The potential for microbial life in the highest-elevation (>6000 m.a.s.l.) mineral soils of the Atacama region

R. C. Lynch, 1 A. J. King, 2 Marié E. Farías, 3 P. Sowell, 4 Christian Vitry, 5 and S. K. Schmidt 1

Received 20 January 2012; revised 3 May 2012; accepted 5 May 2012; published 26 June 2012.

[1] Here we present the first culture-independent microbiological and biogeochemical study of the mineral soils from 6000 m above sea level (m.a.s.l.) on some the highest volcanoes in the Atacama region of Argentina and Chile. These soils experience some of the harshest environmental conditions on Earth including daily temperature fluctuations across the freezing point (with an amplitude of up to 70°C) and intense solar radiation. Soil carbon and water levels are among the lowest yet measured for a terrestrial ecosystem and enzyme activity was near or below detection limits for all microbial enzymes measured. The soil microbial communities were among the simplest yet studied in a terrestrial environment and contained novel Bacteria and Fungi and only one Archaeal phylotype. No photosynthetic organisms were detected but several of the dominant bacterial phylotypes are related to organisms involved in carbon monoxide oxidation on other volcanoes (e.g., Pseudonocardia and Ktedonobacter spp.). Focused studies of a gene responsible for carbon monoxide oxidation, the large subunit of carbon monoxide dehydrogenase (coxL of CODH), revealed several novel lineages and a broad diversity of coxL genes. Overall our results suggest that a unique microbial community, sustained by diffuse atmospheric and volcanic gases, is barely functioning on these volcanoes, which represent the highest terrestrial ecosystems yet studied.


1. Introduction

[2] Studies of microbial life in extremely dry environments have focused mostly on low elevation areas such as the Dry Valleys of Antarctica [Cary et al., 2010] and the Atacama Desert [Connon et al., 2007; Lester et al., 2007]. Due to its status as the driest desert of the planet, the lower elevation regions of the Atacama have served as a natural testing ground for the dry-limit of microbial life [Navarro-González et al., 2003]. In the hyper-arid core of the Atacama, mean annual rainfall is less than 5 mm/year (with decadal periods of no rainfall), which appears to be below the threshold of water availability required to support soil phototrophic life [Warren-Rhodes et al., 2006]. At slightly higher elevations in the Atacama region precipitation allows for sparse vegetation in a zone between 3000 and 4900 m.a.s.l. [Arroyo et al., 1988; Richter and Schmidt, 2002]. At elevations above 5000 m.a.s.l., extreme conditions create a Mars-like landscape (totally devoid of plant-life) that receives intermittent snowfall, most of which sublimates back to the atmosphere [Richter and Schmidt, 2002]. Very little work has been done on un-vegetated soils above 5000 m.a.s.l. [Schmidt et al., 2009, 2011], especially on the large stratovolcanoes that dot the Atacama region [Costello et al. 2009, Halloy 1991]. Volcán Llullailaco (6739 m.a.s.l.) and Volcán Socompa (6051 m.a.s.l.) are part of a chain of stratovolcanoes that comprise the Andean Central Volcanic Zone [Stern, 2004], which rise above the “true desert” zone [Arroyo et al., 1988] and altiplano of the Atacama region. Although these volcanoes receive snowfall, they are at present largely un-glaciated [Richards and Villeneuve, 2001], making their upper reaches some of the highest-elevation exposed soil and lithic environments on Earth (Figure 1).

[3] In spite of the intense interest in the Atacama region [Connon et al., 2007; Lester et al., 2007; Warren-Rhodes et al., 2006], the upper band of unvegetated mineral soil and rock that extends from 5000 to over 6700 m.a.s.l. has received little attention except from archeologists [Wilson et al., 2007]. Initial exploration of the upper unvegetated...
zone on Volcán Socoma in 2005 revealed a low diversity microbial community, and low levels of organic matter (0.03%) in the mineral soils at 5235 m.a.s.l. [Costello et al., 2009]. The present study was undertaken to determine if soils at significantly higher elevations (>6000 m.a.s.l.) in this region are similarly depauperate, or if the increased snowfall at higher elevations counterbalances the harsh conditions in a way that increases either diversity or activity of microbial communities.
[4] Here we report on the results of the first biogeochemical and cultivation-independent exploration of the potential for microbial activity in mineral soils above 6000 m.a.s.l. in the Andean Central Volcanic Zone. These data suggest that a low diversity, low energy ecosystem of unique and previously uncharacterized microbes may function during periodic episodes of favorable conditions. Although oxygenic phototrophs are absent from all samples, we suggest the ecosystem has at most two trophic levels and is subsisting on both aeolian organic carbon inputs as well as chemolithoautotrophic CO₂ fixation and trace gas oxidation.

2. Methods

[5] Soil samples and data logger data were collected during the austral summer in mid-February 2009 at elevations ranging from 5500 to 6330 m.a.s.l. on Volcán Socompa and Volcán Llullaillaco. Soils used for biogeochemical and microbial diversity measurements were collected on February 14 from six spatially separated samples (to four cm depth) in a semi-nested sampling scheme [King et al., 2008, 2010b] at elevations of 6034 m.a.s.l. and 6330 m.a.s.l. on Volcán Llullaillaco. Soil temperatures at four cm depth and the soil surface were recorded every 15 min at two sites on Volcán Socompa and Volcán Llullaillaco using HOBO Pendant data loggers (UA-002-08, Onset Computer Corp., Bourne, Mass.). The data from the loggers were also used to calculate sub-zero rates of soil cooling, a parameter that can profoundly affect microbial survival in soils [Henry, 2007; Lipson et al., 2000; Schmidt et al., 2009]. Rates of sub-zero soil cooling were estimated by using linear regressions of soil temperatures after soils dropped below 0°C. The rates obtained were deemed reliable if the R² value from the regression was greater than 0.96 from at least five data points during the linear cooling period. This sampling expedition was part of a broader global study of biodiversity at high elevation sites in the Andes, Rockies, and Himalayan mountain ranges and more information about sampling protocol and sites has been published previously [Freeman et al., 2009; King et al., 2010a, 2010b; Schmidt et al., 2011].

[6] Dissolved organic carbon (DOC) and nitrogen (DON) and microbial biomass carbon (MBC) and nitrogen (MBN) were determined using a Shimadzu TOC-V CSN Analyzer with a previously described protocol [King et al., 2008]. Total nitrogen (N) and carbon (C) measurements were performed according to the method of Nemergut et al. [2007], wherein soils were dried and sieved to 2 mm then ground to a fine powder and measured for percent C and N by mass using a Carlo-Erba combustion-reduction elemental analyzer (CE Elantech, USA). Soil water content was measured gravimetrically as the difference between the weight of the soils at field conditions and the weight after drying at 80°C for 48 h. Soil pH was determined using a glass pH probe (Oakton Instruments, Vernon Hills, IL, USA) in soil slurries consisting of 2 g soil and 2 ml of water that were shaken for 1 h. Levels of common microbially produced extracellular enzymes were also measured using standard techniques adapted for cold soils as described by King et al. [2008, 2010b]. Enzyme activities assayed were: N-acetylglucosaminidase, cellulase (β-glucosidase), α-glucosidase, β-xylanase, cellulobiosidase, leucine aminopeptidase and phosphatase. For each sample, 2 g of soil was added to 150 ml of buffer (adjusted to the pH of the soil) and homogenized at 3000 rpm for 1 min using an Ultra-Turrax homogenizer (IKA Works Inc., USA). Soil slurries were incubated for 20 h at 14°C using the controls, fluorescent substrates, and volumes as described in King et al. [2008].

[7] DNA was extracted from the soils using the MO BIO Power Soil bead beating kit (MO BIO Laboratories, Inc., Carlsbad, CA, USA). Community small-subunit ribosomal DNA was PCR amplified using the 18S/16S primers 4Fa-short (5′-ATCCGCGTGATCCTGC-3′) and 1492R (5′-GGTTACCTTGTAGCACTT-3′), and 16S primers 8F (5′-AGAGTTTGTATCCTGCGAG-3′) and 1391R (5′-GACGGCGGTGTGCATTA-3′). The large subunit of the carbon monoxide dehydrogenase gene (coxL) was targeted for PCR amplification using the primers Ompf (5′-GCGGCTTYGGSSAASGAAGGT-3′) and O/Br (5′-YTGCAYGATCATTGCGTGA-3′) [King, 2003b]. Amplicons were then gel purified, and cloned as described elsewhere [Schmidt et al., 2011]. Cell pellets were sent to Functional Biotecories (Madison, WI, USA) for plasmid extraction, and bi-directional Sanger sequencing. Sequences were vector-trimmed and assembled into contigs using SEQUENCER 4.6 (Gene Codes Co., Ann Arbor, MI, USA). For the ribosomal small subunit data, the full contigs were then aligned with the SINA aligner tool [Pruesse et al., 2007]. The parsimony insertion function of ARB (5.1) was then utilized to determine the nearest relatives in the Silva 108 database, which formed the basis for taxonomy assignment [Ludwig et al., 2004]. An iterative process of calculating neighbor-joining trees using the Felsenstein correction and a 35% minimum identity per residue filter in ARB, with National Center for Biotechnology Information (NCBI) web-based BLASTN homology tests [Altschul et al., 1990], was used to refine our sequence classifications. We then clustered our sequences with the select database guide sequences into 97% identity operational taxonomic units (OTUs) using the average neighbor algorithm implementation in mothur [Schloss et al., 2009]. For the coxL data set, our multiple sequence alignment (MSA) of translated amino acids was built in ClustalX (2.0) [Larkin et al., 2007], and anchored around the essential active site motifs. This ‘Form I’ (OMP) motif (A/YXSFR) is 100% conserved in the MSA, which seems to be restricted to functional coxL genes [King and Weber, 2007]. A final uncorrected neighbor-joining tree with 1000 bootstrap replicates was calculated in ClustalX (2.0) after top scoring NCBI BLASTP hits were added into the MSA. Comparisons of microbial community beta diversity among sites was done using weighted Unifrac analysis [Lozupone and Knight, 2005].

3. Results

3.1. Microclimate and Edaphic Characteristics

[8] During our expedition in February of 2009 we were able to deploy data loggers at two high elevation sites on Volcán Socompa and Volcán Llullaillaco to gain a preliminary indication of how soil temperatures vary on a diurnal basis. Due to time and weather restrictions, these data loggers were deployed at the highest camps on each volcano at elevations slightly lower than the sampling sites. Nonetheless, the data paint an extraordinary picture of the temperature fluctuations faced by life in these high elevation soils.
Temperatures on Socompa volcano at 5500 m.a.s.l. dropped to overnight lows of \(-10^\circ\)C and reached highs of 56°C by midday on the soil surface (Figure 2a). On Volcán Llullaillaco we were only able to deploy data loggers for 16 h at 5737 m, but they show a similar trajectory of sub-freezing overnight lows (\(-15^\circ\)C) followed by a rapid rise in temperatures in the morning (Figure 2b). Linear rates of subzero temperature decline (at 4 cm depth) were \(1.15^\circ\)C h\(^{-1}\) (R\(^2\) = 0.996) and \(1.50^\circ\)C h\(^{-1}\) (R\(^2\) = 0.991), on Volcán Socompa and Volcán Llullaillaco, respectively.

Our analyses demonstrate for the first time the truly oligotrophic status of these soils, with levels of carbon similar to other almost lifeless soils. In addition, total nitrogen values were below detection limits in all samples, indicating that nitrogen levels in these soils are less than 25 µg N g soil\(^{-1}\). Likewise, microbial biomass C and N were extremely low (Table 1). Levels of common microbial extracellular enzymes were also mostly undetectable despite the fact that methods were employed to increase the sensitivity of these measurements for cold oligotrophic soils.

### 3.2. Microbial Community Analysis

Our comprehensive 16S and 18S targeted surveys of the soil community revealed a microbial community noteworthy for overall low diversity and the phylogenetic uniqueness of the component community members (Figure 3). The species richness Chao1 estimate for bacteria, pooled from five sample sites is 95 OTUs (97% identity). Nearly 75% of that total bacterial diversity is contained within just four OTUs, and our sampling effort recovered representatives from only nine bacterial phyla. All between site community beta diversity tests (weighted Unifrac) were significantly different at both 5 m and 300 m scales (P < 0.05). Of particular interest is the shift in dominance of a *Pseudonocardia*-like OTU at our high camp on Volcán Llullaillaco (5737 m.a.s.l.), but nighttime lows reached \(-14.5^\circ\)C and \(-9.4^\circ\)C at the surface and 4 cm depth, respectively.

---

Table 1. Biogeochemical Properties of the High-Elevation Mineral Soils of Volcán Llullaillaco

<table>
<thead>
<tr>
<th></th>
<th>Low Site</th>
<th>High Site</th>
</tr>
</thead>
<tbody>
<tr>
<td>M.A.S.L.</td>
<td>6034</td>
<td>6330</td>
</tr>
<tr>
<td>UTM coordinates</td>
<td>19J 0548168 7266202</td>
<td>19J 0547614 7266157</td>
</tr>
<tr>
<td>Percent water</td>
<td>0.24 (0.1)</td>
<td>0.25 (0.2)</td>
</tr>
<tr>
<td>TOC (%)</td>
<td>0.017 (0.006)</td>
<td>0.005 (0.005)</td>
</tr>
<tr>
<td>TON (%)</td>
<td>&lt;d.l.</td>
<td>&lt;d.l.</td>
</tr>
<tr>
<td>Extractable DOC (\mu)g g dry soil(^{-1})</td>
<td>1.3 (0.9)</td>
<td>2.0 (1.2)</td>
</tr>
<tr>
<td>Extractable TDN (\mu)g g dry soil(^{-1})</td>
<td>0.7 (0.4)</td>
<td>0.6 (0.5)</td>
</tr>
<tr>
<td>pH</td>
<td>4.2 (0.03)</td>
<td>4.6 (0.1)</td>
</tr>
<tr>
<td>Microbial biomass C (\mu)g g(^{-1})</td>
<td>30.61 (30.61)</td>
<td>58.07 (24.6)</td>
</tr>
<tr>
<td>Microbial biomass N (\mu)g g(^{-1})</td>
<td>2.24 (0.52)</td>
<td>1.15 (0.9)</td>
</tr>
<tr>
<td>BG (\text{nmol h}^{-1} \text{g}^{-1})</td>
<td>0.24 (0.1)</td>
<td>0.25 (0.2)</td>
</tr>
<tr>
<td>NAG (\text{nmol h}^{-1} \text{g}^{-1})</td>
<td>0.02 (0.02)</td>
<td>0.05 (0.006)</td>
</tr>
<tr>
<td>NHO (\text{nmol h}^{-1} \text{g}^{-1})</td>
<td>0.17 (0.16)</td>
<td>0.26 (0.09)</td>
</tr>
</tbody>
</table>

*Activity of \(\alpha\)-glucosidase, \(\beta\)-xylase, cellobiosidase, and leucine aminopeptidase was below detection limit. All values are the means of at least 3 replicates with the standard error of the mean in parentheses.*

*Below detection limit.
lower elevation (6034 m.a.s.l.) sites, to the dominance of a relative of the *Ktedonobacter* genus at the 6330 m.a.s.l. sites.

Eukaryotic diversity was restricted to only seven 18S OTUs (97% identity) and 92% of the total sampling effort (>300 sequences) revealed a single novel OTU. This dominant OTU is most closely related to endolithic and xerotolerant members of the *Cryptococcus-albidus* clade (Figure 4). Archaeal diversity was limited to just one 16S OTU across all sites, which is most closely related to the obligate oligotrophs of the phylum Thaumarchaeota. Absent from our data are any known chlorophyll containing clades of bacteria or algae. The lack of traditional photoautotrophs was partially confirmed by the lack of observable autofluorescence (~680 nm) using the same methods that detected very low levels of chlorophyll containing algae and cyanobacteria in high elevation soils of the Himalayas. Given the lack of evidence for phototrophic primary production, we began a preliminary exploration of other means of carbon and energy acquisition in these soils. Sequences of the large subunit of the carbon monoxide dehydrogenase gene (*coxL* of CODH) from Volcán Llullaillaco soils are at minimum 5% different, and in one instance up to 22% different, compared to their nearest database relatives (Figure 5). These nearest relatives are for the most part uncultured representatives from other oligotrophic volcanic deposits and cultured Actinobacteria (rather than common CO oxidizing Proteobacteria), which re-enforces the general phylogenetic signal from our SSU rDNA data. Additionally, despite these large genetic distances, we are confident in the *coxL* homology of these sequences, due to the 100% conservation of the primary catalytic site motif (as well as four other separate sites) that contact an essential molybdopterin cytosine dinucleotide cofactor.

4. Discussion

Taken together, these results suggest that conditions in the high-mountain mineral soils above 6000 m.a.s.l. are more restrictive to life than nearly anywhere on the surface of Earth. Despite potentially higher water availability due to orographic snowfall compared to the lower elevation portions of the Atacama, high-elevations pose additional challenges to life. The thinness of the atmosphere exposes any surface life to severe solar radiation [Farias et al., 2009], and massive daily temperature cycles across the freezing point (Figure 3). UV exposure for only 1 day, combined with extreme aridity, has been previously shown to sterilize both monolayers of *Chroococcidiopsis*, as well as dormant *Bacillus* endospores at just 1000 m.a.s.l. in the Atacama [Cockell et al., 2008]. Given that UV intensity increases 4–10% every 1000 m in elevation gained [Cabrera et al., 1995], our sites above 6000 m.a.s.l. may be subjected to the most UV exposure of any terrestrial soil environment studied to date.

Daily temperature cycling across the freezing point is considered a key challenge that severely limits net primary productivity in the similarly extreme Dry Valleys of Antarctica [Cary et al., 2010]. At Dry Valley sites temperatures vary more than 20°C per day during the austral summer, resulting in annual net primary productivity (NPP) in the 1–20 g carbon m⁻² yr⁻¹ range [Aislabie et al., 2006; Novis
et al., 2007]. During our 2009 expedition to the mountains of the Atacama region, mineral soils at 5500 m.a.s.l. experienced triple the diurnal temperature fluctuations of Antarctic Dry Valley soils (Figure 2). While winter Antarctic Dry Valley soil temperatures stay well below freezing, with daily minimums of −40 to −60°C, insulating mountain-top snow cover could potentially offer a dark microbial niche [Freeman et al. 2009; Ley et al., 2004], but no data currently exists for the duration and depth of snow cover (or winter-time temperatures) above 6000 m in the Atacama region.

Previous work has also shown that the rate of freezing is an important parameter determining the survivability of microbes in cold terrestrial ecosystems [Henry, 2007; Lipson et al., 2000; Schmidt et al., 2009]. For example, Lipson et al. [2000] showed that alpine tundra microbial biomass levels were significantly depressed by cooling rates of over 1.4°C h\(^{-1}\) (measured at the soil surface) but were largely unaffected by slower rates of soil cooling. The linear cooling rates recorded on Volcán Llullaillaco (1.50°C h\(^{-1}\) at 4 cm depth) were faster than 1.4°C h\(^{-1}\) and were comparable to the highest rates of subzero soil cooling yet reported (1.83°C h\(^{-1}\)), measured during the austral winter at 5400 m.a.s.l. in barren, per-glacial soils of the Peruvian Andes [Schmidt et al., 2009]. The rate of soil freezing on Volcán Llullaillaco is also much faster than that measured in limited studies of high elevation soils (5000 m.a.s.l.) in the Himalayas and Tibetan Plateau [cf. King et al., 2010a, Yang et al., 2003].

The average organic carbon value from our six sites on Volcán Llullaillaco (163 μg C g\(^{-1}\)) classifies these high-mountain soils as highly oligotrophic; at the low end of the range found in other extreme deserts (Figure 6) [Drees et al., 2006; Parsons et al., 2004]. Soils on the hyper-arid desert floor of the Atacama contain organic carbon values consistently below that of the samples studied here. However, pyrolysis-GC-MS analysis of the desert floor organic carbon revealed a much simpler mixture of organic compounds than that released from living microbes [Navarro-González et al., 2003]. This, and other evidence, suggests that life is rarely if ever active in some parts of the soil in the hyper-arid core of the Atacama. Conversely, 18 year old volcanic deposits on Kilauea volcano of the Hawaiian archipelago reportedly contain only slightly more (200 μg C g\(^{-1}\)) organic carbon than the >6000 m.a.s.l soils, yet conclusively demonstrate in situ biological uptake of CO\(_2\), CO, and H\(_2\) [King, 2003a]. Although exact ages for the parent volcanic deposits of our samples are currently undetermined, we know they are much older than 0.048 +/− 0.012 Ma, based on the work of Richards and Villeneuve [2001]. On Volcán Llullaillaco the early colonizers appear to have gained a foothold, but unlike less restrictive environments, are never supplanted by later successional communities even after tens of thousands of years.

In addition to low TOC values for Llullaillaco soils, our estimates of microbial biomass carbon (MBC) were also extremely low (Table 1). These values are similar to those measured (using the same method) in soils of the Dry Valleys of Antarctica (26 μg C g\(^{-1}\)) [Ball et al., 2009] and high elevation soils of the Himalayas (21 μg C g\(^{-1}\)) [Schmidt et al., 2011]. They are also lower than MBC values (140 μg C g\(^{-1}\)) averaged across many sites in a plant-free, recently de-glaciated landscape in the high Andes of Perú [King et al., 2008]. For comparison vegetated soils usually have MBC levels that are two orders of magnitude higher.
than those reported here [Cleveland et al., 2004; Weintraub et al., 2007]. Another indication of the extreme nature of Llullaillaco soils is that the levels of measurable enzyme activities (Table 1) were 3 to 80 times lower than values from the driest sites studied by Zeglin et al. [2009] in the Antarctic Dry Valleys.

[18] Aside from revealing a low diversity community, which lacks obvious phototrophs, our molecular phylogenetic analyses hint at a set of traits necessary for survival in the >6000 m.a.s.l. soil environment. For example, the dominant Actinobacterial OTU is closely related (94% identity) to Pseudonocardia asaccharolytica (Y08536), which can oxidize dimethyl sulfide (DMS) for energy [Reichert et al., 1998]. Nearer un-cultured database relatives are from Icelandic and Azorean volcanic deposits (GQ495403, HM445437). Likewise, the dominant Chloroflexi lineage branches from Ktedonobacter racemifer (AM180159), a putative facultative ‘carboxydovore’ [Chang et al., 2011], which may be able to use carbon monoxide (CO) as an electron donor and carbon source, in addition to wide array of organic carbon substrates [Cavaletti et al., 2006]. Other un-cultured relatives are from dry Antarctic soils (FR749824, FR749772). Both of these distantly related clades seem to share a number of convergent traits that confer success in these oligotrophic environments: a mixotrophic lifestyle, filamentous morphology, and the ability to sporulate.

[19] The extremely limited eukaryotic and archaeal diversity mirrors the organic carbon restriction, which can only support all but the most efficient of secondary trophic consumers. Members of the Cryptococcus-albidus clade (Basidiomycetous yeasts, Figure 4) seem well suited to this role. They have radiated widely into xeric environments, where they can occupy the endolithic niche as highly
competitive heterotrophs due, in part, to abundant carbohydrate capsule production [Vishniac, 2006]. Although knowledge is limited regarding the archaeal phylum Thaumarchaeota [Brochier-Armanet et al., 2008], multiple lines of evidence suggest they can aerobically oxidize trace quantities ammonia for energy [Könneke et al., 2005], and have a broad distribution in soil environments [Bates et al., 2011; Oline et al., 2006].

Overall, our analyses suggest that energy and carbon sources for microbial activity above 6000 m.a.s.l. could be derived from a combination of heterotrophic respiration of aeolian deposited organic carbon, and chemoautotrophic carbon fixation driven by aerobic oxidation of ammonia, DMS, and CO.

Although energy yield from trace gas oxidation is limited, it is a constantly available substrate, even in the dark deeper layers of soil and rock where microbes can avoid the massive diurnal temperature swings, rapid cooling and UV exposure of the surface environment. Additionally, even though global atmospheric CO concentrations are only in the 5–350 ppb range, proximity to fumaroles may increase CO availability on volcanoes [King, 1999; Symonds et al., 1994]. The last un-official reported activity of Volcán Llullaillaco dates to 1887 (www.volcano.si.edu), but it is unknown whether the local atmosphere is currently being enriched with volcanic gases. Either way, our coxL data (Figure 5) are genetic novelties that represent either divergent natural selection driven by this unique environment, or genetic drift by geographic isolation, both of which support the hypothesis that soils above 6000 m.a.s.l. harbor functioning microbial ecosystems.

As discussed above, our results suggest that an endogenous community of novel microbes may be periodically active in this understudied high-elevation setting. However, it is also possible that continuous atmospheric deposition of microbial propagules is responsible for some of the genetic diversity seen in these soils. Microbes are well known to be globally dispersed in the upper atmosphere [Darcy et al., 2011; Mladenov et al., 2011] and it is possible that there is a constant input of ice nucleating [Christner et al., 2008] and other microbes to these soils. But the unique and extreme environmental conditions on Volcán Llullaillaco are likely to be highly selective for specific microbes. Indeed the limited diversity of the microbial

**Figure 6.** Rank order of organic carbon content of select oligotrophic mineral soils from regions similar to the high elevation sites described here. Data for other sites are from Drees et al. [2006], Parsons et al. [2004], Biemann et al. [1977] and Costello et al. [2009].
community on Llullailaco suggests strong selection because the microbial groups present do not match the profiles of atmospheric microbial communities. For example, the Llullailaco soils contain less than 1% of the common groups Betaproteobacteria, Firmicutes and Pseudomonas and 7 out of 10 major groups of bacteria that are abundant in atmospheric samples [Bowers et al., 2012]. Likewise the limited fungal diversity on Llullailaco is very different than the profile of fungal spores found in atmospheric samples; out of the 22 different fungal genera present in high elevation atmospheric samples [Amato et al., 2007], only 2 were present on Volcán Llullailaco. However, studies of the connection between atmospheric and terrestrial microbes are in their infancy and much more work is needed to determine both the origin and function of the microbial communities of high elevation soils [Meyer et al., 2004; Schmidt et al., 2011].

5. Conclusions and Implications

[22] Like the chemosynthetic ecosystems of the deep sea and deep subsurface biosphere [e.g., Connelly et al., 2012; Lin et al., 2006], life on the Earth’s highest volcanoes may not be supported by in situ photosynthesis but rather by the oxidation of gaseous substrates. Our work suggests that the highest sites on Volcán Llullailaco are devoid of photosynthetic primary producers and contain unique microbial communities that may be partially supported by the oxidation of carbon monoxide, but more work is needed to test this hypothesis. Future research at sites above 6000 m.a.s.l. will focus on isolation of the dominant microbes from high elevation sites, and determination of survival and growth under conditions that mimic the extreme temperature fluctuations and low energy inputs of the environment. It is expected that these organisms are reservoirs of uncharacterized biological traits that allow adaptation to the unique challenges of this dynamic and oligotrophic environment. Deeper insight into these outer limits of biological adaptive capacity will inform our understanding of biogeochemical processes under conditions never before examined in terrestrial ecosystems. This work may also be informative for the search for life on other planets, especially in light of recent analyses that suggest seasonal near-surface water flow on Mars [McEwen et al., 2011].

[23] Acknowledgments. We thank J. L. Darcy and M. S. Robeson for technical assistance and Pablo Maciel, G. Jesperson and T. Harris for assistance in the field. This work was supported by grants from the National Science Foundation of the USA, the National Geographic Society Committee for Research and Exploration and a faculty fellowship from the University of Colorado. DNA sequences used in this study are available in GenBank (accession numbers JX098274 - JX099326).

References


9 of 10


