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Seasonal Changes in Nitrogen-Cycle Gene Abundances and in Bacterial Communities in Acidic Forest Soils

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The abundance of genes related to the nitrogen biogeochemical cycle and the microbial community in forest soils (bacteria, archaea, fungi) were quantitatively analyzed via real-time PCR using 11 sets of specific primers amplifying *nifH*, bacterial *amoA*, archaeal *amoA*, *narG*, *nirS*, *nirK*, *norB*, *nosZ*, bacterial 16S rRNA gene, archaeal 16S rRNA gene, and the ITS sequence of fungi. Soils were sampled from Bukhan Mountain from September of 2010 to July of 2011 (7 times). Bacteria were the predominant microbial community in all samples. However, the abundance of archaeal *amoA* was greater than bacterial *amoA* throughout the year. The abundances of *nifH*, *nirS*, *nirK*, and *norB* genes changed in a similar pattern, while *narG* and *nosZ* appeared in sensitive to the environmental changes. Clone libraries of bacterial 16S rRNA genes were constructed from summer and winter soil samples and these revealed that *Acidobacteria* was the most predominant phylum in acidic forest soil environments in both samples. Although a specific correlation of environmental factor and gene abundance was not verified by principle component analysis, our data suggested that the combination of biological, physical, and chemical characteristics of forest soils created distinct conditions favoring the nitrogen biogeochemical cycle and that bacterial communities in undisturbed acidic forest soils were quite stable during seasonal change.

Keywords: ammonia-oxidizing bacteria, ammonia-oxidizing archaea, nitrification, denitrification, climate change, nitrous oxide

Introduction

The nitrogen biogeochemical cycle is an important process in agriculture because nitrogen must be supplied to arable soil by nitrogen fixation and fertilization and denitrification is the major route of nitrogen loss (Freney *et al.*, 1990). Nitrification also can lead to nitrate leaching to ground water. More importantly, nitrous oxide produced during the denitrification process is a very powerful greenhouse gas, causing a greater warming effect than carbon dioxide (Steig *et al.*, 2009). Because public concerns about global warming are increasing, a good understanding of the nitrogen biogeochemical cycle is becoming more important. The nitrogen cycle has been investigated in diverse type of soils such as agricultural fields (Kelly *et al.*, 2011), paddy soils (Bannert *et al.*, 2011), wetlands (Dollhopf *et al.*, 2005) and forest soils (Onodera *et al.*, 2010). The limited scope of many previous studies has not allowed a comprehensive understanding of the nitrogen cycle processes.

We previously investigated the abundance of eight genes including *nifH*, bacterial and archaeal *amoA*, *narG*, *nirS*/*nirK*, *norB*, and *nosZ*, which are related to nitrogen fixation, ammonia oxidation, nitrate reduction, nitrite reduction, nitric oxide reduction, and nitrous oxide reduction, respectively, thereby providing an overall nitrogen cycle gene abundance profile in Antarctic soils and changes in response to warming and nitrogen addition (Jung *et al.*, 2011). The abundance of genes involved in the Antarctic microbial nitrogen cycle could be dramatically altered by environmental changes, which in turn might influence the production of nitrous oxide gas. Here, we hypothesized that seasonal changes in undisturbed forest soil environments may cause changes in the nitrogen biogeochemical cycle and the bacterial communities due to different leaf litter decomposition rates during seasons of the year. To test our hypothesis, we analyzed the abundance of genes related to the nitrogen biogeochemical cycle and the soil characteristics for 7 samples from four seasons. Principle component analysis (PCA) was applied to determine the relationships between environmental conditions and the gene abundance. Clone libraries of 16S rRNA genes were constructed to determine the bacterial community composition during summer and winter seasons.

Materials and Methods

Soil sampling

Soils were collected from Bukhan mountain in Seoul, Republic of Korea (37° 37′ 13N, 126° 58′ 54E, altitude: 425 m). Three soil cores were obtained within a 1 m radius of the sampling site and analyzed as a composite sample. Sampling was performed in September, October, November, and December of 2010, and January, April, and July of 2011. Soils were collected from a depth of 10–18 cm and subjected to immediate analysis and DNA extraction.

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Quantification of soil nitrogen

Analysis of total nitrogen was conducted according to the Kjeldahl method (Bremner, 1996). Soil samples were dried and ground to pass through a 0.5 mm sieve for total nitrogen analysis. One gram of soil, K_2SO_4, and concentrated H_2SO_4 were placed into 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 instructions and titrated using indicator solution and standard H_2SO_4 solution. Ammonium and nitrate concentrations were determined by steam-distillation (Mulvaney, 1996). Sieved soil samples were agitated with 2 M KCl solution and the filtered extract was distilled with MgO. The resultant solution was titrated to determine the ammonium concentration. Additional distillation was conducted with MgO and Devarda's alloy. The second resultant 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.

Nitrification 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 filtrated 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.

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, USA). The soil DNA was extracted from 250 mg of soil 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) 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 sec at 95°C, 45 sec at 60°C, and 45 sec 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 to 95°C, 0.5°C increments) were carried out to ensure PCR specificity. To assess the presence of any 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, USA) and transformed into *E. coli* Top10. Isolated cloned plasmids were digested with a restriction enzyme and used as a template. Three independent experiments were carried out and averages with standard deviations are shown.A standard curve foreach qPCR primer was previously determined (Jung *et al.*, 2011).

Table 1. The PCR primers used for quantitative real-time PCR and clone library construction

Target gene (Reference strain)	Primer	Primer sequence (5′→3′)	Product size (bp)	Reference
Bacterial 16S rRNA gene (*Acinetobacteroleivorans* DR1)	27F 1492R 341F 534R	AGAGTTTGATCMTGGCTCAG GGTTACCTTGTTACGACTT CCTACGGGAGGCAGCAG ATTACCGCGGCTGCTGGCA	1465 193 	Watanabe *et al.* (2001)
Archaeal 16S rRNA gene (*Haloterrigena jeotgali* A29)	Arch 349F Arch 806R	GYGCASCAGKCGMGAAW GGACTACVSGGGTATCTAAT	457	Takai and Horikoshi (2000)
Fungal ITS (*Postia placenta* ATCC 11538)	ITS1F ITS4	CTTGGTCATTTAGAGGAAGTAA TCCTCCGCTTATTGATATGC	420–825[a]	Manter and Vivanco (2007)
Bacterial ammonium monooxygenase; *amoA* (*Nitrosomonas europaea*)	amoA-1F amoA-2R	GGGGTTTCTACTGGTGGT CCCCTCKGSAAAGCCTTCTTC	491	Rotthauwe *et al.* (1997)
Archaeal ammonium monooxygenase; *amoA* (*Haloterrigena jeotgali* A29)	amo196F amo277R	GGWGTKCCRGGRACWGCMAC CRATGAAGTCRTAHGGRTADCC	81	Treusch *et al.* (2005)
Nitrogenase reductase; *nifH* (*Pseudomonas stutzeri* A1501)	nifHF nifHRb	AAAGGYGGWATCGGYAARTCCACCAC TGSGCYTTGTCYTCRCGGATBGGCAT	400	Rösch and Bothe (2005)
Cu-containing nitrite reductase; *nirK* (*Agrobacterium tumefaciens* C58)	nirK 1F nirK 5R	GGMATGGTKCCSTGGCA GCCTCGATCAGRTTRTGGTT	514	Braker *et al.* (1998)
Nitrite reductase; *nirS* (*Ralstonia eutropha* H16)	nirS cd3AF nirS R3cd	GTSAACGTSAAGGARACSGG GASTTCGGRTGSGTCTTGA	425	Michotey *et al.* (2000) Throback *et al.* (2004)
Nitrate reductase; *narG (Escherichia coli* APEC O1) (*Pseudomonas aeruginosa* PAO1)	W9 T38	MGNGGNTGYCCNMGNGGNGC ACRTCNGTYTGYTCNCCCCA	442	Gregory *et al.* (2000)
Nitrate reductase; *norB* (*Pseudomonas aeruginosa* PAO1)	cnorB2F cnorB6R	GACAAGNNNTACTGGTGGT GAANCCCCANACNCCNGC	389	Braker and Tiedje (2003)
Nitrous oxide reductase; *nosZ* (*Pseudomonas aeruginosa* PAO1)	nosZ-F nosZ-R	CGYTGTTCMTCGACAGCCAG CGSACCTTSTTGCCSTYGCG	453	Kloos *et al.* (2001)

[a] PCR product sizesvary by species

Table 2. Characteristics of soils

	Sep	Oct	Nov	Dec	Jan	Apr	Jul
pH	4.7±0.0	4.6±0.0	5.0±0.0	5.3±0.1	4.4±0.0	4.0±0.0	3.1±0.0
Temperature (°C)	21.0±0.0	17.0±0.2	7.3±1.0	7.4±0.5	3.9±0.4	10.2±0.2	20.6±0.3
Water content (w/w %)	36.1±4.9	37.6±0.7	31.8±0.8	33.1±1.5	33.6±2.4	34.2±3.8	43.1±2.4
TOC (μg/g)	6411.7±97.6	6795.0±21.2	6928.0±34.0	6238.0±52.0	6021.2±47.2	7060.3±85.7	7182.8±71.2
Nitrogen (μg/g)							
Total	5614.0±178.2	8566.6±627.6	4301.7±796.0	2585.3±149.7	5131.5±140.9	2900.1±35.0	4863.6±88.2
NH_4^+	8.3±1.8	4.2±1.0	1.1±1.0	12.8±3.0	56.9±4.8	25.6±2.8	22.0±3.5
NO_2^-	ND	ND	ND	ND	ND	ND	ND
NO_3^-	1.0±0.6	8.8±1.5	2.0±1.4	15.6±1.3	22.9±8.7	4.2±1.4	22.8±1.7
Nitrification (ng N/dry g·d)	ND[a]	4.0±1.0	15.8±1.0	7.3±1.9	2.0±0.7	ND	ND

[a] ND, Not Detected

Bacterial community analysis

To investigate the bacterial community, clone libraries containing the 16S rRNA gene were constructed from soil DNA. Bacterial 16S rRNA genes were amplified with 27F and 1492R primers (Table 1) and cloned into pGEM-T Easy vector (Promega). Clones were confirmed to have the desired size of PCR product amplified with T7 and SP6 primers. PCR products were subjected to restriction fragment length polymorphism (RFLP) analysis using *Hae*III. RFLP results were compared via electrophoresis in a 1.5% agarose gel at 100 V for 40 min. Plasmids were isolated from clones having different RFLP patterns and sequenced with T7 primer. Sequences were manually checked to ensure high quality. To determine the taxonomic affiliations of clone libraries, sequences were aligned and closely related sequences were retrieved using the Greengene database

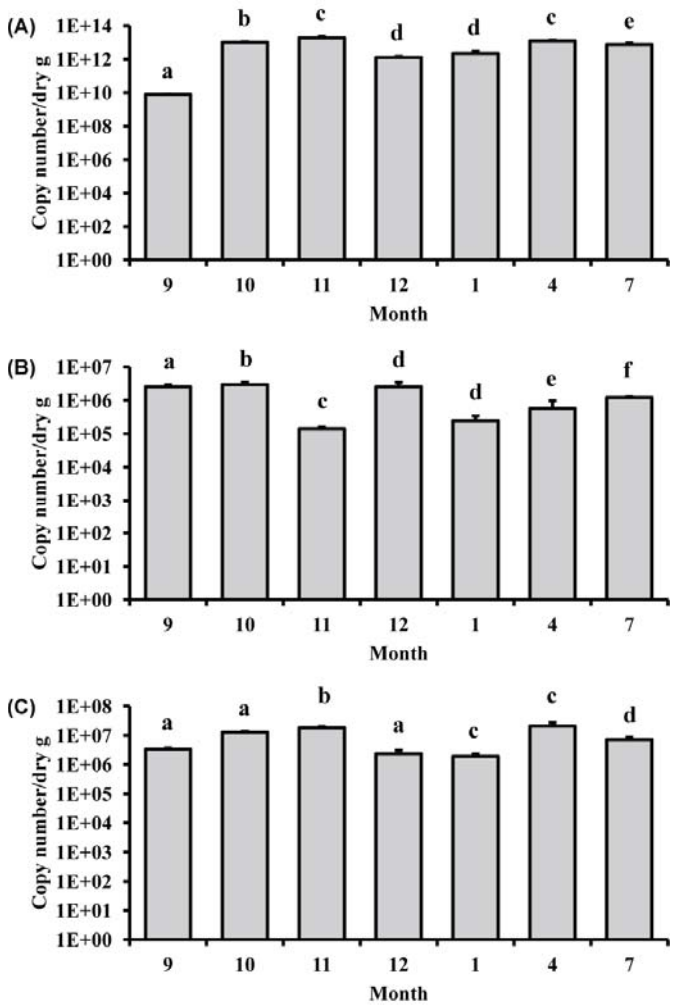

Fig. 1. The abundance of microorganisms from different domains in forest soils. (A) Bacteria (B) Archaea (C) Fungi. The average and standard deviations were calculated from triplicate samples. Bars with different letters are significantly different ($P<0.05$).

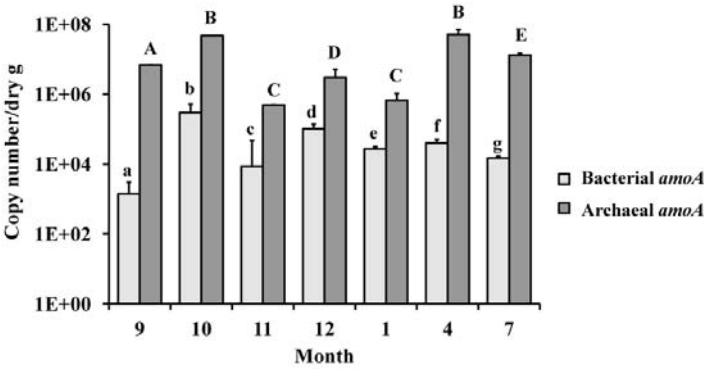

Fig. 2. The abundance of bacterial and archaeal *amoA* encoding ammonia monooxydase subunit A in forest soils. The average and standard deviations were calculated from triplicate samples. Bars with different letters are significantly different (P<0.05).

(http://greengenes.lbl.gov). A phylogenetic tree was drawn using MEGA version 5.0 (Tamura *et al.*, 2011). Clone library sequences were deposited in GenBank under the accession number JQ693103 to JQ693131 and JQ798910 to JQ798941.

Statistical analysis

Gene copy numbers determined by qPCR were analyzed by *t*-tests at a significance level of 5% to confirm statistical differences among samples. Bar graphs with different letters are significantly different. For principle component analysis (PCA), the log value of gene copy number and the soil characteristics were used. For normalization, the logarithms of raw values were divided by their average value to represent the deviation from the average. Data analysis was performed with R software (version 2.13. 1, http://www.R-project.org; R development core team, 2011).

Results

Soil characteristics

The results of pH, water content, total organic carbon (TOC), nitrogen concentration, and nitrification activity were analyzed and summarized in Table 2. Soil texture was sandy clayloam. All soil samples were acidic (pH 3.1–5.3), especially the soil sampled in July, which was pH 3.1. Soil temperature varied with sampling dates (3.9°C–21.0°C). Soil water content was maintained above 30% (w/w) and the highest water content was recorded in July due to frequent heavy rain fall in summer in Korea. Plant litter from deciduous trees is expected to be a major carbon input, sustaining a high TOC level in sampled soils. Differences in TOC were not significant by month, though a slight reduction was observed in winter. In contrast to TOC, total nitrogen content seemed to change continuously according to the

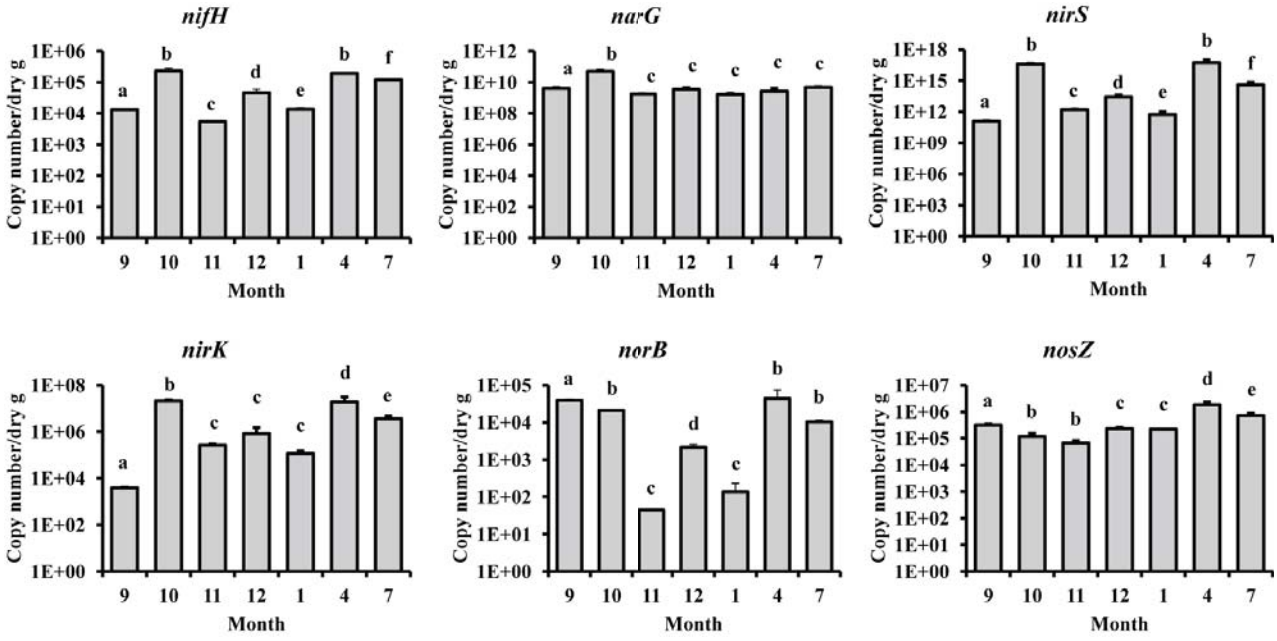

Fig. 3. The abundance of *nifH*, *narG*, *nirS*, *nirK*, *norB*, and *nosZ* in forest soils. The average and standard deviations were calculated from triplicate samples. Bars with different letters are significantly different (P<0.05).

month. Only a very small amount of inorganic nitrogen species such as NH_4^+ and NO_3^- were detected while NO_2^- was not detectable. Nitrification activity peaked in late fall (November, 15.8 ng N/dry g·d) but was not observed in spring and summer (April–September).

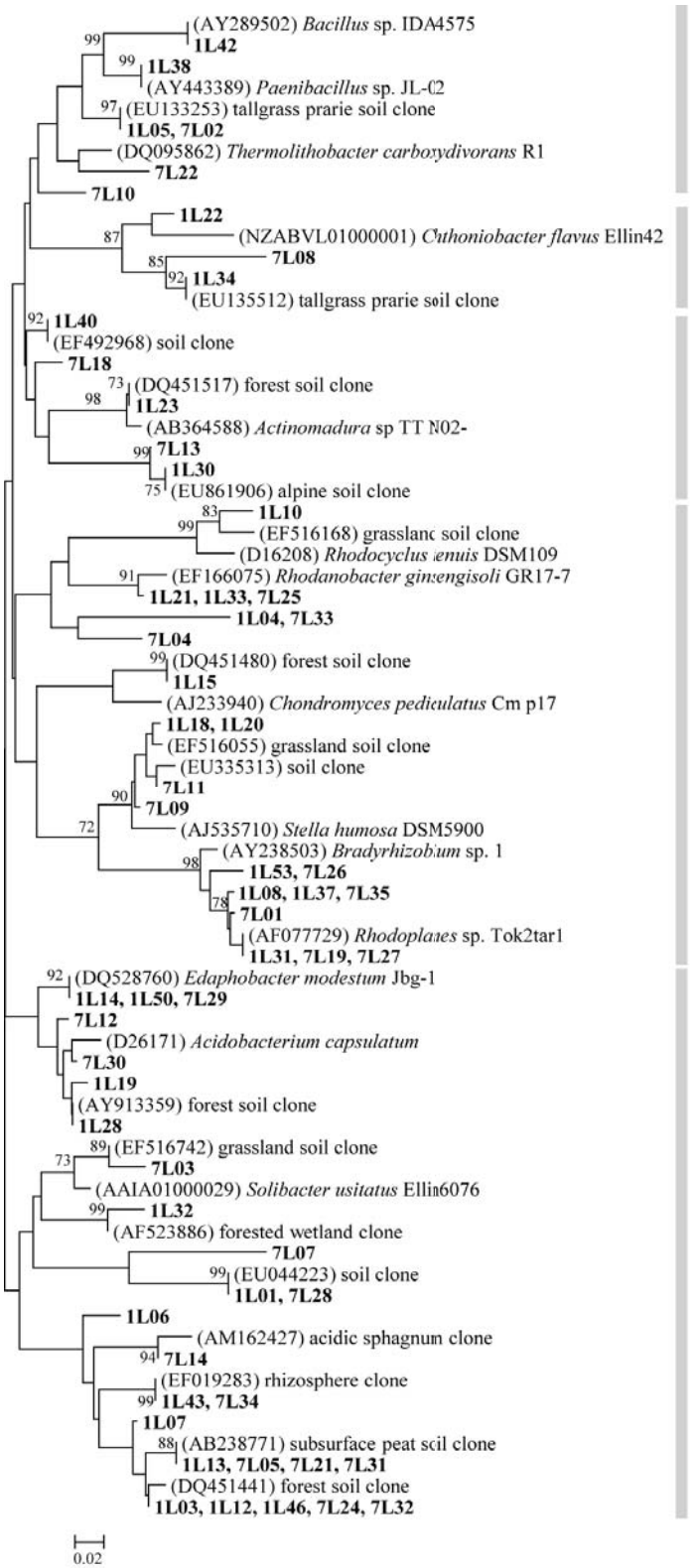

Fig. 4. Phylogenetic tree of 16S rRNA gene clone libraries constructed from DNA in soil samples. Sequences derived from this study are in boldface. Clone name begins with 1 and 7 indicate sampling time, which are January and July, respectively. Bootstrap values more than 70 are shown at nodes. The scale bar indicates 0.02 substitutions per nucleotide position.

Abundance of bacteria, fungi, and archaea in forest soil

The microbial community was quantitatively analyzed using domain specific-primers (Table 1). Bacteria comprised the predominant microbial community in all samples (8.15×10^{9} –1.94×10^{13} copies/dry g) (Fig. 1A). Fungi were the second most dominant microbial community (1.90×10^{6}–2.08×10^{7} copies/dry g) (Fig. 1B), except in December. Archaea were the smallest microbial community (1.41×10^{5}–2.99×10^{6} copies/dry g); however, the copy number was not significantly different from the fungal community (Fig. 1C). Bacteria overwhelmingly outnumbered the two other communities (more than 4–9 orders of magnitude). The archaeal community seemed to be more sensitively reactive to the environment than fungi, as reflected in the ratio of the maximum to minimum copy number (Archaea: 21.2; Fungi: 10.0). These results appear to show a minimal effect of environmental factors on the size of the total microbial community, because there was no clear relationship between soil characteristics and the microbial community.

Predominance of AOA *amoA* over AOB *amoA* in forest soils

The ammonia-oxidizing bacterial (AOB) and archaeal (AOA) communities were quantified by analyzing the ammonia monooxydase subunit A gene, *amoA* (Fig. 2). AOB *amoA* outnumbered by the AOA *amoA* by 1–3 orders of magnitude in all samples. The copy number of AOA *amoA* was almost equal to the copy number of archaeal 16S rRNA genes in most samples, suggesting a high portion of ammonia oxidizers within the total archaeal community. However, only a small portion of ammonia oxidizers was shown to exist in the total bacterial community according to the ratio of AOB *amoA* to bacterial 16S rRNA gene (3.10×10^{-9}–1.72×10^{-7} copies/dry g). Therefore, the potential contribution of AOA to the nitrogen cycle in forest soils could be more important than AOB, regardless of the size of the total community. We could not identify a relationship between environmental factors shown in Table 1 and the change in the size of the ammonia-oxidizer community.

The abundance of nitrogen-fixing and denitrifying communities

The nitrogen-fixing community and denitrifying bacterial community could be categorized into two groups based on the abundance of *nifH*, *narG*, *nirS*, *nirK*, *norB*, and *nosZ* genes (Fig. 3). First, the abundances of *nifH*, *nirS*, *nirK*, and *norB* were characterized by a reduction in the winter season by more than 2 orders of magnitude (November, December, and January), even though the abundance of each gene was different. Therefore, one microorganism, such as a denitrifier, would possess these genes or different microorganisms collectively having these genes could share similar environmental requirements. Unlike *nifH*, *nirS*, *nirK*, and *norB*, the abundances of *narG* and *nosZ* were relatively constant; with the copy numbers of *narG* from November to July not statistically different. These results indicated that the denitrifying bacterial community containing *nirS*, *nirK*, and *norB* was dissimilar to the community containing *narG* and *nosZ*, which appeared insensitive to the seasonal environmental changes. The ratios of two nitrite reductase genes, *nirS* to *nirK*, were 4.74×10^{6}–2.79×10^{9} copies/dry g, indicating the predominance of *nirS* over *nirK* and nitrite reduction is expected to be facilitated by *nirS* rather than *nirK*. The notorious greenhouse gas, nitrous oxide could be emitted when N_2O produced from nitric oxide is not properly reduced to N_2 and this process is mediated by nitrous oxide reductase encoded by *nosZ*. The predominance of *nosZ* over *norB* throughout all seasons provided little evidence at the DNA level of nitrous oxide emission from the forest soils we tested. To assess the contribution of forest soil to nitrous oxide emission, additional studies, such as the measurement of gas production and gene expression would be required.

Bacterial community analysis

Initially, the bacterial community of the forest soils was investigated via denaturing gradient gel electrophoresis (DGGE). However, DGGE band patterns were very similar throughout all seasons (January, February, July, and October samples; data not shown). Therefore, we constructed clone libraries of 16S rRNA genes from the soils of January and July to search for minor bacterial communities, which may not be shown in the DGGE band patterns. A total of 78 clones (43 from January soil and 35 from July soil) were constructed and clones with different RFLP patterns were sequenced. A phylogenetic tree was drawn with the clone sequences and closely related sequences from the Greengene database (Fig. 4). The most abundant bacterial group was *Acidobacteria*, followed by *Proteobacteria*, *Actinobacteria*, *Firmicutes*, and *Verrucomicrobia* (Fig. 5). Closely related sequences retrieved from the Greengene database were the clone sequences derived from diverse soil environments such as forest, grassland, wetland, and rhizosphere. Seasonal changes in the bacterial community were not observed in the clone libraries using the DGGE band patterns.

Principle component analysis (PCA)

PCA was performed based on the abundance of *nifH*, archaeal *amoA*, bacterial *amoA*, *narG*, *nirS*, *nirK*, *norB*, and *nosZ* (Fig. 6A). The months were roughly grouped by seasons, as fall and winter seasons (November, December,

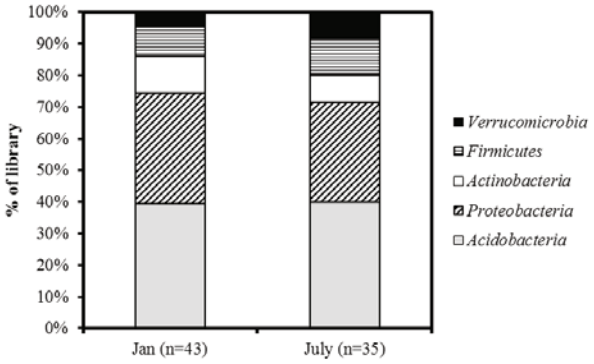

Fig. 5. Taxonomic composition of the bacterial community represented in the clone library sequences. The numbers of clones used in the analysis are in parenthesis.

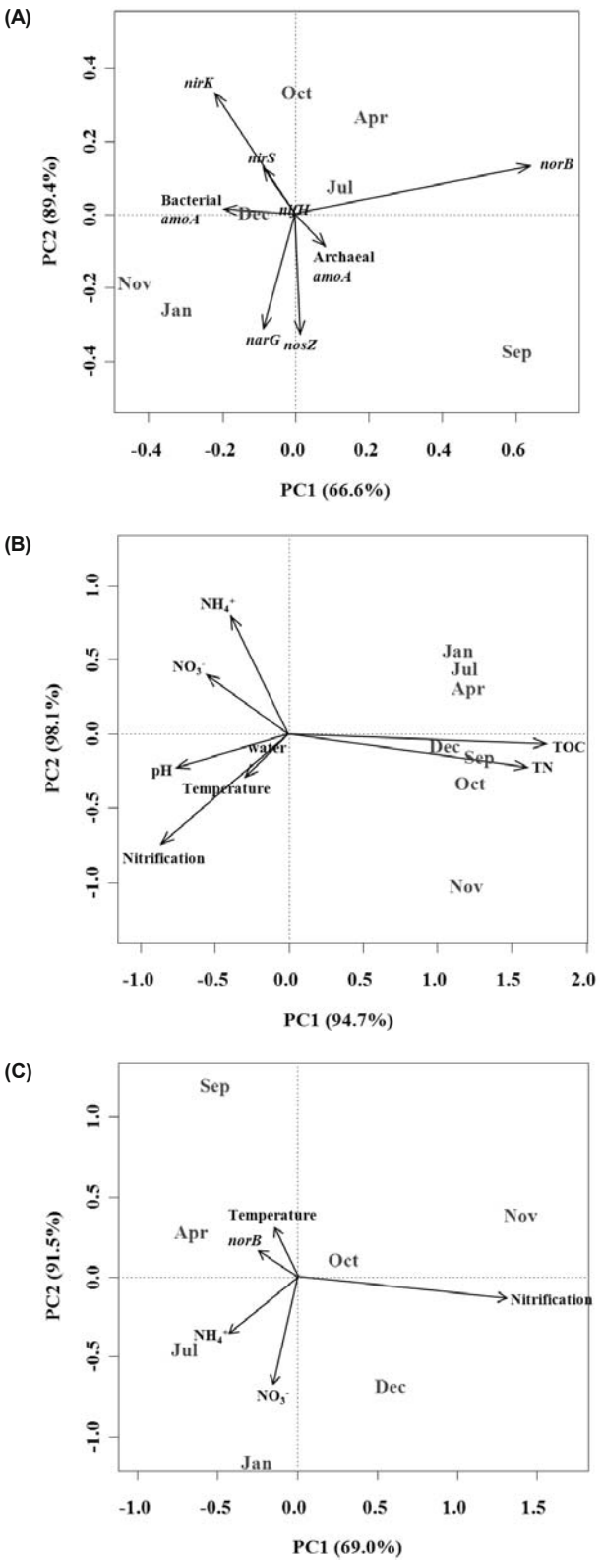

Fig. 6. PCA analysis with (A) the gene abundance, (B) soil characteristics, and (C) the combination of the gene abundance and soil characteristics. Vectors representing other genes and soil characteristics are not shown because they are located in the center of coordination.

January) were place on the third quadrant and spring and summer seasons (April, July, October) were placed on the first quadrant. September was separated from the other samples. The key genes attributed to the differences between months were bacterial *amoA*, *narG*, *nirK*, *norB*, and *nosZ*. Soil characteristics were also analyzed via PCA (Fig. 6B). All months were relatively close, except November, and the factor dividing samples on the PCA plot was not determined. Interestingly, when gene abundance and environmental factors were considered together in PCA, each soil sample was distinguished from each other, indicating that forest soils from each month experienced their own unique biological, physical and chemical conditions (Fig. 6C). A seasonal change pattern was observed in represented 2D space, as shown characteristics of soil samples were located following clockwise directions and environmental conditions seemed to contribute to seasonal changes greater than variance in gene abundance.

Discussion

The predominance of archaeal *amoA* over bacterial *amoA* has been reported in diverse environments such as soils (Leininger *et al*., 2006) and marine ecosystems (Beman *et al*., 2007). Previous studies from other forest soils also identified a higher abundance of AOA *amoA* than AOB *amoA* (Onodera *et al*., 2010; Szukics *et al*., 2010). The predominance of archaeal *amoA* in this study was consistent with other studies; however, the copy numbers of bacterial *amoA* and archaeal *amoA* were lower by approximately one and two orders of magnitudesthan pristine forest soils (pH 5.3) and temperate forest soils (pH 5.8–6.5) (Onodera *et al*., 2010; Szukics *et al*., 2010). Although numerous studies identified a predominance of archaeal *amoA* in various environments, a predominance of bacterial *amoA* has also been observed in several cases, such as Antarctic soils (Jung *et al*., 2011), a subglacial ecosystem (Boyd *et al*., 2011), and estuarial environments (Caffrey *et al*., 2007).

Denitrifying microorganisms do not always participate in all processes of denitrification. For example, some denitrifiers are able to reduce nitrate to nitrous oxide but unable to further reduce (Zumft, 1997) whereas *Escherichia coli* performs reduction of nitrous oxide to dinitrogen gas (Kaldorf *et al*., 1993). This type of denitrifier could be expected to exist in the forest soils we tested, and be the cause of the similar change pattern of *nirS*, *nirK*, and *norB*, while *narG* and *nosZ* maintained a relatively constant gene abundance (Fig. 3). The *nirS* and *nirK* encoding nitrite reductase are distributed in phylogenetically separated groups of microorganisms, and PCR amplification of these two genes did not occur in one microorganism (Rösch *et al*., 2002). Therefore, the similar change pattern observed in *nirS* and *nirK* would be due to the presence of two types of nitrite reducer with a similar preference in environmental characteristics.

Phylogenetic analysis of the 16S rRNA gene clone libraries from summer and winter determined that *Acidobacteria* and *Proteobacteria* were the most abundant bacterial groups. These two groups were frequently observed as the predominant microbial groups in acidic forest soils (Lipson, 2007),

therefore the microbial community structure determined in this study may be a common one. We could not detect any seasonal change in the bacterial community based on the DGGE band patterns and clone library sequences (Fig. 5). The stable bacterial community may be explained by the stable environmental conditions. The constant presence of high levels of organic carbon may also support a stable bacterial community (Giardina and Ryan, 2000; Lipson *et al*., 2000).

Important environmental factors affecting the abundance and activity of genes in the nitrogen biogeochemical cycle are well-documented in numerous studies. For example, temperature, organic carbon, ammonium levels, salinity, dissolved oxygen level, and pH can be important in determining the archaeal *amoA* community (Erguder *et al*., 2009). Moisture and temperature affected the nitrogen turnover rates by switching the ratio of archaeal *amoA* to bacterial *amoA* and shaping the composition of the denitrifier community (Szukics *et al*., 2010). The previously identified relationship between environmental characteristics and the nitrogen biogeochemical cycle prompted us to investigate if there is any connection between soil characteristics and specific gene abundances via PCA analysis (Fig. 6). Our results indicated that the combination of genetic, chemical, and physical parameters created the unique conditions distinguishing every month from another, and these changing conditions could be repeated (Fig. 6C). However, no major environmental feature governing any specific process of the nitrogen cycle was identified. A lack of impact of environmental conditions and gene abundance has often been found (Yergeau *et al*., 2007; Jung *et al*., 2011). The various experimental conditions used by researchers were not standardized, and controlled designs such as microcosm studies would likely be better. Experimental setups allowing manipulation of nutrients, nitrogen source, temperature, moisture, and pH can identify significant correlations of controlled factors and gene abundance reflecting community composition (Dollhopf *et al*., 2005; Szukics *et al*., 2010; Herrmann *et al*., 2011).

In summary, we have provided comprehensive data related to the gene abundance of every process of the nitrogen biogeochemical cycle, varying by month, along with the microbial community in forest soils. Seasonal change in soil conditions were apparent only when the gene abundances and soil conditions were considered together, suggesting the forest ecosystem is closely connected. This pattern of seasonal change is expected to repeat every year.

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References

Bannert, A., Kleineidam, K., Wissing, L., Mueller-Niggemann, C., Vogelsang, V., Welzl, G., Cao, Z., and Schloter, M. 2011. Changes in diversity and functional gene abundances of microbial communities involved in nitrogen fixation, nitrification, and denitrification in a tidal wetland versus paddy soils cultivated for different time periods. *Appl. Environ. Microbiol.* **77**, 6109–6116.

Beman, J.M., Roberts, K.J., Wegley, L., Rohwer, F., and Francis, C.A. 2007. Distribution and diversity of archaeal ammonia monooxygenase genes associated with corals. *Appl. Environ. Microbiol.* **73**, 5642–5647.

Braker, G., Fesefeldt, A., and Witzel, K.P. 1998. Development of PCR primer systems for amplification of nitrite reductase genes (*nirK* and *nirS*) to detect denitrifying bacteria in environmental samples. *Appl. Environ. Microbiol.* **64**, 3769–3775.

Braker, G. and Tiedje, J.M. 2003. Nitric oxide reductase (*norB*) genes from pure cultures and environmental samples. *Appl. Environ. Microbiol.* **69**, 3476–3483.

Bremner, J.M. 1996. Nitrogen-Total. Methods of soil analysis part 3 chemical methods, pp. 1085–1121. *In* Sparks, D.L. (ed.), Soil Science Society of American, Madison, WI, USA.

Boyd, E.S., Lange, R.K., Mitchell, A.C., Havig, J.R., Hamilton, T.L., Lafrenière, M.J., Shock, E.L., Peters, J.W., and Skidmore, M. 2011. Diversity, abundance, and potential activity of nitrifying and nitrate-reducing microbial assemblage in a subglacial ecosystem. *Appl. Environ. Microbiol.* **77**, 4778–4787.

Caffrey, J.M., Bano, N., Kalanetra, K., and Hollibaugh, J.T. 2007. Ammonia oxidation and ammonia-oxidizing bacteria and archaea from estuaries with differing histories of hypoxia. *ISME J.* **1**, 660–662.

Dollhopf, S.L., Hyun, J., Smith, A.C., Adams, H.J., O'brien, S., and Kostka, J.E. 2005. Quantification of ammonia-oxidizing bacteria and factors controlling nitrification in salt marsh sediments. *Appl. Environ. Microbiol.* **71**, 240–246.

Erguder, T.H., Boon, N., Wittebolle, L., Marzorati, M., and Verstraete, W. 2009. Environmental factors shaping the ecological niches of ammonia-oxidizing archaea. *FEMS Microbiol. Rev.* **33**, 855–869.

Freney, J.R., Trevitt, A.C.F., De Datta, S.K., Obcemea, W.N., and Real, J.G. 1990. The interdependence of ammonia volatilization and denitrification as nitrogen loss processes in flooded rice fields in the Philippines. *Biol. Fertil. Soils* **9**, 31–36.

Giardina, C.P. and Ryan, M.G. 2000. Evidence that decomposition rates of organic carbon in mineral soil do not vary with temperature. *Nature* **404**, 858–861.

Gregory, L.G., Karakas-Sen, A., Richardson, D.J., and Spiro, S. 2000. Detection of genes for membrane-bound nitrate reductase in nitrate-respiring bacteria and in community DNA. *FEMS Microbiol. Lett.* **183**, 257–279.

Herrmann, M., Scheibe, A., Avrahami, S., and Küsel, K. 2011. Ammonium availability affects the ratio of ammonia-oxidizing bacteria to ammonia-oxidizing archaea in simulated creek ecosystems. *Appl. Environ. Microbiol.* **77**, 1896–1899.

Jung, J., Yeom, J., Kim, J., Han, J., Lim, H.S., Park, H., Hyun, S., and Park, W. 2011. Change in gene abundance in the nitrogen biogeochemical cycle with temperature and nitrogen addition in Antarctic soils. *Res. Microbiol.* **162**, 1018–1029.

Kaldorf, M., Linne von Berg, K.H., Meier, U., Servos, U., and Bothe, H. 1993. The reduction of nitrous oxide to dinitrogen by *Escherichia coli*. *Arch. Microbiol.* **160**, 432–439.

Kelly, J.J., Policht, K., Grancharova, T., and Hundal, L.S. 2011. Addition of biosolids to an agricultural soil produces distinct responses in ammonia oxidizing archaea and bacteria. *Appl. Environment. Microbiol.* **77**, 6551–6558.

Kloos, K., Mergel, A., Rosch, C., and Bothe, H. 2001. Denitrification within the genus *Azospirillum* and other associative bacteria. *Aust. J. Plant Physiol.* **28**, 991–998.

Leininger, S., Urich, T., Schloter, M., Schwark, L., Qi, J., Nicol, G.W., Prosser, J.I., Schuster, S.C., and Schleper, C. 2006. Archaea predominate among ammonia-oxidizing prokaryotes in soils. *Nature* **442**, 806-809.

Lipson, D.A. 2007. Relationships between temperature responses and bacterial community structure along seasonal and altitudinal gradients. *FEMS Microbiol. Ecol.* **59**, 418–427.

Lipson, D.A., Schmidt, S.K., and Monson, R.K. 2000. Carbon availability and temperature control the post-snowmelt decline of microbial biomass in an alpine soil. *Soil Biol. Biochem.* **32**, 441–448.

Manter, D.K. and Vivanco, J.M. 2007. Use of the ITS primers, ITS1F and ITS4, to characterize fungal abundance and diversity in mixed-template samples by qPCR and length heterogeneity analysis. *J. Microbiol. Methods* **71**, 7–14.

Michotey, V., Mejean, V., and Bonin, P. 2000. Comparison of methods for quantification of cytochrome cd_1-denitrifying bacteria in marine samples. *Appl. Environ. Microbiol.* **66**, 1564–1571.

Mulvaney, R.L. 1996. Nitrogen-inorganic forms. Methods of soil analysis part 3 chemical methods, pp. 1123–1184. *In* Sparks, D.L. (ed.), Soil Science Society of American, Madison, WI, USA.

Onodera, Y., Nakagawa, T., Takahashi, R., and Tokuyama, T. 2010. Seasonal change in vertical distribution of ammonia-oxidizing archaea and bacteria and their nitrification in temperate forest soil. *Microbes Environ.* **25**, 28–35.

R development core team. 2011. R: A language and environment for statistical computing. R foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0.

Rösch, C. and Bothe, H. 2005. Improved assessment of denitrifying, N_2-fixing, and total community bacteria by terminal restriction fragment length polymorphism analysis using multiple restriction enzymes. *Appl. Environ. Microbiol.* **71**, 2026–2035.

Rösch, C., Mergel, A., and Bothe, H. 2002. Biodiversity and dinitrogen-fixing bacteria in an acid forest soil. *Appl. Environ. Microbiol.* **68**, 3818–3829.

Rotthauwe, J.H., Witzel, K.P., and Liesack, W. 1997. The ammonia monooxygenase structural gene *amoA* as a functional marker: molecular fine-scale analysis of natural ammonia-oxidizing populations. *Appl. Environ. Microbiol.* **63**, 4704–4712.

Schinner, F., Öhlinger, R., Kandeler, E., and Margesin, R. 1995. Methods in soil biology, Springer, New York, N.Y., USA.

Steig, E.J., Schneider, D.P., Rutherford, S.D., Mann, M.E., Comiso, J.C., and Shindell, D.T. 2009. Warming of the Antarctic ice-sheet surface since the 1957 international geophysical year. *Science* **457**, 459–462.

Szukics, U., Abell, G.C.J., Hödl, V., Mitter, B., Sessitsch, A., Hackl, E., and Zechmeister-Boltenstern, S. 2010. Nitrifiers and denitrifiers respond rapidly to changed moisture and increasing temperature in a pristine forest soil. *FEMS Microbiol. Ecol.* **72**, 395–406.

Takai, K. and Horikoshi, K. 2000. Rapid detection and quantification of members of the archaeal community by quantitative PCR using fluorogenic probes. *Appl. Environ. Microbiol.* **66**, 5066–5072.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., and Kumar, S. 2011. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.* **28**, 2731–2739.

Throback, I.N., Enwall, K., Jarvis, A., and Hallin, S. 2004. Reassessing PCR primers targeting *nirS*, *nirK* and *nosZ* genes for community surveys of denitrifying bacteria with DGGE. *FEMS Microbiol. Ecol.* **49**, 401–417.

Treusch, A.H., Leininger, S., Kletzin, A., Schuster, S.C., Klenk, H.P., and Schleper, C. 2005. Novel genes for nitrite reductase and Amo-related proteins indicate a role of uncultivated mesophilic crenarchaeota in nitrogen cycling. *Environ. Microbiol.* **7**, 1985–1995.

Watanabe, K., Kodama, Y., and Harayama, S. 2001. Design and evaluation of PCR primers to amplify bacterial 16S ribosomal DNA fragments used for community fingerprinting. *J. Microbiol. Methods* **44**, 253–262.

Yergeau, E., Kang, S., He, Z., Zhou, J., and Kowalchuk, G.A. 2007. Functional microarray analysis of nitrogen and carbon cycling genes across and Antarctic latitudinal transect. *ISME J.* **1**, 163–179.

Zumft, W.G. 1997. Cell biology and molecular basis of denitrification. *Microbiol. Mol. Biol. Rev.* **61**, 533–616.