCR product (81 bp for old AOA *amoA*, 633 bp for new AOA *amoA*), possible unique characteristics of samples and so on, we were unable to pinpoint a specific reason. Our data also indicated that the interpretation of gene abundance using qPCR should be approached cautiously because of the specificity of each primer set. In order to more precisely determine the structure of the microbial community, thorough research such as terminal restriction fragment length polymorphism or denaturing gradient gel electrophoresis will be required. This research provides essential information regarding the nitrogen biogeochemical cycle in Antarctica. To the best of our knowledge, this is the first report to show the abundance of nitrogen cycle genes, and particularly that AOB *amoA* predominated over AOA *amoA* in Antarctic soils. Further detailed research, such as studies into community structures, transcriptional analyses of nitrogen cycle genes, environmental factors, and nitrous oxide gas emissions will promote a greater understanding of Antarctic terrestrial ecosystems.

Acknowledgments

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Appendix. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.resmic.2011.07.007.

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