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Effects of Nutritional Input and Diesel Contamination on Soil Enzyme Activities and Microbial Communities in Antarctic Soils

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Pollution of Antarctic soils may be attributable to increased nutritional input and diesel contamination via anthropogenic activities. To investigate the effect of these environmental changes on the Antarctic terrestrial ecosystem, soil enzyme activities and microbial communities in 3 types of Antarctic soils were evaluated. The activities of alkaline phosphomonoesterase and dehydrogenase were dramatically increased, whereas the activities of β-glucosidase, urease, arylsulfatase, and fluorescein diacetate hydrolysis were negligible. Alkaline phosphomonoesterase and dehydrogenase activities in the 3 types of soils increased 3- to 10-fold in response to nutritional input, but did not increase in the presence of diesel contamination. Consistent with the enzymatic activity data, increased copy numbers of the *phoA* gene, encoding an alkaline phosphomonoesterase, and the 16S rRNA gene were verified using quantitative real-time polymerase chain reaction. Interestingly, dehydrogenase activity and 16S rRNA gene copy number increased slightly after 30 days, even under diesel contamination, probably because of adaptation of the bacterial population. Intact Antarctic soils showed a predominance of *Actinobacteria* phylum (mostly *Pseudonorcarida* species) and other phyla such as *Proteobacteria*, *Chloroflexi*, *Planctomycetes*, *Firmicutes*, and *Verrucomicrobia* were present in successively lower proportions. Nutrient addition might act as a selective pressure on the bacterial community, resulting in the prevalence of *Actinobacteria* phylum (mostly *Arthrobacter* species). Soils contaminated by diesel showed a predominance of *Proteobacteria* phylum (mostly *Phyllobacterium* species), and other phyla such as *Actinobacteria*, *Bacteroidetes*, *Planctomycetes*, and *Gemmatimonadetes* were present in successively lower proportions. Our data reveal that nutritional input has a dramatic impact on bacterial communities in Antarctic soils and that diesel contamination is likely toxic to enzymes in this population.

Keywords: soil, enzyme, biogeochemical cycle, community analysis, Actinobacteria, Proteobacteria

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Introduction

Nutrient cycling of carbon (C), nitrogen (N), phosphorus (P), and sulfur (S) in soils is mediated by microorganisms, plant roots, and soil animals via a variety of biochemical and physiochemical reactions (Tabatabai, 1982). Therefore, various enzymes generated by living organisms play important roles in soil ecology (Bhattacharyya *et al.*, 2008). In particular, the presence of microorganisms and their secreted extracellular enzymes, which are essential for nutrient cycling in soils, regulates vegetation development, ecosystem functioning, and crop productivity (Bauer *et al.*, 1991). Soil microorganisms and enzymes are profoundly sensitive to any environmental pollution, since their diversity and activity change rapidly as a result of such perturbations (Schloter *et al.*, 2003). Microbiological parameters such as soil respiration, microbial biomass, and enzyme activities are used as indexes for soil fertility and can provide useful information regarding soil and ecosystem health (Perucci and Scarponi, 1985; Brohon *et al.*, 2001; Eibes *et al.*, 2006). Thus, the determination of specific enzyme activities (e.g., phosphatases, urease, dehydrogenase) or other parameters (e.g., ATP content and respiration rate) and the use of general soil characteristics is important for understanding the effect of pollution on soil health (Nannipieri *et al.*, 1990).

Many soil enzymes have been extensively studied, and their enzymatic activities can be affected by soil characteristics such as texture, organic matter content, and pH (Labud *et al.*, 2007). β-Glucosidases are linked to the C cycle and hydrolyze β-glucosyl residues, thereby releasing β-D-glucose in the final reaction step (Turner, 2010). Urease catalyzes the hydrolysis of urea into carbon dioxide and ammonia and is closely related to the N cycle (Kandeler and Gerber, 1988). Phosphomonoesterases are enzymes involved in the P cycle and catalyze the hydrolysis of O-P bonds, thereby yielding free phosphate. According to their pH optimum, specific phosphomonoesterases can be found in both acidic and alkaline environments (Margesin, 1995; Turner, 2010). Arylsulfatase is involved in the S cycle and mediates the release of inorganic sulfate from organic sulfate esters, which influence microbial and plant growth (Tabatabai and Bremner, 1970; Turner, 2010). Dehydrogenase is considered to be be involved in the total metabolic activity of live soil microorganisms (Tabatabai, 1982). Fluorescein diacetate (FDA) hydrolysis can be performed by a number of different enzymes, including proteases, lipases, and esterases, and is triggered by the hydrolytic activities of soil bacteria and fungi (Schnürer and Rosswall).

Contamination of soils with nutritional input and pollutants has been reported even in the remote climate of Antarctica

(Aislabie *et al*., 2004). Increased anthropogenic activities in this region because of the extensive use of fossil fuels for resupplying vessels, tourist ships, small research boats, and terrestrial vehicles and as an energy source for the heating and lighting of research stations may well engender pollution (Martins *et al*., 2010). However, the impacts of this pollution on soil microorganisms have been poorly explored in this environment. In this study, we investigated the effect of these environmental changes on the Antarctic terrestrial ecosystem by analyzing soil enzyme activities and the microbial community. Six types of enzyme activities that represented C, N, P, and S cycles and general microbial activities, in addition to the spectrum of soil bacteria present, were examined for their responses to nutritional input and diesel pollution. The copy numbers of functional and 16S rRNA gene in the Antarctic soil were quantitatively evaluated via DNA-based quantitative real-time polymerase chain reaction (qPCR). This is, to the best of our knowledge, the first study to investigate various soil enzyme activities involved in the biogeochemical cycle in the Antarctic terrestrial soil environment.

Materials and Methods

Soil sampling sites and treatment procedures

The Antarctic soils were sampled from the Cape Burks area (Cape Burks 64: 74° 45.464S, 136° 49.024W; Cape Burks 67: 74° 45.273S, 136° 48.950W; Cape Burks 68: 74° 45.326S, 136° 48.743W). All soil samples were collected from topsoil (<10 cm) and subjected to storage below -80°C until analysis. The Korea Polar Research Institute (KOPRI) provided soil samples. In microcosm studies, the Antarctic soils were supplemented with diesel oil and/or nutrients. The following conditions were used: 50 g of soil in a 500-ml conical tube with (1) no treatment, (2) 0.5% yeast extract (3) and 1% (v/w) diesel oil, and (4) 0.5% yeast extract and 1% (v/w) diesel oil. The soils were incubated at 4°C for 30 days. Enzyme activities were measured after 1, 10, 20, and 30 days. During the incubation, microcosms were placed in an injection needle on a bottle cap to maintain aerobic conditions.

Soil enzyme activity assays

β-Glucosidase activity was performed using a modified assay based on that of Taylor *et al*. (2002). One gram of soil was incubated for 2 h at room temperature with 4 ml of 0.5 M Tris buffer (pH 12.0) and 1 ml of 25 mM *p*-nitrophenyl β-D-glucopyranoside. Autoclaved distilled water was used rather than *p*-nitrophenyl β-D-glucopyranoside as the negative control; 0.5 M Tris buffer (pH 6.0) was used as the negative control. At the end of the incubation, 1 ml of 25 mM *p*-nitrophenyl β-D-glucopyranoside was added to the negative controls and autoclaved distilled water was added to the other samples. This mixture was shaken with 1 ml of 0.5 M $CaCl_2$ and 4 ml of 0.5 M NaOH for an additional 30 min. The supernatant was obtained via 5 min of centrifugation at 10,000×*g*. Enzyme activity was determined by measuring the absorbance at 400 nm. Urease activity was measured using the method developed by Kandeler (1996). Alkaline and acid phosphomonoesterase activities were determined via the method developed by Margesin *et al*. (1995). Phosphomonoesterase was used 0.5 M Tris buffer (acid, pH 4.0; alkaline, pH 9.0) and 25 mM *p*-nitrophenyl phosphate. Arylsulfatase activity was determined as described previously (Tabatabai and Bremner, 1970). Arylsulfatase was used 0.5 M sodium acetate (pH 5.8) and 25 mM *p*-nitrophenyl sulfate at room temperature. Dehydrogenase activity was determined using the method developed by Casida *et al*. (1964). One gram of soil was incubated for 24 h at room temperature with 1.5 ml of 0.5 M Tris buffer (pH 7.0) and 2 ml of 2(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyl tetrazolium chloride (INT). Autoclaved distilled water was used rather than INT as the negative control. At the end of the incubation, 2 ml of INT was added to the negative control and autoclaved distilled water was added to the other samples. This mixture was shaken for 1 h with 10 ml of *N*,*N*-dimethylformamide/ethanol (1:1) solution. The supernatant was obtained via centrifugation at 10,000×*g* for 5 min. Enzyme activity was determined by measuring absorbance at 464 nm. The protocol by Schnürer and Rosswall (Schnürer and Rosswall, 1982) was used for fluorescein diacetate (FDA) hydrolysis assay.

Soil DNA extraction and qPCR analysis

Microbial communities were evaluated using qPCR with the iCycler iQ real-time PCR detection system (Bio-Rad, USA). The soil DNA was isolated using the NucleoSpin soil kit (Macherey-Nagel, Germany), in accordance with the manufacturer's instructions. For qPCR of bacterial 16S rRNA gene sequences, the primers used were as follows: 341F (5′-CCTACGGGAGGCAGCAG-3′) and 534R (5′-ATTACCGCGGCTGCTGGCA-3′); these primers amplified a fragment of about 193 bp in length (Watanabe *et al*., 2001). For qPCR of alkaline phosphatase (*phoA*) gene sequences, the primers were designed as follows: phoA F (5′-AAAACCGGGCTGCTCAGGGC-3′) and phoA R (5′-CGCCCGCACCTTCGGCATAA-3′); these primers amplified a fragment of about 186 bp in length. The PCR mixture contained 100 ng of template soil DNA, 5 pmol of primers, and 2× SYBR Green iCycler iQ mixture (Bio-Rad). Fluorescence was measured at the end of each 72°C incubation and analyzed using the iCycler iQ software (version 3.0). PCR specificity was verified by measuring melting curves (60°C to 95°C, 0.5°C increments). For quantification, PCR products from reference strains were cloned into the pGEM-T easy vector (Promega, USA) and transformed in *E. coli* Top10. Isolated cloned plasmids were restricted and used as a template. Four independent experiments were performed, and average values with standard deviation were recorded (Jung *et al*., 2011, 2012).

Construction of 16S rRNA gene clone library and phylogenetic analysis

The two universal 16S rRNA gene primers 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′) (Tamura *et al*., 2007) were used for amplification of the 16S ribosomal RNA gene from the individual Antarctic soils (64, 67, 68). The following PCR protocol was used: 5 min at 95°C and 35 cycles consisting of 45 sec at 95°C, 45 sec at 58°C, and 45 sec at 72°C. The final

extension step was at 72°C for 5 min. PCR products were cloned into the pGEM-T EASY vector (Promega). A total of 113 white colonies for each soil were collected from Luria-Bertani agar plates containing 50 μg/ml of ampicillin and 20 μg/ml of X-gal. White colonies were screened directly for inserts by performing PCR with T7 (5′-TAATA CGACTACTCACTATAGGG-3′) and SP6 (5′-TACGATT TAGGTGACACTATAG-3′) primers. PCR products were purified using the PCR purification kit (Dyne Bio, Korea). The resulting PCR products were digested with *Msp*I for 1 h at 37°C. The clones were grouped according to the resulting restriction fragment length polymorphisms, and then, the representative clones of each group were sequenced (Jung *et al.*, 2012). Sequences were checked for chimerism by using the Chimera Check program available at the Greengene website (http://greengenes.lbl.gov/cgi-bin/nph-index.cgi). All the clones that displayed chimeric profiles were discarded from further analysis. Sequences that were over 97% similar were grouped into an operational taxonomic unit on the basis of manual comparison. The representative GenBank sequences to the clones of interest were extracted from the National Center for Biotechnology Information database and included in further phylogenetic analyses by using MEGA Version 4.0. The nucleotide sequence of the 16S rRNA gene obtained in this study submission in GenBank (JX453497-JX453554).

Results

Characterization of various soil enzyme activities in response to environmental changes

To choose sensitive soil enzyme activities under nutritional input and diesel pollution, control experiments were performed using soil samples collected from Nelson Island (Fig. 1, 62° 14′ 127S, 58° 44′ 467N, altitude 115 m, within 30 cm, collected on January 18th, 2008); the soil samples were obtained from the Korea Polar Research Institute (KOPRI). These soil samples were incubated at 2 different temperatures (4°C and 10°C), and various enzyme activities were measured after 1 day and 7 days. Only a slight increase in β-glucosidase activity was observed within 7 days at both 4°C and 10°C upon nutrient addition (Fig. 2A). Diesel contamination appeared to be toxic to this enzyme, but recovery of β-glucosidase activity was observed when diesel contamination was present together with nutritional input after 7 days (Fig. 2A). In contrast, both treatments increased soil arylsulfatase activities at 10°C after 7 days (Fig. 2B). Urease activity was not detected (data not shown). Total nitrogen measurements showed the presence of nitrogen (1.7243 μg/g), which is a significantly lower amount of total nitrogen than that in other terrestrial forest soil (average, 4849.9 μg/g) (Jung *et al.*, 2012). Significant amounts of phosphomonoesterase activity were observed in intact soil (Figs. 2C and 2D). Acidic phosphomonoesterase is the most insensitive enzyme to environmental changes (Fig. 2C); by contrast, alkaline phosphomonoesterase (AP) activity was more sensitive (Fig. 2D). Diesel contamination appears to be very toxic to this enzyme activity. Although dehydrogenase activity is generally detected wherever there is microbial activity, very little dehydrogenase activity was observed in the tested soil (Fig. 2E). However, experimental nutritional input and temperature change increased dehydrogenase activity, and diesel contamination inhibited it (Fig. 2E). Since FDA hydrolysis can be due to enzymic activity in live and dead cells, we were unable to attribute it to a particular soil condition (Fig. 2F). Our data showed that FDA activities were unsuitable for monitoring microbial activity under the various environmental changes. On the basis of our observations, AP and dehydrogenase activities were selected for further study because they both served as robust indexes for soil health in conditions of nutritional input and diesel contamination.

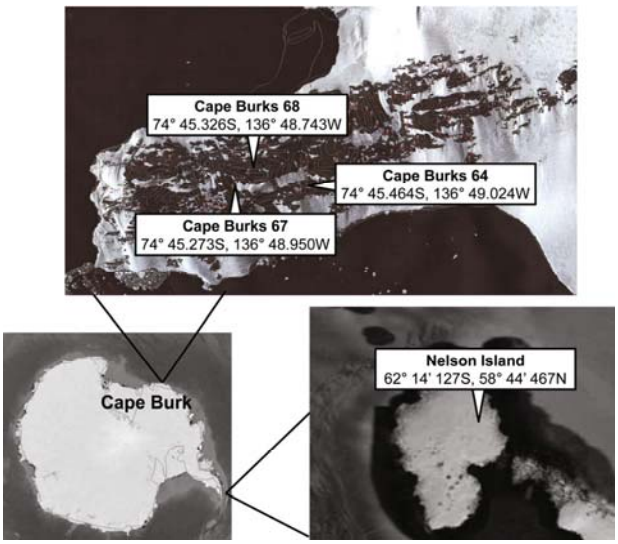

Fig. 1. A map of the sampling sites. Images were captured using Google Earth and edited.

AP and dehydrogenase activities in Cape Burks soils

Cape Burks soils from the northern part of the Antarctic area were analyzed (Fig. 1). Soil texture, water content, pH, total organic carbon (TOC), and electrical conductivity (EC) are summarized in Table 1. Water contents of the 3 soils were very low (0.24–3.27%), and their pH ranges were between 6.5 and 7.17. The texture of the Antarctic soils was sandy, and TOC varied according to the sampling sites. AP and dehydrogenase activities of 3 Cape Burks soils were monitored over 30 days at 4°C. The AP activities of Cape Burks soils were significantly lower than those of the soil from Nelson Island (Fig. 2D intact and Fig. 3 intact). Consistent with previous control experiments, the AP activities were

Table 1. Characteristics of Antarctic soils

Characteristics	Cape Buks 64	Cape Buks 67	Cape Buks 68
Soil Texture	Sand	Sand	Sand
Water content (%)	0.24±0.0	3.78±0.2	3.71±0.0
pH	7.17±0.04	6.93±0.02	6.50±0.05
[a]TOC (mg/kg)	119.42±9.15	97.54±12.39	260.27±25.12
[b]EC (mS/m)	1.36	0.24	8.39

[a] TOC, Total organic carbon
[b] EC, Electrical conductivity

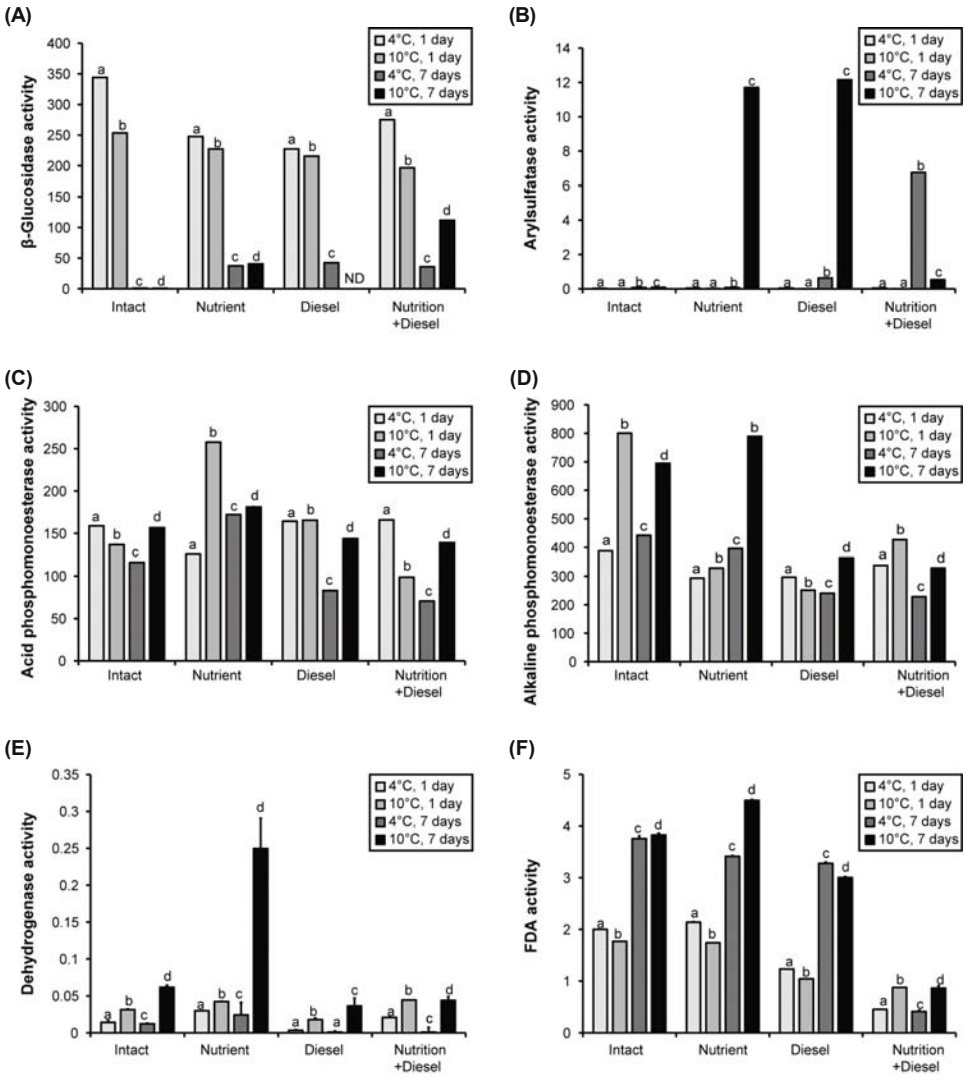

Fig. 2. Six enzyme activities in Nelson Island soil of Antarctica. β-Glucosidase activity (μg pNP·g of soil^{-1}·h^{-1}) (A), arylsulfatase activity (μg pNP·g of soil^{-1}·h^{-1}) (B), acidic phosphomonoesterase activity (μg pNP·g of soil^{-1}·h^{-1}) (C), alkaline phosphomonoesterase activity (μg pNP·g of soil^{-1}·h^{-1}) (D), dehydrogenase activity (μg INT/g of soil) (E), and fluorescein diacetate (FDA) hydrolysis activity (μg·g of soil^{-1}·h^{-1}) (F). Average and standard deviation values were calculated from duplicates. Bars with different letters represent significantly different values ($P<0.05$).

very sensitive to nutritional input and diesel contamination, since they increased 100-fold in all 3 soil samples over 30 days of incubation. By contrast, intact soils showed a 4-fold increase under the same conditions (Fig. 3). Diesel contamination did not increase AP activities; this indicates that diesel toxicity suppresses microbial and AP activities. Furthermore, diesel treatment together with nutritional input showed incremental increase in AP activities, probably since the microbial population adapted over the 30-day experimental period. This result would not have been observed in

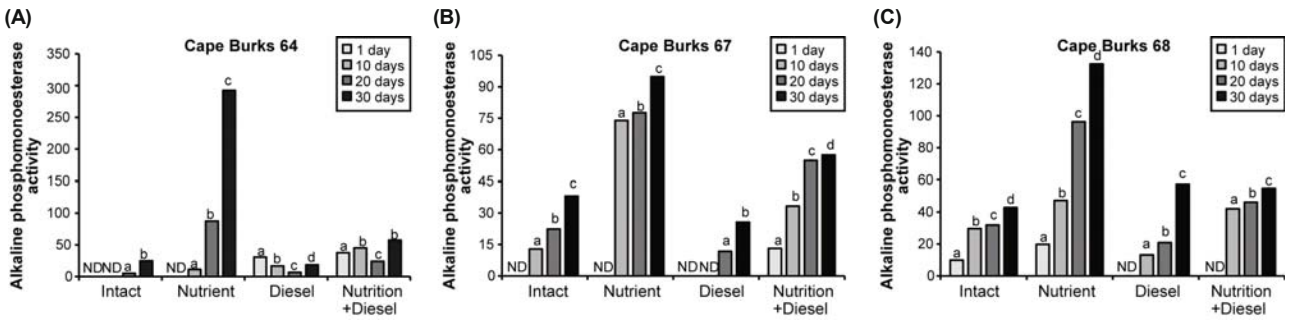

Fig. 3. Alkaline phosphomonoesterase activity in Cape Buks 64 (A), 67 (B), and 68 (C) soils. ND, Not detected. Average and standard deviation values were calculated from duplicates. Bars with different letters are significantly different ($P<0.05$).

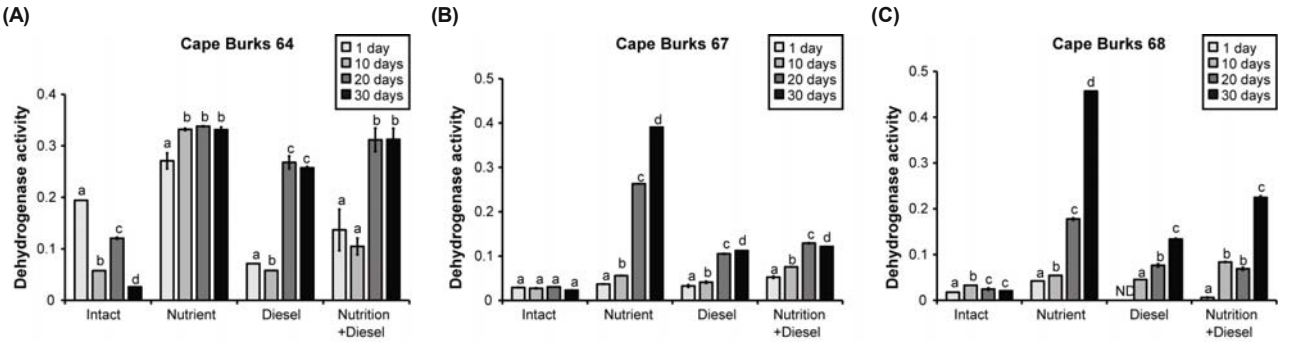

Fig. 4. Dehydrogenase activity in Cape Buks 64 (A), 67 (B), and 68(C) soils. ND, Not detected. Average and standard deviation values were calculated from duplicates. Bars with different letters are significantly different (P<0.05).

our short-term (7 days) control experiment (Fig. 2D). Dehydrogenase activities increased in soils, following nutritional input (Fig. 4). Longer incubation further increased enzyme activities in all 3 soils, whereas intact soil showed no significant variations. Dehydrogenase activity under nutritional input was 12.6- to 21.9-fold and 1.28- to 3.5-fold higher than that of intact and diesel-contaminated soils, respectively (Fig. 4). After 30 days of incubation, increased dehydrogenase activity was observed in both diesel-contaminated and diesel-plus-nutrition conditions, probably because of loss of diesel toxicity through volatilization and biodegradation. These data also suggested that the AP and dehydrogenase activities in Cape Burks soils subjected to those environmental changes were consistent with the activities observed in our previous control experiment with soils collected from Nelson Island.

Copy-number analysis of genes that encode AP and 16S rRNA gene

To understand microbial growth related to AP activity, the copy numbers of the *phoA* gene that encodes an AP and the 16S rRNA gene were analyzed using qPCR (Fig. 5). Soils from Cape Burks 64 showed 2.2–3.1-fold higher AP activity than the other soils under nutritional input after 30 days. Dehydrogenase activities were also significantly increased even on day 1, and upon diesel contamination, the dehydrogenase activity increased after 20 days. Therefore, Cape Burks 64 was chosen for further study of the copy number of the 16S rRNA gene and the microbial community. The copy number of the 16S rRNA gene increased after 30 days in Cape Burks 64 soils under all conditions, but more increases could be observed following nutritional input (Fig. 5A). This inconsistency in the data from the dehydrogenase

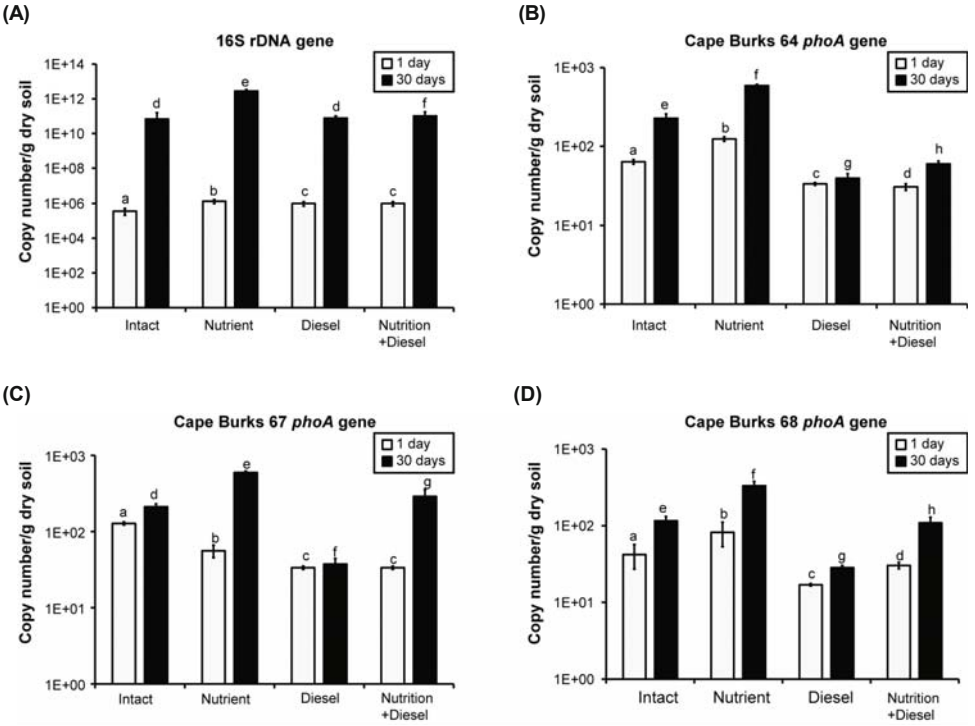

Fig. 5. Abundance of alkaline phosphatase (*phoA*) gene and 16S rRNA gene were verified using quantitative real-time PCR. The copy number of 16S rRNA gene in Cape Buks 64 (A) and that of the *phoA* gene in Cape Buks 64 (B), 67 (C), and 68 (D). Bars with different letters are significantly different (P<0.05).

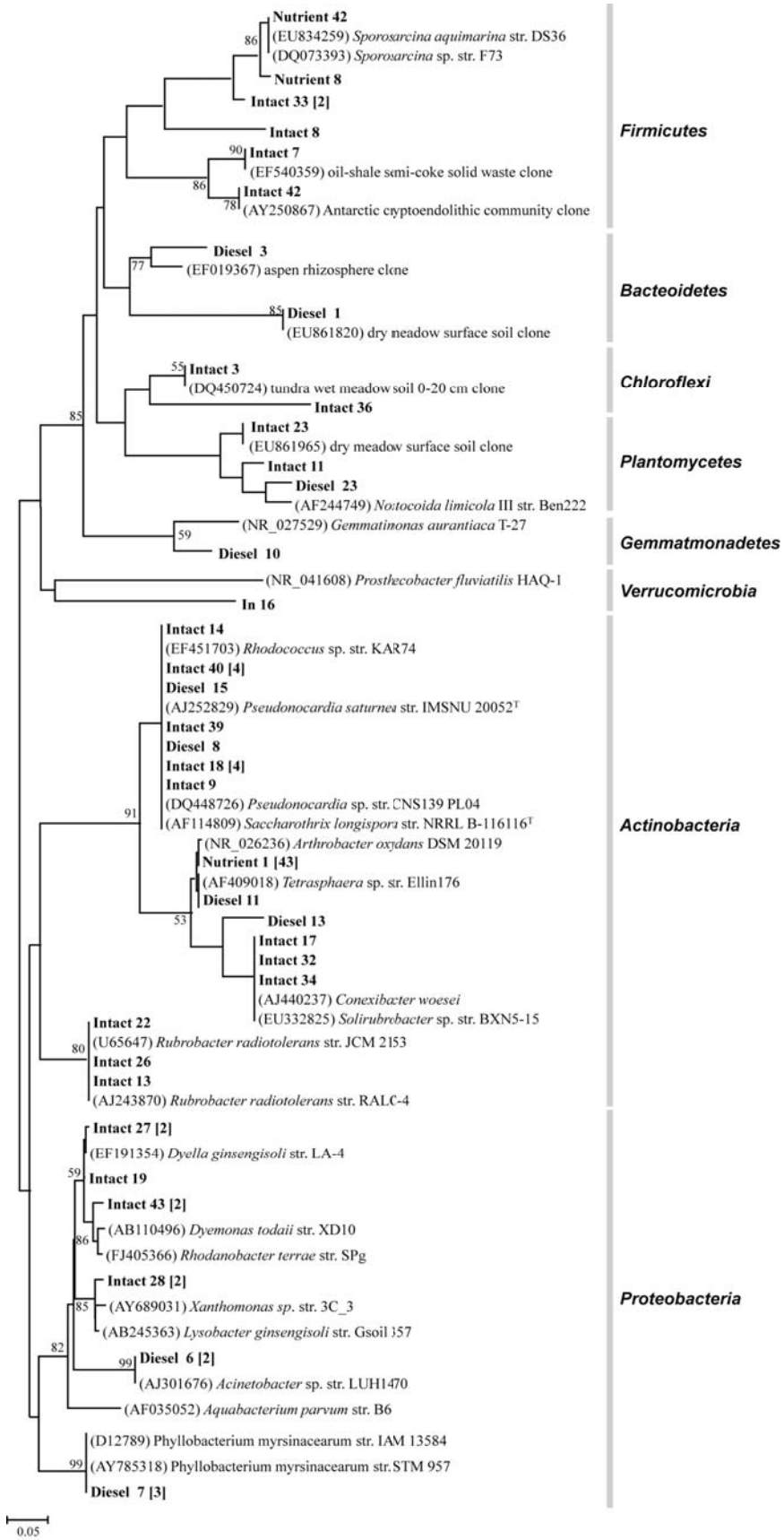

Fig. 6. Phylogenetic tree based on partial 16S rRNA gene sequences from clone libraries. Sequences derived from this study are in boldface. The number of identical RFLP patterns and GenBank accession nos. are in brackets and parentheses and brackets, respectively. Bootstrap values more than 70 are shown at the nodes. The scale bar indicates 0.02 substitutions per nucleotide position.

activity tests with regard to 16S rRNA gene copy number is likely attributable to 2 effects. First, a reduction in enzyme activity may be due to cell death. Second, different experimental temperatures (room temperature for enzyme assay and 4°C incubation for soil DNA extraction) may also influence bacterial growth and therefore copy number. The AP activities and copy numbers of the *phoA* gene were consistent with each other (Figs. 3, 5B–5D), and the copy number of *phoA* in the 3 soils increased between 4.7- and 9.3-fold under nutritional input soils; this was not be observed following 30 days of diesel treatment. Adding nutrition to the diesel-treated soil increased the copy number of the *phoA* gene, probably because of adaptation in the bacterial population (Fig. 5). Our qPCR data supported the enzyme assay data, revealing that nutrition input increased microbial activity and that diesel addition is toxic to the microbial community.

Phylogenetic analysis of the microbial community under different conditions

To determine whether there was a shift in the microbial community upon exposure to environmental changes, we examined the microbial community by making clone libraries with amplified PCR products by using a 16S rRNA gene primer set (see 'Materials and Methods'). Our data revealed that many known phyla (*Actinobacteria*, *Proteobacteria*, *Verrucomicrobia*, *Firmicutes*, *Chloroflexi*, *Planctomycetes*, *Bacteroidetes*, and *Gemmatimonadetes*) were detected in intact, nutritional input, and diesel-contaminated soils. A phylogenetic tree was prepared using the clone sequences and closely related sequences from the Greengene database (Fig. 6). Antarctic intact soils have a predominance of *Actinobacteria* (74.7%) phylum (composed mostly of *Pseudonorcarida* species), followed by other phyla such as *Proteobacteria* (29.5%) phylum (mostly *Gammaproteobacteria* genus), *Firmicutes* (11.4%), *Chloroflexi* (4.5%), *Planctomycetes* (4.5%), and *Verrucomicrobia* (2.3%) (Fig. 7). Nutrient addition might act as a selective pressure on these bacterial communities, resulting in a shift towards *Actinobacteria* (95.6%) phylum (composed mostly of *Arthrobacter* species), whereas *Firmicutes* (4.4%) was rarely isolated from the libraries. For diesel-contaminated soils, the predominance of *Proteobacteria* (37.5%) phylum (mostly *Alphaproteobacteria* and *Phyllobacterium* species) was observed, followed by other phyla such as *Actinobacteria* (16.7%), *Bacteroidetes* (8.3%), *Planctomycetes* (4.2%), and *Gemmatimonadetes* (4.2%) (Fig. 7).

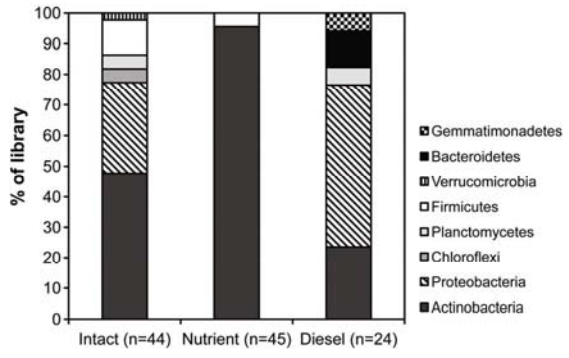

Fig. 7. Taxonomic composition of the bacterial community represented in the clone library sequences. The numbers of clones used in the analysis are within parentheses.

Discussion

Soil enzyme activities are important in terms of ecosystem functioning, and many articles have described relationships between these enzymes and the environmental factors that regulate them (Burns, 1982). Enzyme activities are useful indicators of soil quality because they are related to soil organic matter and microbial biomass and are sensitive and change more quickly than chemical or physical properties (Jordan *et al.*, 1995; Dick and Breakwell, 1996; Roscoe *et al.*, 2000). Many microorganisms utilize low molecular-weight sugars as energy sources (Donderski *et al.*, 1998), and β-glucosidase is the key enzyme in this metabolism. Vegetation is known to be an important factor in soil sugar metabolism where β-glucosidase is involved (De Varennes *et al.*, 2010). We found that lichens were the principal vegetation at the sampling sites. Only 2 plant species grow in the Antarctic regions (personal communication with KOPRI). Therefore, it was reasonable to suspect that sparse vegetation would result in lower β-glucosidase activities in the Antarctic soil. The insensitivity of β-glucosidase upon nutrition addition during the short-term experiment might be related to the composition of added nutrients (Fig. 2A). The yeast extract used in this study has a high amino acid and peptide content, but contains an insufficient amount of sugar (Kurosumi *et al.*, 2008). Urease activity was not detected in our study, probably because the Antarctic soil has only low concentrations of urea-like nitrogen as well as. Other studies have shown that urease activity was relatively lower (0.235 μg N/g) than other enzyme activities in the vegetation field (Hinojosa *et al.*, 2008). Increased phosphomonoesterase activity attributable to nutritional addition was observed in the Antarctic soils. The soil pH level might be an important factor that drives the response of the 2 different phosphomonoesterase activities. The high sensitivity of AP in the neutral-pH Antarctic soils is expected, since this enzyme is known to be very active in neutral pH soils (Dick *et al.*, 2000). Arylsulfatase is involved in the release of inorganic sulfate from organic ester sulfate into the soil and is also important for the S biogeochemical cycle. It has been suggested that approximately half of surface soil S is present in the form of ester sulfate (Freney, 1961; Tabatabai and Bremner, 1970; Tabatabai, 1982). Soil pH may be one of the most important determinants of arylsulfatase activity. The optimal pH for arylsulfatase activity has been shown to be pH 3 or less in tropical rain forest soils (Turner, 2010). Thus, neutral soil pH, low nutritional status, and low microorganism content in the Antarctic soil may explain its low arylsulfatase activity.

Dehydrogenase is a requirement for aerobic bacterial respiration; therefore, many studies of soil enzyme function have used dehydrogenase activity to measure overall soil microbial activity (Casida, 1964; Chendrayan, 1980). Dehydrogenase activity was sensitive to nutritional input and diesel contamination in the Antarctic soils. Our data support the notion that dehydrogenase activity is a good index for the meas-

urement of overall microbial activity in soils. Non-specific esterase, proteases, and lipases have all been shown to hydrolyze FDA (Adam and Duncan, 2001). Thus, the ability to hydrolyze FDA appears to be widespread, particularly among the major decomposers, bacteria and fungi (Schnürer and Rosswall, 1982). The FDA assay proved easy, useful, and convenient for the detection of microbial activity. However, it is not as sensitive as the dehydrogenase assay, largely because the FDA assay generally measures extracellular enzymes from both live and dead cells.

Antarctic areas are characterized by extreme climate conditions, with subzero temperatures almost throughout the entire year, low water availability, and low nutrient concentrations (Ganzert *et al*., 2011), and food webs are relatively simple, with a general absence of insect and mammalian herbivores (Davis, 1981; Heal and Block, 1987). Consequently, nutritional input likely leads to biostimulation of activated microorganisms, thereby increasing AP and dehydrogenase activities in Cape Burks soils. Most bacteria increase their synthesis of alkaline phosphatase when they are grown in P-limiting conditions; it is assumed that these extracellular enzymes release inorganic phosphate (Pi) from organic phosphates into the environment (Torriani, 1990; Rodríguez *et al*., 2008). Among the genes for bacterial alkaline phosphatases, the *phoA* gene has been extensively studied, particularly in relation to AP activity. The findings of our phylogenic study of the Antarctic soil is consistent with those of recent studies on the microbial community of Antarctic soil environments (Shivaji *et al*., 2004; Saul *et al*., 2005; Aislabie *et al*., 2006, 2008; Smith *et al*., 2006; Shravage *et al*., 2007), which indicated that majority of the phyla are associated with *Actinobacteria* and *Proteobacteria*. Other studies also showed that the phosphate enrichment significantly increased bacterioplankton growth, changed particulate organic stoichiometry, and induced shifts in bacterial community composition, including consistent declines in the relative abundance of *Actinobacteria* (Nelson and Carlson, 2011). Furthermore, diesel-contaminated soil shifted the microbial community towards *Gammaproteobacteria*, *Alphaproteobacteria*, and *Actinobacteria* dominance (Yergeau *et al*., 2012). Our data reveal that nutritional input and diesel contamination in Antarctic soils changed various microbial enzyme activities and the composition of the microbial community; this may in turn perturb natural biogeochemical cycling in the Antarctic area.

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