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ORIGINAL PAPER

Microbial activity and diversity during extreme freeze–thaw cycles in periglacial soils, 5400 m elevation, Cordillera Vilcanota, Perú

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Abstract High-elevation periglacial soils are among the most extreme soil systems on Earth and may be good analogs for the polar regions of Mars where oligotrophic mineral soils abut with polar ice caps. Here we report on preliminary studies carried out during an expedition to an area where recent glacial retreat has exposed porous mineral soils to extreme, daily freeze–thaw cycles and high UV fluxes. We used in situ methods to show that inorganic nitrogen (NO_3^- and NH_4^+) was being actively cycled even during a period when diurnal soil temperatures (5 cm depth) ranged from −12 to 27°C and when sub-zero, soil cooling rates reached 1.8°C h^{-1} (the most rapid soil cooling rates recorded to date). Furthermore, phylogenetic analyses of microbial phylotypes present at our highest sites (5410 m above sea level) showed the presence of nitrifying bacteria of the genus *Nitrospira* and newly discovered nitrite-oxidizing Betaproteobacteria. These soils were overwhelmingly dominated (>70% of phylotypes) by photosynthetic bacteria that were related to novel cyanobacteria previously found almost exclusively in other plant-free, high-elevation soils. We also demonstrated that soils from our highest sites had higher potential for mineralizing glutamate and higher microbial biomass than lower elevation soils that had been more recently covered by ice. Overall, our findings indicate that a diverse and robustly functioning microbial ecosystem is present in these previously unstudied high-elevation soils.

Keywords Freeze–thaw cycles · Freezing rates · Glacial retreat · Unvegetated soils

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Introduction

Research on life in extreme soil environments has accelerated in recent years, especially with regard to the diversity and function of microbes in cold, plant-free soils of the high Arctic and in Antarctica (Aislabie et al. 2006; Cowan et al. 2002; Kaštovská et al. 2005; Parsons et al. 2004). Less well studied are high-elevation soils, which can be even harsher than high-latitude soils due to lower atmospheric pressures, higher UV irradiance and drier conditions that occur above 5000 m elevation (e.g., Costello et al. 2009; Nemergut et al. 2007). High-elevation soils also can be more extreme than polar soils because even during the summer they experience dramatic freeze–thaw cycles on a daily basis. This is especially true in high-elevation sites in the tropics that are characterized by small seasonal but very large diurnal changes in temperature (Cuatrecasas 1968; Hedberg and Hedberg 1979). In a sense, tropical alpine sites experience "summer every day and winter every night" (Hedberg and Hedberg 1979).

This temperature variability causes daily freeze–thaw cycles that are often cited as a major disruptor of microbial communities in soil (references in Henry 2007). However, microbial biomass and activity is actually minimally affected when laboratory experiments are done with realistic rates of freezing (Elliott and Henry 2009; Grogan et al. 2004; Henry 2007; Lipson et al. 2000). To our knowledge little work has been done in systems that undergo the extreme high amplitude freeze–thaw cycles typically experienced by tropical high-elevation soils.

High-elevation tropical soils are increasing in extent globally due to the rapid melting of ice caps and glaciers (Barry 2006), especially in the high Andes (Bradley et al. 2006; Mark and Seltzer 2005; Seimon et al. 2007). One extensively studied area of expanding barren soils is in the Sibinacocha watershed in the Cordillera Vilcanota of Southern Perú (King et al. 2008; Nemergut et al. 2007; Schmidt et al. 2008a, b; Seimon et al. 2007). This watershed has been impacted by the melting of both ice caps and glaciers resulting in the exposure of large tracts of barren soils (Nemergut et al. 2007; Seimon et al. 2007). To date, published work on microbial activity and communities in high-elevation soils has been restricted to elevations of around 5000 m above sea level (m.a.s.l.) and below (King et al. 2008; Nemergut et al. 2007; Zhang et al. 2007). However, areas of exposed soils extend much higher in the Sibinacocha watershed, up to approximately 5410 m.a.s.l. These high-elevation sites consist of mostly barren mineral soils surrounded by towering ice cliffs. Therefore, these soils are some of the best analogs on Earth for near-pole Martian soils because they present areas where frozen ice caps intersect with cold, dry soils (Skidmore et al. 2000; Jakosky et al. 2003). These "barren" soils are also of scientific interest because their high-elevation raises the potential for rare soil-microbial community interactions, yet soils of this type are under-sampled. In this study we present evidence that the highest exposed soils in the Sibinacocha watershed contain active microbial communities despite the elevation and extreme diurnal fluctuations in soil temperatures observed at these sites.

Methods

Study sites and sampling strategy

We studied soils in a recently deglaciated valley of the Laguna Sibinacocha watershed, Cordillera Vilcanota, Perú (Fig. 1). This isolated drainage basin is near the Quelccaya Ice Cap (Thompson et al. 1986) in southern Perú and contains a number of large glaciers that have been receding at rapid rates since the early 1900s (Seimon et al. 2007). Soils (3 or 4 spatial replicates per site) were collected in

Fig. 1 Aerial photograph of the study sites taken in March 2005. The highest mountain on the horizon (Ausangate, el. 6372 m.a.s.l.) and the Puca Glacier in the foreground are labeled for reference. The 100-m site is just outside the picture and is obscured by the wing of the airplane. The extent of the Little Ice Age is clearly visible just to the right of the Boundary site (labeled LIA extent). Photo Credit: Anton Seimon

September 2001 from five different sites in the Sibinacocha watershed. The aim of our study was to compare some of the highest exposed soils in the watershed (Pass and Spit soils) to several recently deglaciated soils (0 and 100 m soils) and to soils that were not covered by ice during the Little Ice Age (Boundary soils, Seimon et al. 2007). The Pass site was 5408 m.a.s.l. (UTM coordinates 274710, 8479104, zone 19 S) and located within the pass in the northwest corner of the watershed (Fig. 1). The "Spit" site is a de-glaciated piece of land to the east of the pass that is boxed in on three sides by ice cliffs and represents some of the highest soils in this region at 5410 m.a.s.l. (UTM coordinates 274794, 8479171). The 0-m site was at 5230 m.a.s.l. (UTM coordinates 274411, 8476050) and the 100-m site was at the same elevation and located 100 m directly to the west of the 0-m site. Both the 0- and 100-m sites were recently covered by the extension of the glacial ice cap on Nevado Japujapu. We also sampled a reference vegetated area ("Boundary" site, 5220 m, UTM coordinates 275491, 8477726) that lies just outside the maximum extent of the ice sheet that covered most of the upper reaches of the Laguna Sibinacocha watershed during the Little Ice Age (Fig. 1).

Soil temperatures

Soil temperature readings at 5 cm depth were recorded every hour during our stay in the Sibinacocha watershed from 9 September through 17th September 2001 using HOBO Pro Series data loggers (Model H08-031-08, Onset Computer Corp., Bourne, MA, USA) installed at the Pass

(5408 m.a.s.l.) and Boundary sites (5220 m.a.s.l). In addition to showing the extreme diurnal cycles of temperature at these sites, the datalogger data were also used to calculate rates of soil cooling which have been shown to be an important environmental variable affecting microbial activity and survival (Lipson et al. 2000; Henry 2007). Rates of soil cooling were estimated for each night by using a linear regression of soil temperatures once soils dropped below 0°C. Rates were used only if the R^2 value from the regression was greater than 0.96 and there were at least three data points during the linear cooling period. Using these criteria, soil cooling data from 6 of the 7 nights sampled were usable; only data from the night of September 14th had too short of a linear period to yield a reliable rate estimate.

Nitrogen turnover

We measured gross nitrogen (N) mineralization and nitrification rates in soils at the Pass site on 14–15 September 2001, using an in situ core pool dilution technique (Stark 2000). Prior to the ^{15}N tracer addition, 4-cm-diameter PVC cores ($n = 4$) were installed in triplicate to a depth of 5 cm. The replicate sets of cores (3 cores at each of 4 sites) were spaced approximately 2 m apart within a 50-m^2 area. Two cores in each triplicate set were injected with 3 ml of a 0.1 mM solution of either ^{15}N–NH_4^+ (99% atom enrichment) or ^{15}N–NO_3^- (99% atom enrichment) at a concentration of 0.06 μg ^{15}N/g soil. The unlabeled core and the first labeled core were extracted immediately (<5 min after labeling) in 2 M KCl in the field, for determination of initial ambient and initial ^{15}N-labeled pools. The second labeled core within each triplicate set was harvested and extracted in the field 24 h later. All extracts were filtered in the field within 12 h of collection using pre-rinsed Whatman #1 filters, and were transported on ice in sterile centrifuge tubes back to the lab.

Soil extracts were diffused to collect ^{15}N–NH_4^+ and ^{15}N–NO_3^- on acidified quartz filter disks (Whatman QMA), following the methods of Stark and Hart (1996). The ^{15}N enrichment of diffused filter disks was determined by EA-MS at the University of California, Davis. Gross N production and consumption rates for NH_4^+ and NO_3^- were calculated using the isotope dilution equations of Kirkham and Bartholomew (1954), as outlined in Stark (2000). Initial ^{15}N–NH_4^+ and ^{15}N–NO_3^- pool sizes were estimated from KCl extracts of unlabeled cores at the time of the ^{15}N addition.

Soil samples

Soil samples (3 or 4 spatial replicates per site) for laboratory analyses and DNA extraction were collected from the top 5 cm of soil using sterile spatulas. Samples were immediately placed on ice and were kept that way until they arrived in Boulder, Colorado, USA. Information about general properties of soils at our sites (pH, moisture, N, P, C, soil structure, and enzyme activities) has been published elsewhere (King et al. 2008; Nemergut et al. 2007; Schmidt et al. 2008a).

DNA extraction, clone libraries, and phylogenetic analyses

DNA was extracted from the Spit and 0-m soil samples using a modification of the protocol of Moré et al. (1994) as described by Nemergut et al. (2007). DNA extractions were pooled and purified over Sepharose 4B (Sigma, St. Louis, MO, USA) packed columns as described by Jackson et al. (1997). Approximately 30 ng of DNA were amplified with the primers 515f and 1492r, cloned and sequenced as described by Nemergut et al. (2007).

Sequences were edited in Sequencher 4.1 (Gene Codes Co., Ann Arbor, MI, USA) and were used to search the GenBank database using BLAST (Altschul et al. 1990). Sequences were subjected to chimera check in Bellerophon (Huber et al. 2004) and aligned using the NAST aligner available from the Greengenes website (DeSantis et al. 2006). Phylogenetic identity was assigned using both BLAST matches and the Greengenes alignment.

Next, alignments were imported into ARB (Ludwig et al. 2004). Reference sequences were selected using a recent cyanobacterial phylogeny (Hoffmann et al. 2005) as well as cyanobacterial sequences from other high-elevation studies (Freeman et al. 2009; Costello et al. 2009; Nemergut et al. 2007). We used DOTUR (Schloss and Handelsman 2005) to designate sequences to operational taxonomic units (OTUs) and unique OTUs were defined as sequences at least 3% different from all others. OTUs were then used to construct phylogenetic trees in PAUP using both distance methods with the best-fit model as determined by Modeltest (Posada and Crandall 1998) and parsimony. Sequences were deposited in GenBank with the accession numbers GQ306005–GQ306147.

Microbial carbon-mineralization kinetics

The kinetics of carbon mineralization were measured in soils using previously described approaches (Colores et al. 1996; Lipson et al. 1999; Schmidt et al. 2004). Briefly, soils (5 g dry weight equivalent) were placed in sterile 250 ml biometer flasks (Bellco, Vineland, NJ, USA) and glutamate was added in a small amount of sterile water along with tracer concentrations of uniformly ^{14}C-labeled glutamate to yield a final concentration of 50 μg C per g of soil for all soils except the Boundary soil, which received

150 μg C per gram of soil. Boundary soils received more glutamate because preliminary studies indicated that the standing biomass of these soils was so high that more carbon was needed to measure the non-carbon-limited initial rate of respiration. Soils were incubated at 22°C and the evolved CO_2 was trapped in 1 ml of 0.5 M NaOH in the sidearm of the biometer flask. Periodically, the NaOH was removed, mixed with 2.5 ml of ScintiVerse scintillation cocktail (Fisher Scientific, Pittsburgh, PA, USA) in a scintillation vial, and radioactivity was counted with a liquid-scintillation counter. Fresh NaOH was immediately added back to the sidearm after each sampling. To estimate the biomass we used methods based on the well-established principle that the production of CO_2 by a microbial population is directly proportional to the biomass of respiring microorganisms (Anderson and Domsch 1978; Colores et al. 1996; Schlegel 1992). Thus, the CO_2 evolution curves obtained were analyzed using non-linear regression (KalidaGraph, Synergy Software, Reading, PA, USA) and the substrate-induced respiration (SIR) and the substrate-induced growth response (SIGR) methods as described in detail elsewhere (Cleveland et al. 2003; Colores et al. 1996; Lipson et al. 1999).

Statistics

A two-way unbalanced ANOVA with interaction and corrected for repeated measures (Cnaan et al. 1997) was performed on the CO_2 flux data using the program R (version 2.8.1, 12/22/2008, R Foundation for Statistical Computing http://www.r-project.org/index.html). Time was set as a quantitative variable and site was set as a categorical variable. Rate of CO_2 flux was the dependent variable. To account for repeated measures, sample ID was included as a random variable in the linear mixed effect model used to generate the input for the ANOVA (Cnaan et al. 1997). Tukey's Honestly Significant Difference Test was performed in R 2.8.1 as a post hoc assessment of significant differences (Devore 2004).

Two-tailed *t*-tests were preformed to evaluate the difference in the gross N production and consumption for both NH_4^+ and NO_3^- using the statistics package MVPstats (MVP Programs, Vancouver, WA).

Results and discussion

Soil temperatures exhibited extreme diurnal variation at both the Boundary site (5220 m.a.s.l.) and the Pass site (5408 m.a.s.l.) (Fig. 2). Soil temperatures (at 5 cm depth) varied from a nighttime low of −12°C to a daytime high of 25°C in the Pass (compared to a range of −11 to 27°C at the Boundary site) over the 7 days that the data loggers were in place. These values are similar to maximum and minimum soil temperatures recorded by Halloy (1991) near the summit of Socompa Volcano (6030 m m.a.s.l.) over a 1-week period in early summer. Such extreme diurnal swings in temperature reinforce the assertion that high-elevation tropical ecosystems experience "summer every day and winter every night" (Hedberg and Hedberg 1979 and references therein). Therefore, organisms that function in these ecosystems must be adapted to withstand these fluctuations, or else remain dormant for much of the year.

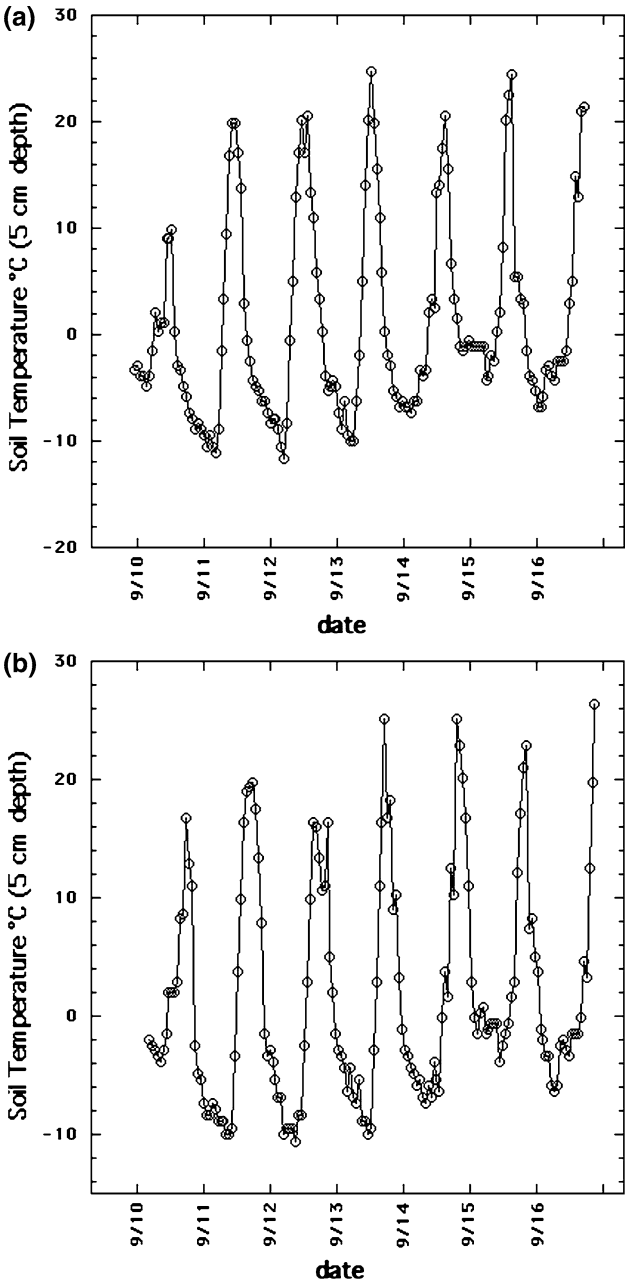

Fig. 2 Diurnal fluctuations of soil temperature (5 cm depth), during the week of 9 September to 16 September 2001, at the Pass site (**a**) and the Boundary site (**b**)

Perhaps the most important aspect of freeze–thaw cycles in terms of lethality to microbes is the rate of cooling (Lipson et al. 2000; Mazur 1980). Lipson et al. (2000) showed that tundra microbial biomass levels were unaffected by cooling rates of 0.8 and 1.1°C h^{-1}, but were significantly decreased by cooling rates of over 1.4°C h^{-1}. They concluded that field measured freezing rates of from 0.2 to 0.9°C h^{-1} (measured at the soil surface) were not rapid enough to affect functioning of tundra microbial communities (Lipson et al. 2000). In the present study we measured much faster cooling rates of 1.83°C h^{-1} (S.E. = 0.12) at the pass and 1.28°C h^{-1} (S.E. = 0.12) at the boundary site (measured at 5 cm depth). Thus, microbes in these soils not only have to withstand extreme diurnal temperature fluctuations, but also the highest soil freezing rates yet documented in the field (cf Lipson et al. 2000; Lipson and Monson 1998; Yang et al. 2002).

Despite the temperature extremes experienced at our sites we found ample evidence that a diverse and active microbial community is present and that soil microorganisms were functioning during the week of our visit in 2001. Ambient NH_4^+ (0.04 ± 0.01 μg N/g soil) and NO_3^- (0.05 ± 0.01 μg N/g soil) pools were low, but in situ gross production of nitrate (nitrification) and ammonia (ammonification) occurred at measurable rates in the Pass soils over a 24-h incubation period (Fig. 3). Although these rates are an order of magnitude lower than those seen in productive alpine tundra soils (cf. Fisk et al. 1998; Miller et al. 2009), they are relatively high compared to the amount of microbial biomass present in these soils (see below). Thus, these soils produced about 1.5 ng of inorganic nitrogen per μg of microbial biomass per day compared to approximately 3.6 ng N μg^{-1} day^{-1} and 6 ng N μg^{-1} day^{-1} for the alpine tundra soils studied by Miller et al. (2009) and Fisk et al. (1998), respectively. Mean residence time of

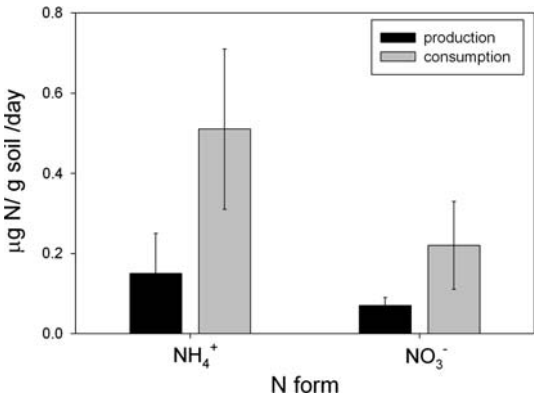

Fig. 3 Gross N production and consumption rates measured at the Pass site over a 24-h period, 14–15 September 2001. Means are ±1 standard error of the mean ($n = 4$). Production and consumption rates for both NH_4^+ and NO_3^- using were not significantly different as indicated by two-tailed *t*-tests

^{15}N–NH_4^+ and ^{15}N–NO_3^- (extractable N pool divided by gross N production rate; Booth et al. 2005) was approximately 0.25 and 0.50 day, respectively, comparable to rates measured in dry alpine tundra (Miller et al. 2009).

Gross N consumption can be stimulated by ^{15}N additions (Davidson et al. 1990), particularly when soils are severely N-limited, and this may explain why microbial N consumption was so high in these soils (Fig. 3). Indeed, we added 0.06 μg ^{15}N g^{-1} to soils that contained 0.1 μg N g^{-1}. Nevertheless, that microbial immobilization (consumption) of N exceeded production could indicate very tight cycling of nitrogen in these soils, as has been observed in other soils that are nitrogen limited (Schmidt et al. 2007 and papers cited therein). For example, Fisk et al. (1998) found that the rate of inorganic N consumption was always higher than the rate of production in soils of the three dominant plant communities in an alpine tundra ecosystem. Plant communities at these tundra sites have been shown to be N-limited in a number of other studies (Bowman et al. 1993; Fisk and Schmidt 1996). Our findings of higher N consumption than production in high-elevation microbial communities could indicate that the Sibinacocha high-elevation microbial communities are also N-limited, and that this N limitation is potentially maintained by rapid N turnover associated with the nearly 40°C diurnal fluctuation in soil temperatures. Other data (e.g., high rates of N-fixation) obtained at lower elevations (5000 m.a.s.l.) in the Sibinacocha watershed also points towards possible N limitation of microbial growth in periglacial soils (Schmidt et al. 2008a).

The makeup of the bacterial community in these high-elevation soils is further indirect evidence that there is a functioning microbial ecosystem at this site. The dominance of the clone library by cyanobacteria indicates that photoautotrophy is probably occurring at least intermittently in these soils (Table 1). Efforts to measure in situ CO_2 uptake using sensitive methods we have employed in other plant-free soils (Freeman et al. 2009) failed in the present study due to the very high carbonate content of these soils (Nemergut et al. 2007). However, the unusually high percentage of cyanobacterial sequences (Table 1) and the diversity of sequences present (Fig. 4) are indicative of a robust cyanobacterial community (cf. Freeman et al. 2009). In addition, many of the sequences obtained from these soils are particularly unique to high-elevation, barren soils. For example, many of the Spit clones fell into an apparently novel and deeply divergent group of cyanobacteria that is basal to the Oscillatoriophycidae, Order Chroococcales (OC, cf. Hoffmann et al. 2005). Members of this newly discovered clade have only been previously identified in clone libraries from the highest elevation sites sampled in Colorado (Freeman et al. 2009) and in very recently deglaciated sites near our study sites (Nemergut

Table 1 The percentage of major bacterial groups in clone libraries for the Spit ($n = 78$) and 0 m Glacier ($n = 65$) sites

Bacterial group	Spit site	0 m Glacier site
Acidobacteria	4	3
Bacteroidetes	8	8
Deinococcus	1	0
Cyanobacteria	72	19*
Firmicutes	0	2
Nitrospira	1	0
Planctomycetes	0	3
Proteobacteria		
Alphaproteobacteria	1	3
Betaproteobacteria	12	37
Gammaproteobacteria	0	20
Deltaproteobacteria	1	2
Verrucomicrobia	0	3

* Cyanobacteria at the 0-m site included chloroplasts from *Euglena* and *Chlorella* (17%) and cyanobacteria sensu stricto (2%). All cyanobacterial sequences from the Spit site were cyanobacteria sensu stricto

et al. 2007). The other major cyanobacterial clade detected in the Spit soils falls within the large Nostocophycidae, Order Nostocales group (Hoffmann et al. 2005) and shares members with the highest elevation (5824 m.a.s.l.) soils sampled anywhere to date (SB sequences in Fig. 4; Costello et al. 2009). This group is related to organisms that produce both heterocysts (for nitrogen fixation) and akinetes (desiccation-resistant spores) which makes members suited to life in high-elevation barren soils. Overall, the phylogenetic position of the Spit cyanobacterial sequences in groups that are for the most part unique to very high alpine soils suggests that these photosynthetic bacteria are probably uniquely adapted to life in plant-free high-elevation soils.

The presence of *Deinococcus* and *Nitrospira* sequences are relatively rare in soil clone libraries from plant-dominated ecosystems (Janssen 2006; Nemergut et al. 2005), but their presence in the Sibinacocha libraries may shed more light on the ecology of these unique high-elevation soils. *Deinococcus* species are among the most radiation resistant organisms known (Mattimore and Battista 1996) and their presence at this site may indicate adaptation to the high UV radiation levels that occur at high elevations in the South America Andes (Piacentini et al. 2003). The presence of sequences related to *Nitrospira* in these soils supports our biogeochemical measurements of nitrification in these soils (Fig. 3). *Nitrospira* species are chemoautotrophic soil bacteria (Bartosch et al. 2002) that obtain energy by carrying out the second step in nitrification, i.e., the oxidation of nitrite to nitrate. Additionally, we obtained one sequence in the 0-m soils that was closely related to a nitrite-oxidizing bacterium isolated from Siberian permafrost (Alawi et al. 2007). Although we did not detect sequences from organisms known to carry out the first step of nitrification, this could be because ammonia oxidation can be carried out by a wider array of soil chemoautotrophs including members of the domain Archaea (Leininger et al. 2006; Nicol and Schleper 2006), which are often in low abundance in soils relative to bacteria (Nemergut et al. 2008; Kemnitz et al. 2007).

Finally, we estimated the activity of the heterotrophic biomass using standard methods for laboratory incubations to measure rates of microbial respiration and to estimate the biomass of microbes that could utilize glutamate in these soils. The initial rate of glutamate mineralization is indicative of the potential activity of heterotrophic biomass in a given soil and has been shown to be directly proportional to the biomass of microorganisms in the soil (Anderson and Domsch 1978; Lipson et al. 1999). The Pass and Spit soils showed significantly higher rates of glutamate mineralization than recently deglaciated soils from lower elevations sites in the Sibinacocha watershed (Fig. 5). This indicates that the Pass and Spit soils are probably further along the successional trajectory than soils that we know have only been ice-free for from less than 1 year (0 m soils) to 5 years (100 m soils) (Schmidt et al. 2008a) and further supports our hypothesis that functioning microbial communities exist in these soils. We can also use the initial rates of CO_2 production and the subsequent growth curves (data not shown) from glutamate additions to estimate the glutamate consuming biomass of these soils (Colores et al. 1996; Lipson et al. 1999). Doing this we obtained a biomass estimate of about 6 μg C g^{-1}, which we used to estimate a total biomass of 180 μg C g^{-1} using the conversion factor of 30 from King et al. (2008). This value falls in the expected range (80–260; mean of 140 μg C g^{-1}) of total microbial biomass (chloroform-fumigation method) for 21 soil samples collected in the Sibinacocha watershed in 2003 (King et al. 2008). These biomass levels are almost twice as high as those for microbial communities in wetter high-elevation, plant-free soils in Colorado (King et al. 2008) that show significant photosynthetic uptake of CO_2 (Freeman et al. 2009). We take this as further evidence for a functioning photosynthetic community in the Pass and Spit soils.

Surprisingly, the initial rates of glutamate mineralization in our reference vegetated (Boundary) soils (~4 μg C g^{-1} h^{-1}, Fig. 5) were similar to rates seen in productive tundra soils (3–13 μg C g^{-1} h^{-1}; Lipson et al. 1999) and a variety of tropical rain forest soils (3–15 μg C g^{-1} h^{-1}; Cleveland et al. 2003). The vegetated soils we sampled are outside of the Little Ice Age boundary and have been ice-free for at least several hundred years and therefore have had time for soil development to occur.

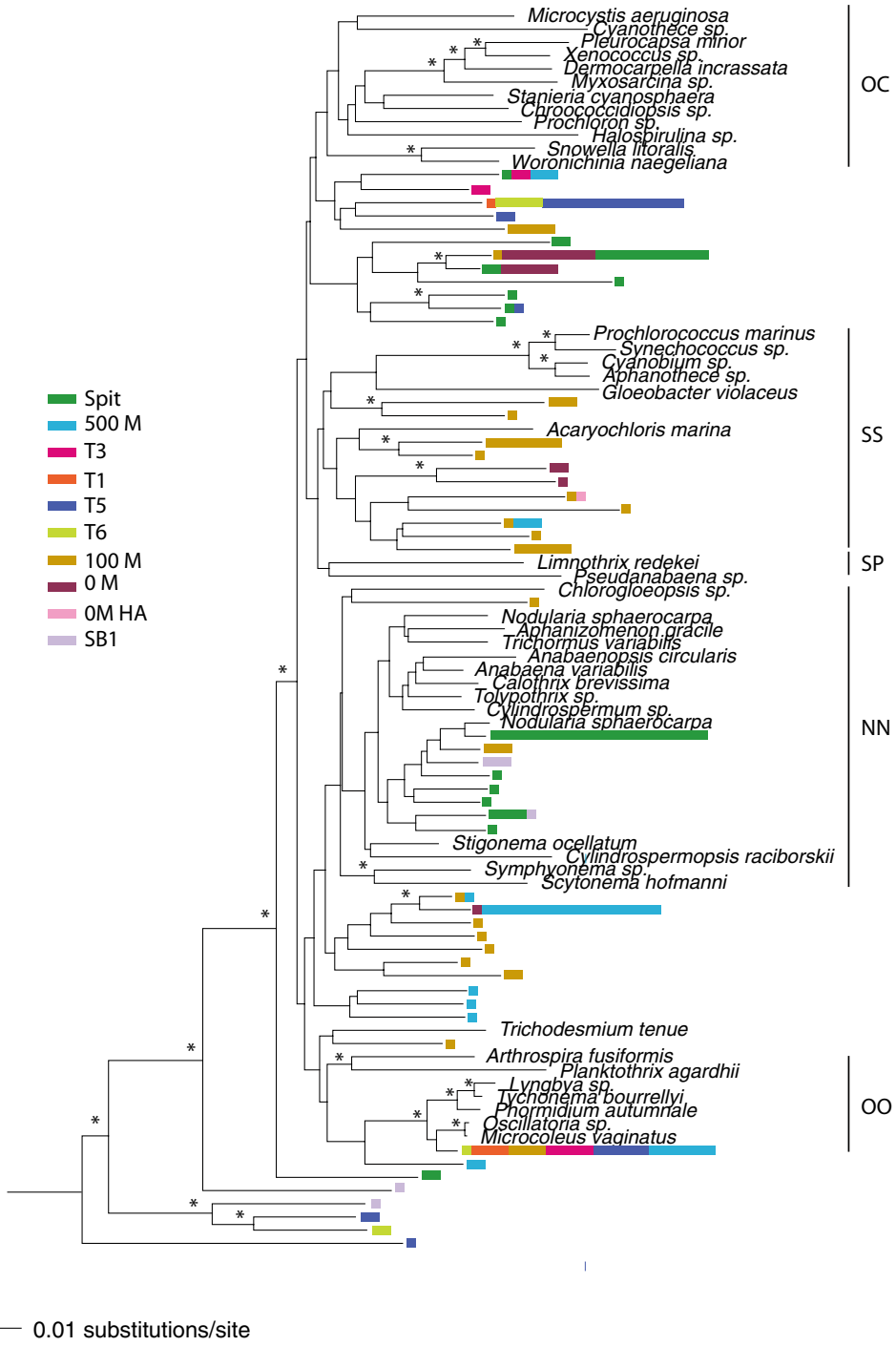

Fig. 4 Phylogenetic tree showing the diversity and closest relatives of the cyanobacteria from the Spit and High Alpine Glacier sites (0M HA). Clades indicated are as described by Hoffmann et al. (2005). Most of the Spit sequences fell into two large clades, one that is just basal to the Oscillatoriophycidae, Order Chroococcales (*OC*) and the other a large clade within the Nostocophycidae, Order Nostocales (*NN*). The Nostocales are mostly heterocystous nitrogen fixing species. Other major identified nodes on the tree are the Synechococcales (*SS*), the Pseudoanabaenales (*SP*) (both in the Synechococcophycidae), and the Oscillatoriophycidae, Order Oscillatoriales (*OO*). Sequences from other studies: T1, T3, T5, T6 (Freeman et al. 2009); 0 M, 100 M, 500 M (Nemergut et al. 2007); SB1 (Costello et al. 2009)

Conclusion

It is very likely that microbial life exists anywhere on the earth where there is free water available for even short periods of time. Thus, it is not surprising that there are active microbes in high-elevation soils; but, our data are the first that we know of to demonstrate this in soils above 5400 m in elevation. Most other studies of periglacial soils have been done at much lower elevations (circa 2500 m or less), in areas with seasonal climates (Bardgett et al. 2007; Jumpponen 2003; Sigler and Zeyer 2004; Tscherko et al. 2003). Perhaps the most unique climatic variable at high-elevation tropical sites, such as ours, is the extreme diurnal temperature fluctuation (Fig. 2) throughout the year. Organisms that function in such environments need to be able to take advantage of the higher temperatures during the day and yet survive the extreme cold temperatures at night. Here we showed that nitrogen was being actively

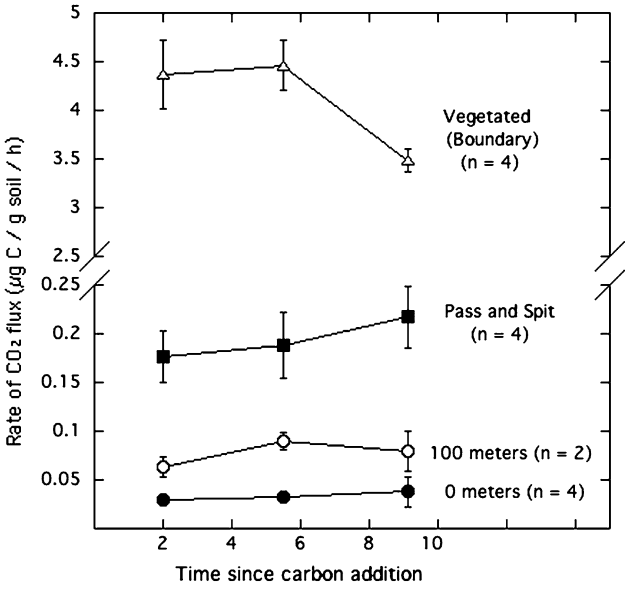

Fig. 5 Rates of glutamate mineralization to CO_2 during the first 10 h of laboratory soil incubations. Rates for each soil type were significantly different ($P < 0.001$) using a two-way unbalanced ANOVA corrected for repeated measures

cycled in the field during one 24-h period (Fig. 3) and that the organisms present in soil clone libraries are indicative of a community capable of nitrification and photosynthesis. Furthermore, the cyanobacterial phylotypes in our highest elevation soils are phylogenetically related to photosynthetic microbes from other high-elevation soils (Table 1 and Fig. 4). Finally, we demonstrated that soils from our highest sites had higher potential for mineralizing glutamate and higher microbial biomass than lower elevation soils that had been more recently covered by ice (Fig. 5). These interactions give us an idea of the types of communities and functions we might find in a Martian Polar Ice freeze/thaw zone. However, much more work is needed to understand the diversity and functioning of microorganisms in these unique high-elevation soils.

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