

# Soil microbial carbon and nitrogen transformations at a glacial foreland on Anvers Island, Antarctic Peninsula

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**Abstract** Microbial communities can play a critical role in soil development and succession at glacial forelands through their contribution to soil carbon (C) and nitrogen (N) cycling. Using a combination of molecular fingerprinting techniques and metabolic rate measurements, we examined the soil microbial community composition and key transformations in the C and N cycles at a glacial foreland on Anvers Island along the Antarctic Peninsula. Soils were sampled along transects representing a chronosequence of <1 to approximately 10 years since deglaciation. The soil microbial community was active adjacent to the receding edge of the glacier, where soil had been ice-free for <1 year. A survey of the microbial community composition identified typical soil bacterial species such as *Arthrobacter* and *Sphingomonas*, as well as known Antarctic heterotrophs, cyanobacteria and fungi. The soil C cycle over this zone was dominated by phototrophic microbial activity, while the N cycle was dominated by heterotrophic N<sub>2</sub>-fixation and not cyanobacterial N<sub>2</sub>-fixation as found at other recently deglaciated forelands. Other N transformations such as ammonia oxidation and denitrification appeared to be of limited relevance.

**Keywords** Glacial foreland · Soil development · Nitrogen cycle · Nitrogen fixation · Succession

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## Introduction

Some of the classic studies examining soil and plant community development have been conducted on glacial forelands, the newly exposed terrain left by receding glaciers (Cooper 1923, 1937; Crocker and Major 1955; Chapin et al. 1994), where distance from the glacier can serve as a proxy for time since deglaciation (i.e., a chronosequence; Jenny 1941, 1980). Studies of soil and plant community development at glacial forelands document a general pattern of increasing soil carbon (C), nitrogen (N), organic matter, and vascular plant cover over time spans of centuries since deglaciation (Matthews 1992). Microbes may play a critical role in soil development at these glacial forelands. For example, rates of microbial C (Freeman et al. 2009) and N<sub>2</sub>-fixation (Hodkinson et al. 2003; Sorensen et al. 2006) at alpine glacial forelands can be high enough to contribute significantly to soil C and N pools. In addition, microbes (e.g., cyanobacteria) can stabilize soils by producing extracellular polymers (de Caire et al. 1997), reducing the potential of erosion (Vincent 1988; Matthews 1992; Wynn-Williams 1996).

The soil microbial communities immediately following deglaciation can be quite active and also change rapidly through time. For example, at alpine glacial forelands, proteobacterial abundance (Nemergut et al. 2007; Duc et al. 2009) can decrease while cyanobacterial (Sattin et al. 2009) and Archaeal abundance (Nicol et al. 2005) can increase within the first 20 years after deglaciation. The contribution of soil microbes to element cycling in glacial forelands is less clear. At the Mendenhall Glacier, Alaska, N<sub>2</sub>-fixation increased over 8 years following deglaciation (Sattin et al. 2009). However, there was little or no measurable N<sub>2</sub>-fixation in soils that were deglaciated for approximately the same time at the Damma Glacier,

Switzerland (Duc et al. 2009; Brankatschk et al. 2010). Both denitrification (Deiglmayr et al. 2006; Brankatschk et al. 2010) and nitrification (Brankatschk et al. 2010) rates were low in soils deglaciated for longer than 25 years at the same glacial foreland. Even less is known about the contribution of microbes to soil C cycling. Cyanobacteria are well documented at glacial forelands (Stibal et al. 2006; Nemergut et al. 2007; Schmidt et al. 2008; Sattin et al. 2009) along with relatively low rates of heterotrophic respiration (Ohtonen et al. 1999; Kastovska et al. 2005), but studies examining soil C and N turnover concurrently at a single glacial foreland are lacking.

Global temperatures have increased since the mid-twentieth century, and the majority of glaciers, on a global basis, are receding (IPCC 2007). One area where this is particularly apparent is along the west coast of the Antarctic Peninsula (Vaughan and Doake 1996; Cook et al. 2005), where 87 % of the glaciers have receded, and where retreat rates have been accelerating since the 1940s (Cook et al. 2005). While the soil microbial community composition has been characterized at some sites on the Antarctic Peninsula (Yergeau et al. 2007; Chong et al. 2010; Bölter 2011), few studies have examined both soil microbial C and N cycling (Bölter 2011).

In this study, we examined microbial C and N cycling along transects at a recently deglaciated foreland on an island along the Antarctic Peninsula. Our experimental transects extended 20 m from the edge of the glacier, representing a chronosequence of approximately 10 years. We have previously reported on patterns of soil parameters, including total C, N, nitrate ( $\text{NO}_3^-$ ), ammonium ( $\text{NH}_4^+$ ), and moisture, along these transects (Strauss et al. 2009). Here, we extend our investigation to the microbial community composition and key biogeochemical transformations in C and N. By combining functional gene analyses with metabolic rate measurements, we provide an assessment of which microbial functional groups were responsible for dominant C and N transformations.

## Materials and methods

### Field sites

Our study site was on Anvers Island off the west coast of the Antarctic Peninsula, 2.5 km east of Palmer Station (64°46'S, 64°04'W). The climate is maritime Antarctic (Smith 1996) with a mean annual air temperature of  $-1.7^\circ\text{C}$  and monthly means ranging from  $-6.6^\circ\text{C}$  in September to  $2.4^\circ\text{C}$  in January, based on Palmer Station records from 1989 to 2002. Annual precipitation (melted) over this period averaged 75 cm. The site was on a gently (5 %) sloping terrace at an unnamed point referred to by

Palmer Station personnel as “Point 8.” The Marr Ice Piedmont that covers much of Anvers Island extends to this terrace. Based on satellite and aerial images and observations by Palmer Station personnel, this terrace appears to have first become ice-free in the mid-1980s and we estimate that the glacier at Point 8 has retreated an average of  $2\text{ m year}^{-1}$  since this time, based on total distance melted divided by time (years). Although there were no vascular plants on this terrace in January 1995, about 90 individuals of *Deschampsia antarctica* (a prostrate perennial tussock grass) were present in January 1999 (Day and Ruhland pers obs). During the 2005–2006 field season (November–March), when our first set of samples was collected, the terrace extended 50 m from the glacier, where it ended at the edge of a 15-m high ledge that dropped abruptly to the ocean. At that time, there were several thousand *D. antarctica* plants concentrated 35–50 m from the glacier. The area within 20 m of the glacier was dominated by plant-free mineral soil or rock, with only occasional *D. antarctica* plants (<1 % total foliar cover). Seabird colonies or nests were not present. Soil along the transects we report on consisted of a mineral horizon of glacial till 3–8 cm deep of sandy loam texture having a pH of 8.2–8.8 overlying granitic parent material (Strauss et al. 2009). Total C and N concentrations and C:N ratios were low, ranging from 0.05–0.15 %, 0.009–0.024 %, and 6.1–7.2, respectively, as were inorganic N concentrations (12.9–56.4 mg g soil $^{-1}$ ), and values of these parameters did not significantly change with distance from the glacier along transects (Strauss et al. 2009). For more details on study site characteristics and location, please see Strauss et al. (2009).

### Sampling approach

Samples were collected along 5 parallel linear transects that ran from the glacier's edge toward the shoreline during two field seasons: 2005–2006 and 2007–2008. Our rationale for sampling along these transects was that distance from the glacier would represent a proxy for time since deglaciation, and points along these transects would generally represent a young chronosequence in terms of soil development (Jenny 1941, 1980).

During the 2005–2006 field season, soil samples were collected approximately every 5 m from 5 transects extending 20 m from the glacier's edge. Sampling was on 21 December 2005, approximately 20 days after snowmelt. All samples were collected from soils that did not contain rooted vascular plants within at least 1 m in all directions, with a microsite slope of less than  $5^\circ$  and a mineral soil (i.e., glacial drift) depth of 5–8 cm. No plant roots were present in the samples. Each sample contained the upper 2 cm of soil and had a ground surface area of  $2.5\text{ cm}^2$ . These samples were air-dried at  $20^\circ\text{C}$  for 5 days before

transport to Arizona State University. An additional set of samples was collected on 15 February 2006 and used to determine substrate-induced respiration (SIR) measurements in the laboratory at Palmer Station.

During the 2007–2008 field season, samples were collected on 18 February 2008, 80 days after snowmelt in the same manner from a new set of 5 transects and transported to Palmer Station, where they were frozen ( $-80^{\circ}\text{C}$ ) and shipped to ASU. Upon arrival, they were divided into sub-samples for use as described below.

Logistic constraints prevented us from measuring all parameters in both field seasons, and we did not have the capabilities to conduct molecular or additional metabolic analyses on station. Soil microbial DNA content, as well as bacterial and cyanobacterial community composition, was assessed for both seasons. However, SIR rates and the fungal community composition were determined only on 2005–2006 samples. All other rates (net photosynthesis, dark respiration,  $\text{N}_2$ -fixation, aerobic ammonium oxidation, denitrification, and anaerobic ammonium oxidation), along with the soil chlorophyll *a* content, were assessed only on 2007–2008 samples.

#### Acetylene reduction assay for potential $\text{N}_2$ -fixation rates

Potential  $\text{N}_2$ -fixation rates were measured on  $32\text{ cm}^3$  of soil following Belnap (2002) as modified by Johnson et al. (2005) to avoid diffusion limitation. Soil from the previously frozen sub-samples was placed in clear plastic tubes that were closed with rubber stoppers at both ends and preconditioned by leaving the samples wet and in the light (at an intensity of  $350\text{ }\mu\text{mol m}^{-2}\text{ s}^{-1}$  PAR) at  $12^{\circ}\text{C}$  for 2 h before incubation. Soil samples were then incubated with added acetylene in the light for 8 h at  $12^{\circ}\text{C}$ . Headspace gas samples were taken after 4 h for ethylene evolution analyses. Ethylene was measured using a gas chromatograph (Hewlett-Packard 5890 Series II) equipped with a thermal ionization detector and a  $6' \times 1/8''$  SS Porapak N 80/100 column (Ohio Valley Specialty Chemical). The nitrogenase enzyme may not catalyze the reduction of acetylene to ethylene as efficiently as it does  $\text{N}_2$ . Conversion factors are used to correct for this (Hardy et al. 1973) and range from 1 to 3 in soils (Nohrstedt 1983), although it is unclear what factor would be the most appropriate in many cases (Belnap 2002). We used a conversion factor of 1. Areal rates of  $\text{N}_2$ -fixation were obtained by dividing total production by the original surface area of the sample and incubation time.

#### Ammonium oxidation rates

Unamended aerobic ammonium oxidation (AAO) rates were measured on  $32\text{ cm}^3$  of soil following Johnson et al.

(2005). Frozen soil sub-samples were placed into double chamber Petri dishes previously designed and described by Johnson et al. (2005), which preserve the community architecture and were allowed to thaw at  $12^{\circ}\text{C}$  for 6 h. The samples were then incubated under approximately  $350\text{ }\mu\text{mol m}^{-2}\text{ s}^{-1}$  PAR with 20 ml of 75 mM sodium chlorate ( $\text{NaClO}_3$ ) for 24 h at  $12^{\circ}\text{C}$ . Aliquots of the pore water were collected at 0 and 24 h, mixed with 500 ml potassium chloride (KCl), and frozen until analysis. Once thawed, samples were centrifuged at  $13,000g$  for 10 min to pellet debris. The supernatant was incubated at  $20^{\circ}\text{C}$  for 15 min with 60 mM ammonium chloride ( $\text{NH}_4\text{Cl}$ ; pH 8.5) and the color reagent *n*-(1-naphthyl)-ethylenediamine dihydrochloride for nitrite ( $\text{NO}_2^-$ ) determination. The absorption at 520 nm was compared to a set of standards. Areal rates were obtained by dividing total production by the original surface area of the sample.

Anaerobic ammonium oxidation (anammox) rates were measured using a series of tracer incubations to determine whether  $\text{N}_2$  was being produced by anammox or denitrification (Thamdrup and Dalsgaard 2002). Anammox organisms couple  $\text{NO}_3^-$  reduction to ammonia oxidation, therefore  $\text{N}_2$  is formed with one N from  $\text{NO}_3^-$  and one from  $\text{NH}_4^+$  (van de Graaf et al. 1995). This allows the use of  $^{15}\text{N}$  tracer incubations to distinguish the  $\text{N}_2$  produced by anammox from that produced by denitrification. Three different combinations of labeled sodium nitrate ( $\text{NaNO}_3^-$ ) and  $\text{NH}_4\text{Cl}$  were used to test whether anammox and/or denitrification was occurring: (a)  $\text{Na}^{15}\text{NO}_3^- + ^{14}\text{NH}_4\text{Cl}$ , which would result in  $^{29}\text{N}_2$  through anammox or  $^{30}\text{N}_2$  through denitrification, (b)  $\text{Na}^{14}\text{NO}_3^- + ^{15}\text{NH}_4\text{Cl}$ , which would result in  $^{29}\text{N}_2$  through anammox and  $^{28}\text{N}_2$  through denitrification, and (c)  $\text{Na}^{15}\text{NO}_3^- + ^{15}\text{NH}_4\text{Cl}$ , which would result in doubly labeled  $\text{N}_2$  for both anammox and denitrification.

Soil samples of  $1.46\text{ cm}^3$  were homogenized and divided into three 12-ml Labco Exetainer (Labco Limited) vials. Vials were purged with helium (He) for 10 min. Solutions in combinations of labeled and unlabeled 100 mM  $\text{NaNO}_3^-$  and  $\text{NH}_4\text{Cl}$  were flushed with He before being injected into the vials (2  $\mu\text{l}$  of each solution; natural abundance or  $^{15}\text{NH}_4\text{Cl}$  (98 atm %),  $\text{Na}^{15}\text{NO}_3^-$  (98 atm %; Isotec)). Headspace  $^{29}\text{N}_2$  and  $^{30}\text{N}_2$  concentrations were determined every 12 h using a mass spectrometer (Thermo Finnigan Delta V Advantage) equipped with a gas bench and an autosampler (CTC Combi PAL). Using a  $^{29}\text{N}_2$  and  $^{30}\text{N}_2$  calibration curve, the amount of  $^{29}\text{N}_2$  and  $^{30}\text{N}_2$  produced in excess of natural abundance was determined. Areal rates were obtained by dividing total production by the original surface area of the sample. A sample collected from the anoxic portion of a Winogradsky column served as a positive control and had an unamended denitrification rate of  $0.2\text{ }\mu\text{mol m}^{-2}\text{ h}^{-1}$  and an anammox rate of  $0.10\text{ }\mu\text{mol m}^{-2}\text{ h}^{-1}$ .

## Acetylene-inhibition assay for potential denitrification rates

Potential denitrification rates were assessed following Groffmann and Tiedje (1989) on 32 cm<sup>3</sup> of soil placed in 125-ml glass bottles with 50 ml of sterile denitrification medium (per liter: 100 mg of potassium nitrate and 100 mg of dextrose). Each bottle was purged with N<sub>2</sub> gas to attain anoxia, injected with 10 ml of acetylene through a septum, and incubated in the dark at 12 °C. Gas samples were drawn at 10 min and 4 h after addition of acetylene using 2 ml purged and capped scintillation vials and double-ended needles. Nitrous oxide (N<sub>2</sub>O) concentration was quantified using a gas chromatograph (Shimadzu GC-14A) equipped with an electron capture detector and a 6' × 1/8" SS Poropak Q 50/80 (Ohio Valley Specialty Chemical). The positive control was a sediment sample from a retention basin from the Arizona State University campus with a denitrification rate of 7.21 μmol N m<sup>-2</sup> h<sup>-1</sup>. Areal rates were obtained by dividing total production by the original surface area of the sample.

## Net photosynthesis and dark respiration measurements

Rates of net photosynthesis and dark respiration of intact soil samples were measured using an open IRGA system (Li-COR Li-6400) equipped with a gas chamber made of transparent acrylic tubing (inner diameter, 10.2 cm; height, 12 cm) lined inside with Teflon tape. Frozen samples (19.5 × 16 × 5 cm) were thawed and placed in incubation chambers having a diel cycle of 14 h in the light (350 μmol m<sup>-2</sup> s<sup>-1</sup> PAR) at 12 °C and 10 h in the dark at 10 °C. Samples received deionized water every 3 days to keep them moist. After 4 weeks in the chambers, a stainless-steel ring (inner diameter, 10.4 cm; height, 2 cm) was pushed 1 cm into the soil sample immediately prior to measurements, thus delimiting a sealed area for measurement. During measurements, the chamber received air with a carbon dioxide (CO<sub>2</sub>) concentration of 370 μl l<sup>-1</sup>, supplied by a CO<sub>2</sub> injector system (Li-COR 6400-01), at a flow rate of 250 μmol s<sup>-1</sup>. Relative humidity was maintained at 80 %. Preliminary determinations found that 5 min was sufficient to reach steady state CO<sub>2</sub> concentrations after sealing, and 3 min sufficient to reach steady state after subsequent darkening. Based on this, we estimated net photosynthetic rates by averaging the CO<sub>2</sub> flux (μmol m<sup>-2</sup> s<sup>-1</sup>), measured every second, over a 5-min period after the sample had been in the light for 5 min. Dark respiration rates then were estimated over a 2-min period after samples had been in the dark for 3 min. Net photosynthetic rates were expressed as a net uptake of CO<sub>2</sub> by the soil and are presented as positive fluxes, while dark respiration rates

were expressed as a net release of CO<sub>2</sub> from the soil and are also presented as positive fluxes. Gross photosynthetic rates were calculated as the sum of dark respiration and net photosynthetic rates. Rates are expressed on an areal or surface area basis.

## Substrate-induced respiration measurements

Substrate-induced respiration rates were measured to provide an estimate of potential heterotrophic respiration rates, following Dilly (2001). Within 4 h of collection, 20 g (field wt) of homogenized soil from each sample was placed in acid-washed glass Petri dishes, covered with plastic wrap, and incubated in the dark at 20 °C. Approximately 1 ml of deionized water was added every 2 days. After 5 days, soil dark respiration rates (CO<sub>2</sub> flux) were measured using the IRGA system. A glucose solution (5 mg glucose g<sup>-1</sup> dry soil) was then added to the sample, and dark respiration was re-measured after 30 min. All measurements were made in the dark at 20 °C.

## Chlorophyll a concentrations

Chlorophyll *a* was extracted from 32 cm<sup>3</sup> of soil in the dark for 24 h at 4 °C using a 1:10 volume ratio of soil: 90 % acetone. Chlorophyll *a* concentrations were estimated with a fluorometer (Turner Designs TD-700) previously calibrated with a primary standard (Sigma) in 90 % acetone at 685 nm. Areal concentrations were obtained by dividing total concentration by the original surface area of the sample.

## DNA extraction

DNA was extracted from approximately 10 g of soil (field weight) with the commercial UltraClean Mega Soil DNA Isolation Kit (MoBio Laboratories). Three additional freeze–thaw cycles using liquid N<sub>2</sub> (1 min each) and a 65 °C water bath (15 min each) were performed before isolating the DNA according to the manufacturer's protocol. DNA was assessed for quality by standard agarose gel electrophoresis followed by ethidium bromide staining. DNA concentrations in the extracts were quantified from the agarose gels using a Fluor-S Multi-Imager system (BioRad Laboratories) with an EZ Load Precision Molecular Mass Standard (BioRad Laboratories).

DNA was extracted from each sampling point along the 5 transects in both field seasons. However, 2005–2006 samples collected at a given distance from the glacier were pooled, providing a single consolidated extract for analysis at each distance. Samples in the 5 transects from 2007 to 2008 were analyzed individually providing 5 replicates for each distance.



### PCR amplification of 16S rRNA gene

For the bacterial community fingerprint, universal eubacterial primers GM5f(GC) and 907r (Muyzer et al. 1995) were used to amplify approximately 590-bp-long 16S rRNA gene fragments from the community DNA. Each 50- $\mu$ l PCR mix contained 5  $\mu$ l Takara ExTaq PCR buffer, 4  $\mu$ l Takara dNTP mixture (2.5 mM each), 50 pmol of each primer (synthesized by Operon Biotechnologies), 5–10 ng template DNA, 1 unit Takara ExTaq DNA polymerase (Takara Bio), and autoclaved sterile Milli-Q water. PCR was modified from the procedure described by Garcia-Pichel et al. (2003). All reactions used a thermocycler (BioRad iCycler).

For the cyanobacterial community fingerprint, universal cyanobacterial primers CYA106f (GC) and CYA781r (Nübel et al. 1999) were used for amplification of approximately 600-bp-long 16S rRNA gene fragments from the community DNA. Each 50- $\mu$ l PCR mix contained the same mixture used for the universal eubacterial primers. PCR was performed according to the methods described by Garcia-Pichel et al. (2002).

For the fungal community fingerprint, fungi-specific primers NL1f (GC) (O'Donnell 1993) and LS2r (Cocolin et al. 2001) were used for amplification of approximately 250-bp-long LSU rRNA gene fragments from the community DNA, and PCR was performed according to the program described by Bates and Garcia-Pichel (2009).

Separate bacterial and cyanobacterial community fingerprints were generated using community DNA from each field season, but only one fungal community fingerprint was made using the 2005–2006 community DNA. All primers were obtained commercially (Operon Biotechnologies). All PCR products were checked for quality on 1 % agarose gels. Quantification of all PCR products was performed as described above for DNA quantification.

### Denaturing gradient gel electrophoresis

Each heterogeneous mixture of PCR-amplified 16S rRNA gene alleles was separated by denaturing gradient gel electrophoresis (DGGE) using a 30–60 % chemical gradient of denaturants (urea and formamide) on a 6 % polyacrylamide gel. Gels were run according to Gundlapally and Garcia-Pichel (2006). For the 2005–2006 samples, one DGGE was run for each of the bacterial, cyanobacterial, and fungal PCR amplifications. For the 2007–2008 samples, PCR amplifications for two transects were loaded per gel, and bacterial and cyanobacterial PCR amplifications were run separately. Approximately 200 ng of PCR product was loaded in each lane. Gels were electrophoresed for 6 h at 150 V in a D-code Universal Mutation Detection System (BioRad Laboratories). Standard lanes, containing

known mixtures of 3 16S rRNA alleles from cultivated isolates, were run to ensure correct gel properties. The gels were stained for 30 min with 3  $\mu$ l of SYBR Gold Nucleic Acid Gel Stain (Invitrogen) in 15 ml of TAE buffer and de-stained in TAE buffer for 5 min. The gels were visualized under a Fluor-S Multi-Imager. The visible bands were excised with sterile scalpels and allowed to elute for 3 days at 4 °C in 10 mM Tris buffer.

### Sequencing and phylogenetic analysis

The eluted DGGE bands were amplified using the unclamped versions of the original primers. PCR products were purified using the QIAquick PCR Purification Kit (Qiagen) and sequenced commercially using both forward and reverse primers. Similarity searches were performed using the BLASTN (Altschul et al. 1998) function available from GenBank (<http://www.ncbi.nlm.nih.gov>). The sequences found from DGGE analysis included in this study have been deposited with GenBank (Table 1). The 2005–2006 DGGE images are available in Online Resources 1, 2, and 3.

### Quantification and analyses of DGGE fingerprints

The DGGE fingerprints were analyzed according to the methods described by Gundlapally and Garcia-Pichel (2006) using Quantity One software (BioRad Laboratories). Phylotype richness was calculated based on the number of bands per lane, and the optical density of each band was used to calculate the relative abundance of each band. This information was used to calculate the Shannon–Weaver diversity indices ( $H'$ ; Nübel et al. 1999).

### NifH gene clone library

To determine which microorganisms had the potential to fix  $N_2$ , we constructed a clone library of the *nifH* gene. The *nifH* gene was amplified by PCR from a pool of all sample points of both the 2005–2006 and 2007–2008 community DNA using the Polf and Polr primers (Poly et al. 2001). Each 50- $\mu$ l PCR mix contained 5  $\mu$ l Takara ExTaq PCR buffer, 4  $\mu$ l Takara dNTP mixture (2.5 mM each), 50 pmol of each primer (synthesized by Operon Biotechnologies), 5–10 ng template DNA, 1 unit Takara ExTaq DNA polymerase (Takara Bio, Madison), and sterile Milli-Q water. PCR followed Poly et al. (2001), and products were purified using the QIAquick PCR Purification Kit (Qiagen).

An aliquot of each PCR product was ligated into the prepared vector (pCR 2.1) supplied with a TA cloning kit (Invitrogen) following the manufacturer's recommendations. Putative insert-bearing *E. coli* colonies were picked and

**Table 1** Unique sequences obtained in this study with GenBank accession number, nearest BLAST match in GenBank and associated similarity, and the phylum for the closest relative

Accession number	Band ID	Closest relative	Similarity (%)	Phylum
HQ020422	0D (2006)	<i>Arthrobacter</i>	98	Actinobacteria
HQ020423	10A1 (2006)	<i>Spirosoma</i>	94	Bacteroidetes
HQ020424	10B4 (2006)	<i>Pseudanabaena</i>	95	Cyanobacteria
HQ020425	10C1 (2006)	<i>Nannocystis</i>	91	Proteobacteria
HQ020426	10C3 (2006)	<i>Deinococcus</i>	96	Deinococcus-Thermus
HQ020427	10D2 (2006)	<i>Phormidium</i>	94	Cyanobacteria
HQ020428	10E1 (2006)	<i>Herbaspirillum</i>	90	Proteobacteria
HQ020429	20A (2006)	<i>Sphingobacteria</i>	99	Bacteroidetes
HQ020430	20B (2006)	<i>Phormidium</i>	99	Cyanobacteria
HQ020433	Fungi 1B (2006)	<i>Geomyces</i>	96	Ascomycota
HQ020434	Fungi 1C (2006)	<i>Geomyces</i>	93	Ascomycota
HQ020435	Fungi 3B (2006)	<i>Geomyces</i>	97	Ascomycota
HQ020436	Fungi 3C (2006)	<i>Geomyces</i>	91	Ascomycota
HQ020437	Cya 1F (2008)	<i>Microcoleus antarcticus</i>	92	Cyanobacteria
HQ020438	Cya 1G (2008)	<i>Microcoleus antarcticus</i>	96	Cyanobacteria
HQ020439	Cya 2E (2008)	<i>Phormidium autumnale</i>	97	Cyanobacteria
HQ020440	Cya 2F (2008)	<i>Phormidium autumnale</i>	98	Cyanobacteria
HQ020441	Cya 2I (2008)	<i>Phormidium autumnale</i>	96	Cyanobacteria
HQ020442	Cya 4D (2008)	<i>Oscillatoria</i>	94	Cyanobacteria
HQ020443	Cya 5A (2008)	<i>Phormidium autumnale</i>	99	Cyanobacteria
HQ020444	Cya 8B (2008)	<i>Oscillatoria</i>	93	Cyanobacteria
HQ020445	Cya 8C (2008)	<i>Phormidium autumnale</i>	99	Cyanobacteria

transferred to a multiwell plate containing Lauria–Bertani medium and 7 % glycerol. Colonies were sequenced as described for sequencing DGGE bands.

Similarity searches were performed using the BLASTN (Altschul et al. 1998) function available from GenBank (<http://www.ncbi.nlm.nih.gov>). Consensus sequence data were submitted to GenBank (Fig. 4 for accession numbers). Publicly available sequences of high similarity with our unknowns were retrieved from GenBank to provide robust phylogenetic context. All sequences were aligned using the CLUSTAL W module in the MEGA 4 software package (Tamura et al. 2007). A phylogenetic tree was constructed using the maximum parsimony algorithm. Clade stability in the phylogenetic tree was assessed using bootstrap analysis with 1,000 replicate data sets. *Nostoc* sp. PCC7120 was used as an out-group to root the phylogenetic tree.

#### Statistics

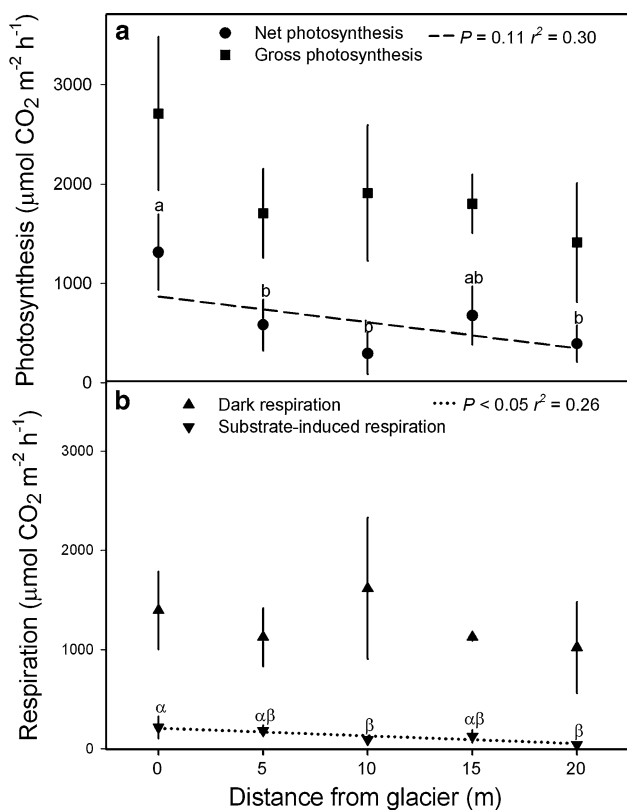
The effect of distance from the glacier on soil parameters was examined using 2-way ANOVA (distance and transect effects). There were no significant transect effects for any parameter. When significant distance effects were found, Tukey's HSD test was used to compare individual means along transects, along with linear regressions to assess the

strength of correlation with distance. Comparisons between net photosynthetic and dark respiration rates were made using a paired *t* test. Unless otherwise stated, differences were considered significant at the  $P < 0.05$  level. All data sets gave Ryan-Joiner test normality values of  $>0.97$ . Analyses were done using the Minitab statistical package (Minitab 15 Statistical Software 2006).

## Results

### Biogeochemical transformation rates

Net photosynthetic rates ranged from 73 to 1,693  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ h}^{-1}$ , gross photosynthetic rates ranged from 554 to 3,817  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ h}^{-1}$ , and dark respiration rates ranged from 475 to 2,388  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ h}^{-1}$  (Fig. 1). Net photosynthetic rates at 0 m were significantly greater than those at 5, 10, and 20 m ( $P < 0.05$ ; Fig. 1a), although there was no significant trend in net photosynthetic rates with distance from the glacier ( $P = 0.11$ ,  $r^2 = 0.15$ ; Fig. 1a). Gross photosynthetic (Fig. 1a) and dark respiration (Fig. 1b) rates also did not change with distance from the glacier. Net photosynthetic and dark respiration rates were similar at a given distance from the glacier. Neither net nor gross photosynthetic rates were correlated with chlorophyll

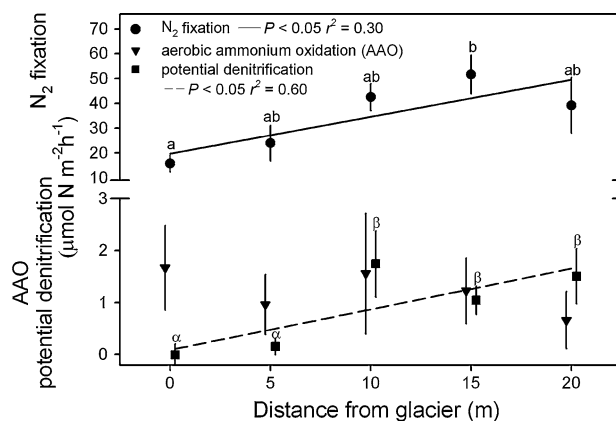


**Fig. 1** a Net photosynthetic (circle), gross photosynthetic (square), b dark respiration (triangle) and substrate-induced respiration (SIR; inverted triangle) rates as a function of distance from the glacier. Values are mean  $\pm$  SE ( $n = 5$ ). Means with different letters are significantly different at  $P < 0.05$ . Positive photosynthetic rates refer to a net uptake of  $\text{CO}_2$  by samples. Positive respiration rates refer to a net release of  $\text{CO}_2$  from samples

$a$  concentrations ( $P > 0.40$ ). Neither net, nor gross photosynthetic, nor dark respiration rates were correlated with total microbial DNA ( $P > 0.47$ ).

Substrate-induced respiration (SIR) rates, a measure of potential heterotrophic respiration, ranged from 10 to  $362 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ h}^{-1}$ . SIR rates were eightfold less than dark respiration rates and were negatively correlated with distance from the glacier ( $P < 0.05$ ,  $r^2 = 0.26$ ; Fig. 1b), with rates at 0 m greater than those at 10 and 20 m ( $P < 0.05$ ).

$\text{N}_2$ -fixation rates ranged from 12.32 to  $59.32 \mu\text{mol N m}^{-2} \text{ h}^{-1}$  and were positively correlated with distance from the glacier ( $P < 0.05$ ,  $r^2 = 0.30$ ; Fig. 2), with rates at 15 m greater than those at 0 m ( $P < 0.05$ ).  $\text{N}_2$ -fixation rates were tenfold greater than those of aerobic ammonia oxidation (AAO), denitrification, or anaerobic ammonia oxidation (anammox). AAO rates ranged from 0.11 to  $2.47 \mu\text{mol N m}^{-2} \text{ h}^{-1}$  and did not vary significantly with distance from the glacier (Fig. 2). Heterotrophic denitrification rates, assessed with the acetylene-inhibition method,



**Fig. 2**  $\text{N}_2$ -fixation (circle), aerobic ammonia oxidation (AAO) (inverted triangle) and potential (amended) denitrification (square) rates as a function of distance from the glacier. Values are mean  $\pm$  SE ( $n = 5$ ). Means with different letters are significantly different at  $P < 0.05$

ranged from 0.15 to  $2.38 \mu\text{mol N m}^{-2} \text{ h}^{-1}$  and were positively correlated with distance from the glacier ( $P < 0.05$ ,  $r^2 = 0.60$ ; Fig. 2), with rates at 10–20 m greater than those at 0 and 5 m ( $P < 0.05$ ). Heterotrophic denitrification and anammox rates, assessed using tracers, were all below our detection limit of  $0.005 \mu\text{mol N m}^{-2} \text{ h}^{-1}$  (data not shown). We found no correlations between chlorophyll  $a$  concentrations and  $\text{N}_2$ -fixation rates,  $\text{N}_2$ -fixation, and AAO rates, or between  $\text{N}_2$ -fixation and total soil DNA concentrations.

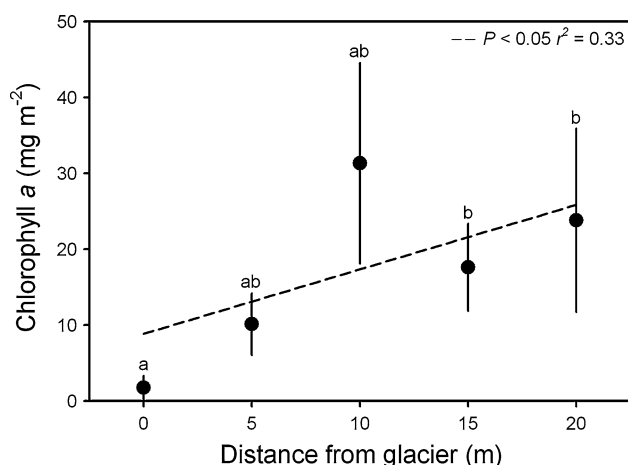
### Soil microbial parameters

Chlorophyll  $a$  was detectable in all samples collected during the 2007–2008 field season, indicating the presence of phototrophs at all distances from the glacier. Concentrations were positively correlated with distance from the glacier ( $P < 0.05$ ,  $r^2 = 0.33$ ; Fig. 3), with concentrations at 10 and 20 m greater than those at 0 m ( $P < 0.05$ ).

Total microbial DNA was extracted and quantified from samples collected during both field seasons, and concentrations were between 0.4 and  $1.4 \text{ ng g}^{-1}$ . Total microbial DNA concentrations did not vary with distance from the glacier in either field season (data not shown).

Bacterial, cyanobacterial, and fungal richness assessed with PCR-DGGE ranged from 15–25, 10–15, and 5–8 phylotypes, respectively. Bacterial, cyanobacterial, and fungal diversity, measured using the Shannon–Weaver diversity indices, ranged from 2.4–3.0, 2.0–2.5, and 1.4–2.0, respectively. Richness and diversity for bacteria, cyanobacteria, and fungi did not vary with distance from the glacier in either field season (data not shown).

Putative microbial  $\text{N}_2$ -fixers were identified using clone libraries of the *nifH* gene (Fig. 4) after specific PCR



**Fig. 3** Soil chlorophyll *a* concentrations as a function of distance from the glacier for samples from the 2007–2008 field season. Values are mean  $\pm$  SE ( $n = 5$ ) and means with *different letters* are significantly different at  $P < 0.05$

amplification. A total of 15 unique clones were examined; 14 clones could be confidently assigned as belonging to members of the proteobacteria, and one could be traced to the Spirochetes (Fig. 4). Of the proteobacteria clones, 12 were 91–95 % similar to sequences previously retrieved from soils at a foreland of the Damma Glacier, Switzerland (Duc et al. 2009; Fig. 4) and to that from an Antarctic heterotrophic bacterium, *Poloromonas* (Foght et al. 2004). The remaining two proteobacterial sequences were closely related to those of symbiotic  $N_2$ -fixing *Bradyrhizobium* species. No clones could be directly traced by similarity as being cyanobacterial in origin.

## Discussion

### Soil microbial C cycle driven by phototrophs

During the first 10 years following deglaciation, the microbial community was dominated by phototrophs (Figs. 1, 3; Table 1). Most of the respiration appeared to be from internal C reserves of phototrophs rather than heterotrophs, as SIR rates were eightfold less than dark respiration rates without addition of C (Fig. 1). Our SIR rates provide an estimate of heterotrophic activity because the majority of cyanobacteria are unable to use externally supplied C (Stal and Moezelaar 1997). In contrast, our dark respiration rates are an estimate of total respiration because they were made immediately following light measurements (i.e., net photosynthesis), which would provide cyanobacteria with abundant polyglucose reserves that they could subsequently respire in the dark. The rates of potential heterotrophic activity at our site are similar to those documented at recently deglaciated alpine (Ohtonen et al. 1999)

**Fig. 4** Diversity of the functional gene *nifH*. The phylogenetic relationship was determined by neighbor-joining analysis of *nifH* sequences obtained from two clone libraries created with soil collected either in 2005–2006 or 2007–2008. Consensus bootstrap confidence levels are indicated if  $>60$  %. The scale bar represents 5 % sequence divergence. The light gray bar indicates clades within the proteobacteria. The dark gray bar indicates clades within the spirochetes, and the black bar indicates clades within the cyanobacteria. GenBank accession numbers are given in parentheses. Sequences with 95–91 % identity matches are indicated by a single asterisk (\*); all others were greater than 95 % identical to known sequence

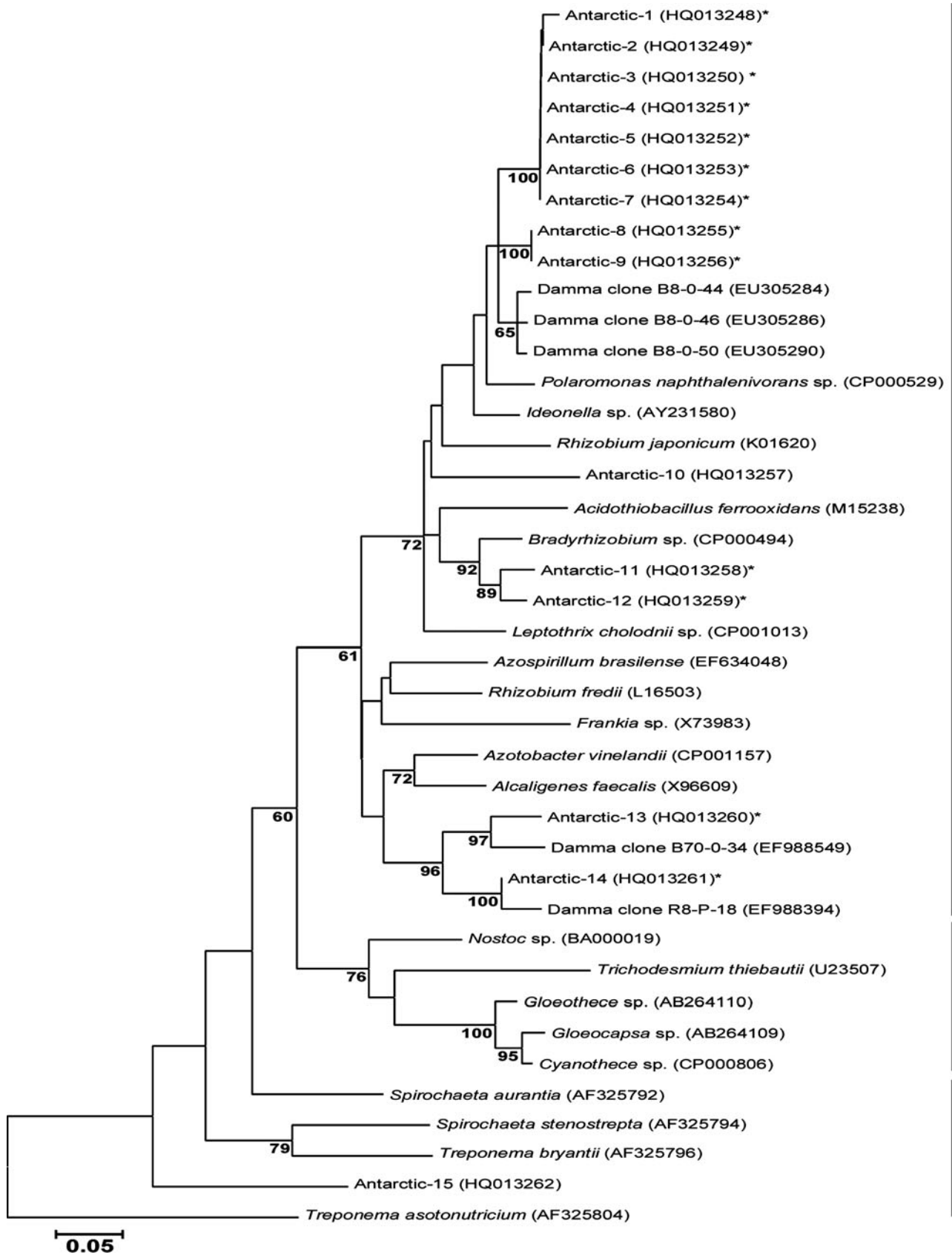
and Arctic forelands (Kastovska et al. 2005), as well as at sites along the Antarctic Peninsula (Wynn-Williams 1982; Bölter et al. 2002), and the rates of net photosynthesis are similar to those found at an Arctic glacial foreland (Yoshitake et al. 2010).

Rates of both phototrophic and heterotrophic activity declined with distance from the glacier, and we suspect that soil moisture played an important role in this. While the climate at our site is maritime, being relatively humid with frequent precipitation events (rain and snow), we observed that occasional dry periods of 2–3 days were sufficient to dry the upper 5 cm of the young soils. Additionally, soil moisture deficits were probably particularly acute during our first sampling season (2005–2006), since precipitation from November through February was atypically low, and soil moisture limited both the productivity of vascular plants and the abundance of soil microarthropods at a nearby, more developed site on Anvers Island (Day et al. 2009).

We should note that our rates of net photosynthesis and dark respiration might be the overestimates of field rates because our measurements were made at temperatures greater than typically occur at the site. During the growing season, diurnal air temperatures near the soil surface at a site approximately 1 km SE of our sample site averaged 4.3 °C (Day et al. 1999). However, due to the constraints of our incubation chambers, net photosynthetic and dark respiration rates were measured at 12 °C. The higher temperature could result in greater photosynthetic and dark respiration rates as the activity of Antarctic cyanobacteria can increase with temperature (Pandey et al. 2004; Novis et al. 2007). However, Novis et al. (2007) found that the  $Q_{10}$  of net photosynthesis and dark respiration of Antarctic cyanobacteria were similar between 5 and 15 °C. Therefore, while the rates we measured in our chambers were probably higher than typical field rates, the magnitude of the difference between these two processes was probably similar.

We found no correlations between soil chlorophyll *a* concentrations and net photosynthetic rates and suspect this to be the result of different sampling dates for these parameters. Specifically, soil chlorophyll *a* was extracted





from sub-samples shortly after they thawed, while net photosynthetic rates were measured after samples were incubated for the following 28 days. It is likely that cyanobacterial biomass (and chlorophyll *a* concentrations) increased over the 28-day incubation period.

#### Soil microbial N cycle dominated by N-fixation

The microbial N cycle, like the C cycle, was also dominated by one process during the first 10 years after deglaciation. N<sub>2</sub>-fixation rates were tenfold greater than those of AAO, denitrification, or anammox rates (Fig. 2) and increased with distance from the glacier (Fig. 2). N<sub>2</sub>-fixation rates were similar to those measured at an alpine glacial foreland (Duc et al. 2009) and frost-heaved subarctic soils (Sorensen et al. 2006). The strength of microbial N<sub>2</sub>-fixation at this site is not surprising given the competitive advantage of N<sub>2</sub>-fixers in low-N environments (Vitousek et al. 2002). Soil N concentrations along our transects were indeed very low, ranging from 0.009 to 0.024 % (Strauss et al. 2009).

Because of their ability to fix both C and N, N<sub>2</sub>-fixing cyanobacteria are generally thought to be common pioneer species at glacial forelands where C and N are typically limited (Wynn-Williams 1996; Hodkinson et al. 2003; Schmidt et al. 2008). Surprisingly, we did not find any evidence for the presence of heterocystous cyanobacteria in our 16S rRNA survey (Table 1). Similarly, we recovered no cyanobacteria *nifH* genes in the analysis of this functional gene marker. Instead, all of the *nifH* phylotypes retrieved from the site were associated with N<sub>2</sub>-fixing heterotrophs, including *Azotobacter vinelandii*, *Leptothrix cholodnii*, and *Bradyrhizobium* sp. (Fig. 4). It is unclear why N<sub>2</sub>-fixing cyanobacteria are absent from our site, particularly since cold-adapted N<sub>2</sub>-fixing cyanobacteria are present in other Antarctic soils (Davey 1982; Christie 1987; Brinkmann et al. 2007; Yergeau and Kowalchuk 2008) and lakes (Taton et al. 2003).

Rates of potential denitrification increased with distance from the glacier, a trend that Deiglmayr et al. (2006) and Brankatschk et al. (2010) also found at alpine glacial forelands. Rates of AAO and anammox did not vary with distance, and all of these rates were very low compared to those of N<sub>2</sub>-fixation (Fig. 2), suggesting that inputs dominate the N cycle at our site. Consistent with our findings, others have found little or no measurable rates of AAO at alpine glacial forelands (Tscherko et al. 2003; Brankatschk et al. 2010). Despite the presence of N<sub>2</sub>-fixation, and therefore a potential source of NH<sub>4</sub><sup>+</sup> for AAO and anammox organisms, N<sub>2</sub>-fixing bacteria may retain their fixed N for cellular growth. The retention of this fixed N is supported by the constant, rather than increasing, soil total N concentrations with distance from the glacier (Strauss et al.

2009). Therefore, the low rates of AAO and anammox at our site were not surprising.

#### Soil microbial community composition

The dominance of soil C and N cycling by a few species is not unexpected given the low DNA concentrations and phylotype richness and diversity. The concentrations of DNA extracted were similar to those at an alpine glacial foreland (Duc et al. 2009), but over three orders of magnitude less than in agricultural soils (1.2–1.3 μg g<sup>-1</sup>; Taylor et al. 2002). We found no differences in bacterial, cyanobacterial, or fungal richness and diversity with distance from the glacier, matching the patterns found at the Dama Glacier (Sigler and Zeyer 2002). In contrast, microbial richness and diversity doubled during the 4 years after colonization at the Puca Glacier (Nemergut et al. 2007). Hence, after initial colonization, microbial community development appears to be more variable at glacial forelands. This is not surprising considering the differences in conditions among foreland sites. For example, the rapid development of the microbial community at the Puca Glacier foreland may stem from greater organic C inputs from pollen deposition that in turn can support a predominantly heterotrophic community (Nemergut et al. 2007). While there is a possibility of similar inputs from glacial surface runoff at Point 8, we found no evidence of elevated C or N in soils near the glacier, suggesting this was not a significant process (Strauss et al. 2009). These different patterns could also result from the different techniques used to determine diversity and richness. DGGE assessments of richness and diversity may not have the taxonomic resolution of clone libraries (e.g., Nemergut et al. 2007) or newer pyrosequencing techniques, but they are useful for an initial assessment of relatively common species.

Sequences revealed the presence of only 24 different relatively common phylotypes (Table 1). These included typical soil bacteria, such as *Arthrobacter* and *Sphingomonas* (Janssen 2006), and an Antarctic heterotrophic bacterium, *Poloramonas* (Foght et al. 2004). Among the 5 cyanobacteria phylotypes identified were two known Antarctic inhabitants, *Phormidium autumnale* (Casamatta et al. 2005) and *Microcoleus antarcticus* (Casamatta et al. 2005; Chong et al. 2009). The latter is a very close relative of *Microcoleus vaginatus*, a known pioneer colonizer of arid soils worldwide (Garcia-Pichel and Wojciechowski 2009). An Antarctic fungus, *Geomyces* (Connell et al. 2006), was also identified from excised DGGE bands.

We note that the different sample storage methods used in each field season (air-dried vs. frozen) due to logistical constraints may have affected DNA extraction, subsequent DGGE analysis, and metabolic measurements. However,

comparisons of air-dried and frozen grassland soils (Tzeneva et al. 2009) and agricultural compost (Klammer et al. 2005) found the storage method resulted in small differences in DNA concentrations, but not the overall community composition. In our study, despite the different storage methods, there were no significant differences in concentrations of total DNA extracted between the field seasons (data not shown). While microbial metabolic measurements are often made using recently collected soils, there is precedent for making these measurements on frozen soils, particularly in studies of remote alpine and Arctic soils (Tscherko et al. 2003; Kastovska et al. 2005; Deiglmayr et al. 2006). As discussed above, our metabolic rates were comparable to those measured in other glacial foreland soils, regardless of sample storage method.

## Conclusions

We found an active soil microbial community in soils exposed from the glacier for only 10 years. Photosynthetic microbes dominated this community, while heterotrophic  $N_2$ -fixing bacteria dominated the soil N cycle. These bacteria released very little  $NH_4^+$  into the soil, limiting rates of AAO, denitrification, and anammox, as opposed to what is seen in soils lacking vascular plants in many arid systems where cyanobacterial  $N_2$ -fixation support an active AAO community (Strauss et al. 2012). We did not detect any changes in concentrations of soil total C and N over this period (Strauss et al. 2009), suggesting these inputs did not lead to appreciable soil C and N accumulation during the first 10 years after glacial recession.

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