

Microbial Diversity of Impact-Generated Habitats

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Abstract

Impact-generated lithologies have recently been identified as viable and important microbial habitats, especially within cold and arid regions such as the polar deserts on Earth. These unique habitats provide protection from environmental stressors, such as freeze-thaw events, desiccation, and UV radiation, and act to trap aerially deposited detritus within the fissures and pore spaces, providing necessary nutrients for endoliths. This study provides the first culture-independent analysis of the microbial community structure within impact-generated lithologies in a Mars analog environment, involving the analysis of 44,534 16S rRNA sequences from an assemblage of 21 rock samples that comprises three shock metamorphism categories. We find that species diversity increases ($H=2.4\text{--}4.6$) with exposure to higher shock pressures, which leads to the development of three distinct populations. In each population, Actinobacteria were the most abundant (41%, 65%, and 59%), and the dominant phototrophic taxa came from the Chloroflexi. Calculated porosity (a function of shock metamorphism) for these samples correlates ($R^2=0.62$) with inverse Simpson indices, accounting for overlap in populations in the higher shock levels. The results of our study show that microbial diversity is tied to the amount of porosity in the target substrate (as a function of shock metamorphism), resulting in the formation of distinct microbial populations. Key Words: Microbial diversity—Endoliths—Impact melt-rocks—Mars—Astrobiology. *Astrobiology* 16, 775–786.

1. Introduction

THE COLONIZATION OF ROCKS was first noted over a century ago (Diels, 1914); however, it was not until the discovery of extensive endolithic (within-rock) colonization in the Dry Valleys of Antarctica that widespread interest in these environments was encouraged (Friedmann and Ocampo, 1976). Previously thought to be uninhabited, the Dry Valleys are characterized by extreme cold and dry conditions, where any precipitation generally sublimates, providing very minimal water input for organisms (Pointing *et al.*, 2009). Since the initial discovery, microbial endolithy has been widely recognized as a beneficial adaptation to environmentally harsh conditions and is generally found to be the primary mode of survival for microorganisms in either cold or hot desert environments (Friedmann, 1980). Rocks are an ideal substrate in such environments in that they can function as a UV shield, protect from large temperature swings, act as a concentrating mechanism for air-blown nutrients and water, and generally provide a higher relative humidity than the

surrounding environment (Cockell *et al.*, 2005). Typically, only rocks with a high level of porosity and translucency are suited for endolithic colonization, such as sedimentary rocks or evaporites (*e.g.*, Wierzchos *et al.*, 2011), whereas more opaque rocks, such as granites and gneisses, will only accommodate chasmoendolithic or epilithic (surficial) growth (Cockell and Stokes, 2006).

Since the initial survey in the late 1970s, much work has also been done to attempt to characterize the microbial diversity of endolithic communities in polar and nonpolar deserts around the world (*e.g.*, Friedmann, 1980, 1982; Friedmann and Kibler, 1980; Friedmann *et al.*, 1993; Wierzchos and Ascaso, 2001; de la Torre *et al.*, 2003; Wierzchos *et al.*, 2006, 2011; Omelon, 2008; Pointing *et al.*, 2009). In a review by Walker and Pace (2007) that focuses on rRNA gene-based studies, the authors point out that these communities are extremely simple in terms of diversity and share compositional similarities with other desert environments. One such example can be found in the genus *Chroococcidiopsis*, a cyanobacterium that is common in endolithic communities

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worldwide (Friedmann, 1980). Other members of the Cyanobacteria are also prevalent in both Arctic and Antarctic endolithic ecosystems, such as *Leptolyngbya*, *Gloeocapsa*, *Trebouxia*, *Oscillatoria*, and *Nostoc* spp. (Omelson *et al.*, 2006; Pointing *et al.*, 2009; Jungblut *et al.*, 2010). Given these similarities, it is plausible that the physical and chemical conditions of the rock—such as porosity, translucency, hygroscopic properties, and rock type—have a higher influence on the composition of the microbial community than geography (Walker and Pace, 2007; Fierer and Lennon, 2011).

In the last decade, a new habitat has been added to the list of substrates capable of hosting endolithic communities, in the form of impact-generated lithologies. Meteorite impacts are a fundamental, though often overlooked, geological process that affects all rocky and icy planetary bodies. The extreme heat and pressure generated by meteorite impact events results in the shock metamorphism of the rock on both a micro (*i.e.*, mineral) and macro (*i.e.*, whole-rock) scale, the formation of microfractures, changes in density, as well as partial to complete melting of the rock and the generation of glass phases (Kieffer *et al.*, 1976). A subsequent increase in the transmission of light through the substrate due to increased porosity is also seen (Cockell *et al.*, 2002; Pontefract *et al.*, 2014). Of particular interest is the impact generation of endolithic habitats in crystalline rocks, specifically gneisses, which before the impact were not capable of being internally colonized (Cockell *et al.*, 2002, 2005). The impacting process can further result in the generation of transient hydrothermal systems that can lead to the formation of various types of evaporites, which can also provide a viable substrate for microbial colonization (*e.g.*, Pellerin *et al.*, 2009; Rhind *et al.*, 2014). Given that microorganisms capable of endolithic growth have developed this strategy as an adaptation to harsh environmental conditions here on Earth, similar microhabitats may be good places to search for life on Mars, if it had adapted to similarly extreme conditions in the martian environment after 3.8 Ga. The extensive impact cratering that Mars experienced, both during the Late Heavy Bombardment (Kring and Cohen, 2002) and afterward, reveals a planet where the dominant lithology is both shocked and brecciated, creating an excellent environment for microbial colonization (Newsom, 1980; Schultz and Mustard, 2004; Cannon and Mustard, 2015).

Early work on these types of substrates showed the prevalence of endolithic communities in shocked gneisses from the Haughton impact structure, Canada (Cockell *et al.*, 2002, 2005; Cockell, 2004). Similarly, work specifically on impact glasses from the Ries impact structure, Germany, has revealed the presence of putative microtubules, which are likely related to earlier hydrothermal activity within the crater (Sapers *et al.*, 2014). Pontefract *et al.* (2014) showed that biomass levels increase with increasing shock level as a function of porosity, with the unshocked rocks (crystalline)—mainly hosting an epilithic community—having intact cell counts at $\sim 10^6$ cells/g of rock, whereas moderately (~ 30 GPa) and highly shocked samples (largely amorphous glass, >50 GPa) reached levels of $\sim 10^8$ and $\sim 10^9$ cells/g, respectively. The biodiversity of these impact-created habitats, however, has yet to be characterized fully. Fike *et al.* (2003) performed a preliminary study of the heterotrophic organisms inhabiting shocked versus unshocked lithologies based on

rock isolates and showed that most of the organisms within this habitat shared similar metabolic strategies. Community complexity increased in the shocked gneisses, relative to host impact breccias from the same area, which were dominated by three genera: *Arthrobacter*, *Pseudomonas*, and *Stenotrophomonas*. Alternatively, shocked samples contained organisms from the bacterial genera *Arthrobacter*, *Pseudomonas*, *Bacillus*, *Stenotrophomonas*, *Planococcus*, *Caulobacter*, and *Janthinobacter* with approximately equal levels of abundance. This initial research suggested that the impact event created an environment with its own unique selectional pressures, affecting the species abundance and distribution of microorganisms within the endolithic community. In this study, we show that shock metamorphism affects the composition of the endolithic community, especially with reference to phototrophic bacteria. Here, the first culture-independent analysis of microbial diversity within shocked gneisses from the Haughton impact structure is presented, expanding on a new type of environment within the Arctic tundra as well as elucidating the selective pressures related to shock metamorphism and the importance of such an environment with respect to the search for life on Mars.

2. Methods

2.1. Field site

Samples were collected from multiple sites within the Haughton impact structure (Fig. 1), located in the northwestern region of Devon Island, Nunavut ($75^{\circ}08'N$, $87^{\circ}51'W$), in the Canadian High Arctic archipelago (Osinski *et al.*, 2005). (See Supplementary Table S1 for sample locations, where samples with the HMP tag were collected in July/August 2000, DI08 tags were collected in July/August 2008, and all other samples were collected in July/August 2010. Supplementary Data are available online at www.liebertonline.com/ast.) Hand-sized samples of shocked gneiss from a range of different shock levels (*i.e.*, exposure to different pressure ranges depending on distance from the epicenter) were collected, placed in sterile sample bags, and stored at $4^{\circ}C$. Endolithic banding was occasionally visible to the naked eye, but this was not a criterion for sample selection. It should be noted that determination of shock level in the field is difficult owing to the high level of variation in the types of gneisses present as well as the range of porosity experienced within each shock level (Singleton *et al.*, 2011). Estimations of density as well as visual observations of mafic banding or complete loss of mafic minerals aided sample selection. A more stringent characterization was then made in the laboratory through the use of petrographic thin sections to characterize shock effects at the microscopic level (see Supplementary Table S1 for characterization). Once characterized, samples were then stored at either $-20^{\circ}C$ for DNA extraction or at $4^{\circ}C$ for culturing or various imaging techniques. It should be noted that very little DNA degradation was expected for these samples. Pontefract *et al.* (unpublished data) showed similar levels of viability (via Live/Dead stain) between rocks of the same shock level regardless of the year collected. Furthermore, Millar and Lambert (2013) calculated the half-life of DNA at $25^{\circ}C$ to be 100 years. Though some cell lysis and degradation due to DNase activity is expected, the

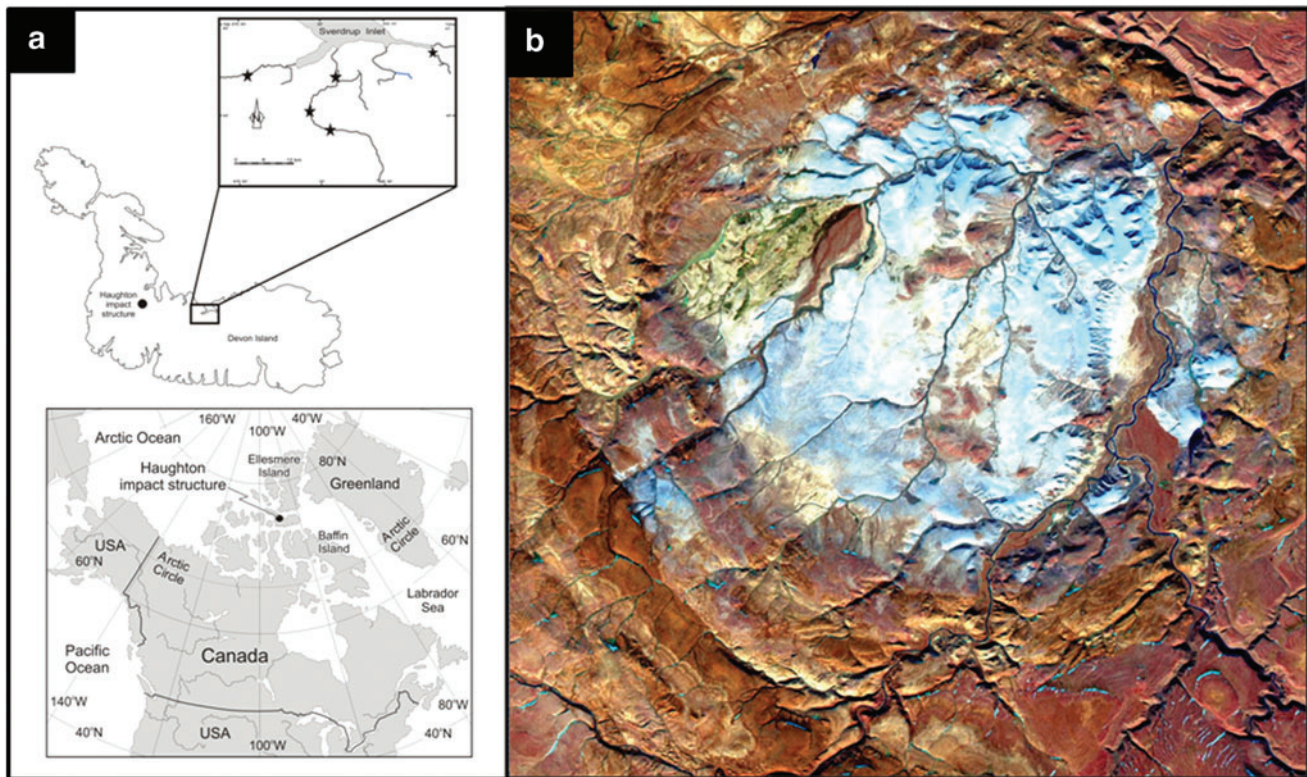


FIG. 1. (a) Map detailing location of Devon Island in the Canadian High Arctic (bottom) as well as location of collected unshocked basement samples (top) and (b) Landsat true-color image of the Haughton impact structure. Adapted from Pontefract *et al.* (2014) and Osinski *et al.* (2005). (Color graphics available at www.liebertonline.com/ast)

environment from which these specimens were collected generally yields communities with significant desiccation tolerance. To further confirm this statement, the collected sequence and diversity data (see Results section) were also used to ensure no significant bias due to storage conditions.

2.2. DNA extraction, sequencing, and statistical analysis

An assemblage of 21 samples—divided into three populations—that comprised unshocked (0), moderate shock (levels 3 and 4), and high shock (levels 6 and 7) were examined for bacterial 16S rRNA gene analysis; three samples (1 per population) were examined for fungal and eukaryal SSU and 23S rRNA gene analysis. Ten grams of sample was weighed out and powdered with a sterile alumina mortar and pestle in a laminar flow hood. DNA extraction was then performed using the PowerMax Soil DNA Isolation Kit (MoBio, Carlsbad, CA, USA) according to the manufacturer's instructions. Samples were sequenced using the Roche 454 FLX and Genome Sequencer with TITANIUM sequencing chemistry at Research and Testing Laboratories (Austin, TX, USA). Primer sets used by Research and Testing were as follows: Bacterium-based primers 28F: 5'-GAG TTT GAT CNT GGC TCA G-3' and 519R: 5'-GTN TTA CNG CGG CKG CTG-3' (Fan *et al.*, 2012); Algal-based primers (23S plastid, V5) p23SrV_f1: 5'-GGA CAG AAA GAC CCT ATG AA-3' and p23SrV_r1: 5'-TCA GCC TGT TAT CCC TAG AG-3' (Sherwood and Presting, 2007); and Fungal-based primers SSUf: 5'-TGG AGG GCA AGT CTG GTG-3' and SSUr: 5'-TCG GCA TAG TTT ATG GTT AA-3' (Hume *et al.*, 2012).

The MOTHUR software suite v.1.34.4 was used for the analysis of the 454 data (Schloss *et al.*, 2009), and standard operating procedures were followed with regard to sequence trimming, sequencing error removal, chimera checking, and generation of operational taxonomic units (OTUs), with a 97% cutoff. OTU data were normalized to 541 for each sample, corresponding to the smallest sample size. At this stage, sample S1001A was discarded from further analysis as it was well below the cutoff, containing only 30 OTUs. Alpha diversity indices were calculated for the remaining 20 samples: Shannon's index, Simpson's index, and Chao's index. Good's coverage values were calculated for OTUs at the 0.03 level of definition (Good, 1953). MOTHUR was also used to run an analysis of molecular variance (AMOVA) and to generate a nonmetric multidimensional scaling (NMDS) plot using Bray-Curtis similarities.

2.3. Culturing and microscopy

To determine the viability of the phototrophic endolithic community, small, 1 cm³ rocks from varying shock levels were placed in BG-11 media for enrichment at 24°C for 1 month using a 16 h daylight: 8 h dark cycle. Enrichments were then plated on BG-11 agar to isolate species, which were imaged with a Nikon Eclipse LV100POL compound microscope. Identification of isolates was made in accordance with the work of Castenholz and Waterbury (1989) or Wehr (2003). Isolates were also plated on NO₃-deficient agar plates to identify nitrogen fixers within the community [for a list of all nitrogen fixers, see Raymond *et al.* (2004)]. For visualization of *in situ* colonization, samples of shocked gneiss were either fractured into small pieces by using a

sterile technique and imaged directly with secondary electrons or were embedded in plastic for back-scattered electron microscopy (SEM-BSE) and energy-dispersive X-ray spectroscopy (EDX). Fractured pieces were fixed in glutaraldehyde, dehydrated in ethanol and critical-point dried to preserve cell structure, platinum-coated by a Denton Vacuum Desk II sputter coater at 12 mA for 150 s, and imaged. For embedded samples, fresh faces were fixed in 2% glutaraldehyde, stained with a 0.1 M OsO_4 -cacodylate buffer, and embedded with EmBed following the protocol from Electron Microscopy Sciences, Hatfield, Pennsylvania (Dykstra and Reuss, 2003).

2.4. Density and porosity

Of the 21 samples used for DNA extraction, 15 were chosen for porosity analysis. Grain density was determined by helium pycnometry (Wilkison *et al.*, 2003; Tamari, 2004) and bulk density determined from volume analyses by X-ray micro-computed tomography 3-D images (McCausland *et al.*, 2010), which could then be used to calculate sample porosity. The advantage of these methods is that they are nondestructive, so the measured properties most directly represent the rock porosity encountered by the microbial community. A Quantachrome Multipycnometer was used to obtain the grain

volume for each sample via the ideal gas law through a minimum of five repeat measurements (Tamari, 2004). Each selected sample was also imaged with X-ray microcomputed tomography using a GE eXplore speCZT scanner at the Roberts Research Institute, Western University (London, Canada). The scanner operated with a peak X-ray voltage of 90 kV and a current of 40 mA. In an angular increment of 0.4 degrees, 900 radiograph views were acquired around the sample in about 5 min. These data were reconstructed into a 3-D image volume with an isotropic voxel size of 50 μm . Using the image grayscale values of air and water, we calibrated all the image data into standard Hounsfield units. CT images were analyzed with GE Microview software to obtain sample bulk volumes using a thresholding method (McCausland *et al.*, 2010). Sample porosity was calculated from the grain and bulk densities rather than from the volumes because of slight changes in sample mass between the applications of the methods, especially for friable samples.

3. Results

3.1. Culturing and microscopy

Samples of shocked gneiss placed in BG-11 nutrient media showed considerable growth after 1 month. In most cases,

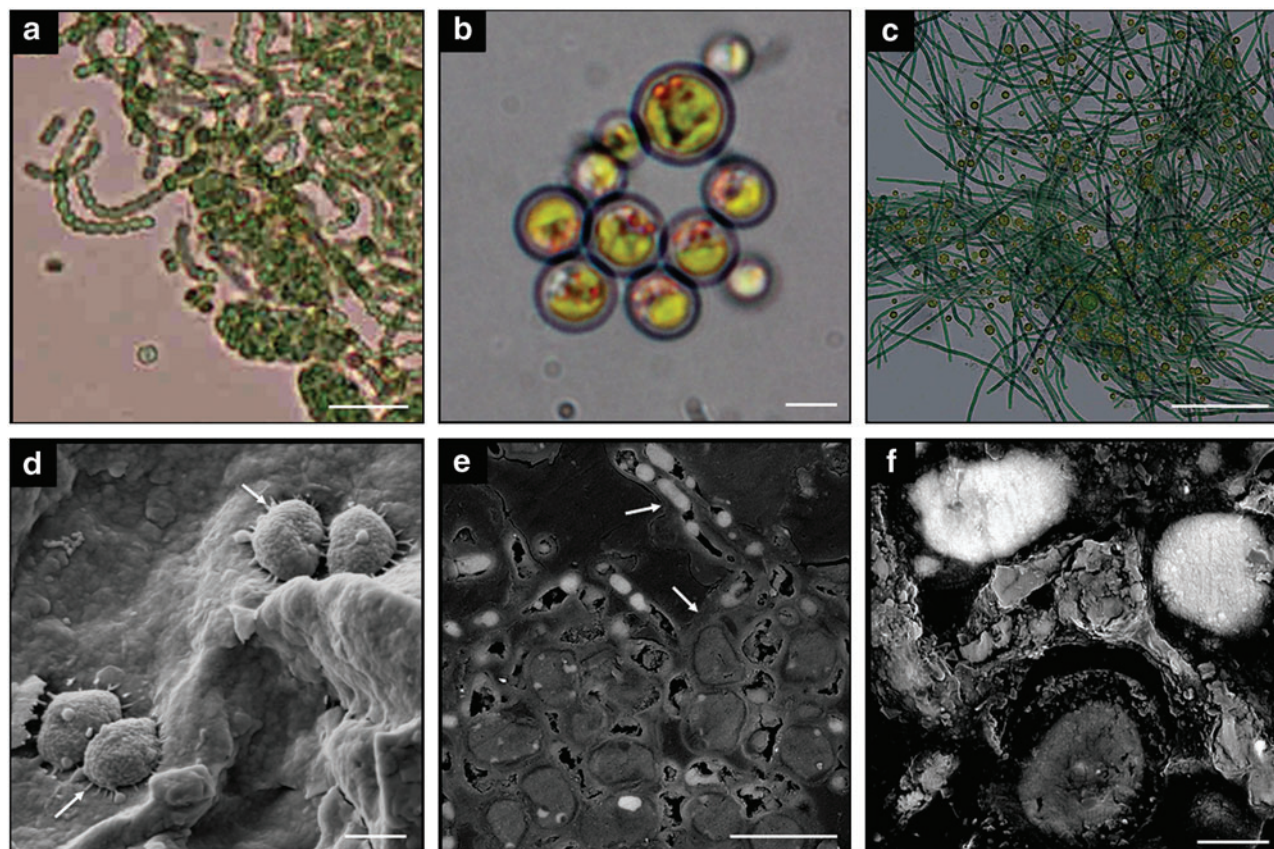


FIG. 2. Micrographs of cultured isolates from shocked gneisses (upper panels) and SEM micrographs of *in situ* colonization within highly shocked gneisses (lower panels). (a) Micrograph of *Anabaena* spp.; bar is 25 μm . (b) Micrograph of coccoid algae, *Chlorella* spp.; bar is 10 μm . (c) Micrograph of culture from shocked gneiss in BG-11 with *Chlorella* and a filamentous cyanobacterium; bar is 100 μm . (d) Secondary electron image of bacterium with EPS (arrows); bar is 600 nm. (e) SEM-BSE image of an endolithic colony consisting of large coccoid organisms and rod-shaped bacteria; EPS is delineated by arrows; bar is 10 μm . (f) SEM-BSE image of endolithic bacterium fossilized in calcium; bar is 2 μm . (Color graphics available at www.liebertonline.com/ast)

growth of cyanobacteria and algae within the flasks completely engulfed the rock, which was frequently no longer visible. Culturing methods revealed several species of cyanobacteria, which could be determined to the genus level: *Oscillatoria* spp., *Nostoc* spp., *Anabaena* spp. (Fig. 2a), *Gloeocapsa* spp., *Chroococciopsis* spp., *Leptolyngbya* spp., and one species of alga, *Chlorella* sp., which was also detected through pyrosequencing (Fig. 2b). Analysis of 454 sequence

reads showed 22 species present within the populations belonging to genera that are known to have N₂-fixing representatives, such as *Rhizobium*, *Chroococcales*, *Nostoc*, *Oscillatoria*, *Calothrix*, *Prochlorales*, *Rhodospirillales*, *Burkholderiales*, and *Chromatiales*. Isolated colonies from the rocks grown in BG-11 were all plated on nitrogen-deficient BG-11 agar and grown for 3 weeks. Growth of *Leptolyngbya* and *Anabaena* were both observed, but no other isolate showed growth,

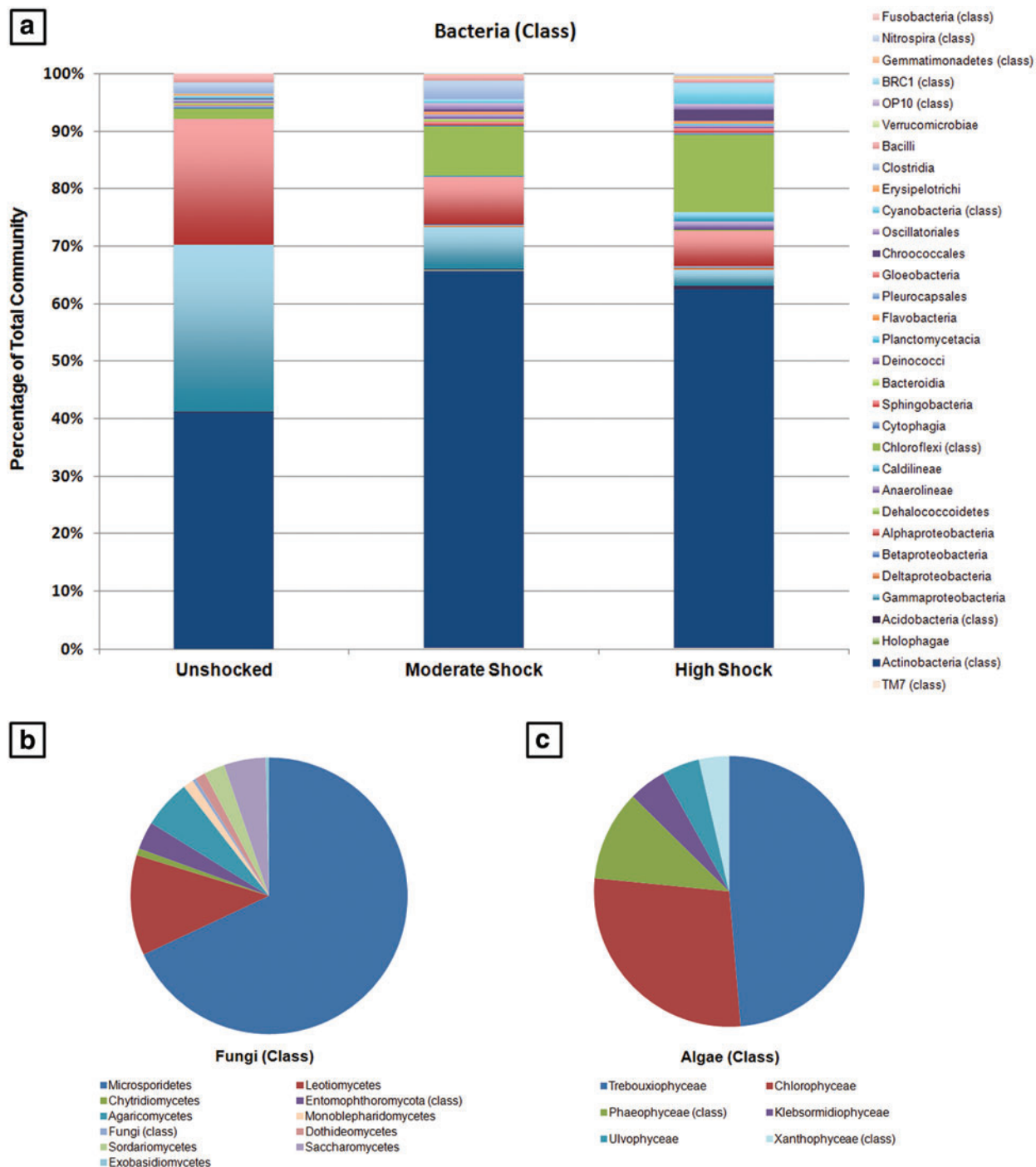


FIG. 3. (a) Percentage abundances of 16S rRNA for the bacterial communities from unshocked, moderate shock, and high shock gneisses. (b) Percentage abundance of SSU RNA for the fungal community of a highly shocked gneiss. (c) Percentage abundance of algal 18S rRNA for the highly shocked gneiss. All data are represented at the class level and were pooled together for each shock level. (Color graphics available at www.liebertonline.com/ast)

indicating that the species of *Oscillatoria* in culture was not capable of N₂ fixation.

Scanning electron microscopy imaging of field samples revealed extensive endolithic colonization, associated with pore spaces within the rocks. Microbes were seen either directly bound to the substrate (Fig. 2d), as a massive colony contained within exopolymeric substance (EPS) (Fig. 2e), or fossilized within calcite (Fig. 2f), a prevalent depositional material within the Haughton impact structure (Osinski *et al.*, 2005). See Supplementary Figs. S1 and S2 for EDX data.

3.2. 16S rRNA analysis and diversity calculations

A total of 2,795 OTUs were generated from 44,534 sequences, with a mean length of 244 nucleobases and a Good's coverage >90%. Analyses of the total bacterial 16S rRNA showed marked statistical differences between the unshocked and shocked populations, revealing some general trends. A total of 45% of OTUs belonged to the high shock community, 36% to the moderate shock community, and 18% to the unshocked community (see Supplementary Fig. S3 for a Venn diagram of un-sampled sequences). The unshocked samples consisted primarily of three classes of bacteria: Actinobacteria (40%), Gammaproteobacteria (29%), and Alphaproteobacteria (21%), with a small contribution from the Clostridia and the Bacilli. Among the different shock levels, Actinobacter and Fusobacteria classes were ubiquitous throughout, with the Actinobacteria having the largest population among all communities (as high as 65%). A small number of OTUs (65) were shared between all three communities, and in general, species that were largely present in the unshocked communities were poorly represented within the shocked samples and vice versa. For example, with the exception of a few OTUs, both the Alphaproteobacteria and Gammaproteobacteria decrease by almost an order of magnitude with increasing shock (*i.e.*, 21% and 29%, down to 5% and 2.5%, respectively), whereas the Betaproteobacteria are largely present only within the shocked samples (Fig. 3a).

Algal and fungal sequencing was completed for only three samples (one from each shock community) to serve as an indicator of species present (Fig. 3b, 3c). Within the algae, only the moderate and high shock samples could be amplified (BE009A13 and AH003A, respectively), and both low and moderate shock samples failed to generate amplicons for fungal analysis. The moderately shocked sample analyzed for algae was composed entirely by an OTU belonging to the Chlorophyta, most closely affiliated (97%) with *Rosenvingiella radicans*. The algal community in the highly shocked sample showed more taxonomic diversity, consisting of 9 OTUs within the Chlorophyta (though still dominated by *R. radicans*) and 1 of Streptophyta: *Klebsormidium flaccidum*, a filamentous green algae. The fungal OTUs (Fig. 3b) from sample AH003A were dominated by the Ascomycota, consisting of sequences most closely affiliated with *Thelidium decipiens* and *Placidiopsis cinerascens*, and the Microsporidia (*Systemostrema alba*), a parasitic fungus of animals, with a small contribution from the Basidiomycota in the form of mushroom-forming spores such as *Cortinarius sodagnitus*. Focusing solely on the bacterial phototrophic community, the largest contributor within the rocks came from the Chloroflexi (class), which represented 2% of sequence reads in the unshocked lithologies, increasing to 16% in the high shock samples (Fig. 4). The dominant order at all shock levels was Chloroflexalus, with small contributions from the Caldilineales, a strictly anaerobic order (Yamada *et al.*, 2006). The Cyanobacteria were not largely present (maximum of 6% of sequences in the highly shocked samples, though counts did increase by an order of magnitude between each of the unshocked, moderately shocked, and highly shocked samples), having a minor contribution from the Chroococcales and Oscillatoriales, the latter of which was only present within the shocked lithologies.

Alpha diversity analyses using the inverse Simpson index (*D*), Shannon-Weiner index (*H*), and Chao index all showed an increase both in species richness and diversity with increasing shock (see Supplementary Table S3 for confidence

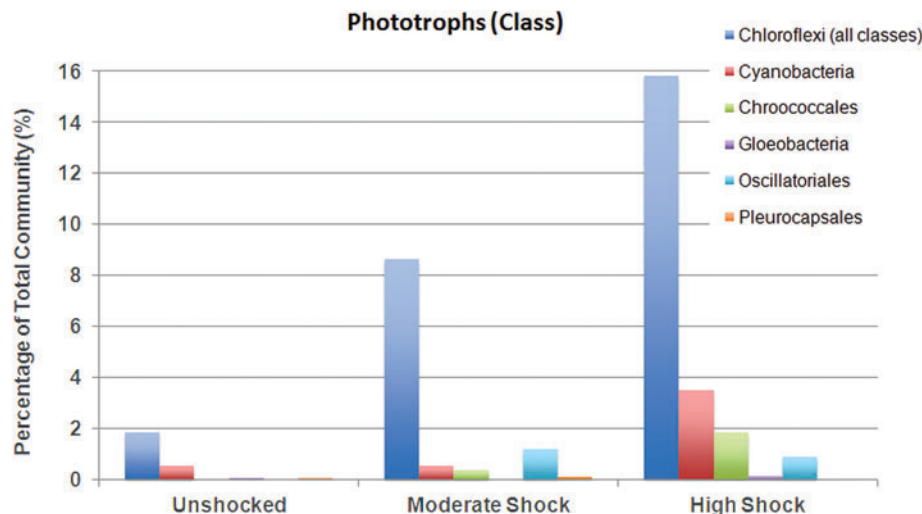


FIG. 4. Percentage abundance of the phototrophic bacterial communities from the unshocked, moderately shocked, and highly shocked gneiss pools, presented at the class level as a function of the percentage of the total bacterial community. Chloroflexi classes have been grouped for the sake of clarity, as all Chloroflexi classes from the sequence reads (other than the class Chloroflexi) had only a small contribution. (Color graphics available at www.liebertonline.com/ast)

TABLE 1. LIST OF DIVERSITY STATISTICS AND PHYLUM ABUNDANCES FOR UNSHOCKED, MODERATE SHOCK, AND HIGH SHOCK POPULATIONS

	<i>Unshocked</i>	<i>Moderate shock</i>	<i>High shock</i>
Alpha Diversity Indices*			
Shannon's index	2.4 ± 1.1	4.1 ± 0.2	4.6 ± 0.2
Inverse Simpson	7.4 ± 5.8	29.6 ± 1.6	48.6 ± 21.4
Chao's richness	79.2 ± 48.3	235.5 ± 78.2	354.3 ± 39.8
Bacterial Phylum Abundance (%)			
Actinobacteria	41.1	65.2	59.4
Acidobacteria	0.1	0.4	0.8
Proteobacteria	51.1	16.9	13.9
Firmicutes	3.3	4.3	0.7
Bacteroidetes	1.0	1.6	1.4
Deinococcus	0.5	0.5	0.5
Planctomycetes	0.1	0.1	0.2
Verrucomicrobia	0	0	0.1
Armatimonadetes	0	0	0
BRC1	0	0	0
Gemmatimonadetes	0	0.1	0.3
Nitrospirae	0	0.1	0.4
Fusobacteria	0.2	0	0
Chloroflexi	1.9	8.7	15.9
Cyanobacteria	0.7	2.2	6.4
Algal Phylum Abundance (%)			
Phaeophyceae	—	0	10.8
Xanthophyceae	—	0	3.6
Chlorophyta	—	100	81.1
Streptophyta	—	0	4.5
Fungal Phylum Abundance (%)			
Microsporidia	—	—	22.2
Ascomycota	—	—	74.0
Chytridiomycota	—	—	0.7
Entomophthoromycota	—	—	1.1
Basidiomycota	—	—	2.0
Fungi (phylum)	—	—	0.1

Standard deviations are provided for all diversity indices.

*Alpha indices calculated only for the bacterial 16S rRNA genes.

intervals). H increased from 2.4 to 4.6, and the inverse Simpson gave a diversity coefficient of 7.4 in unshocked samples, to 29.6 in moderate shock and 48.6 for the high shock community (Table 1). An AMOVA was conducted to test whether the centers of the clouds for each population were statistically separated. All comparisons, except for that of high shock–moderate shock revealed a statistically separate population, where high shock versus unshocked had a p value of 0.01, moderate shock versus unshocked had $p < 0.005$, and a three-way comparison of all communities yielded a p value of 0.002. The NMDS plot was conducted using three dimensions in order to reduce stress on the system and had a final stress value of 0.24 and an R^2 of 0.46. The plot (Fig. 5) only showed clear grouping between the unshocked and shocked populations, with the moderately shocked group clustering within the highly shocked group, though having its own distinct center. Samples of the same shock level group, but from different years and different storage conditions, yielded comparable numbers of sequence reads, while also maintaining similar levels of observed species diversity. For example, within the unshocked sample group, the average number of sequence reads was 2395 for samples collected in 2000 ($D = 8.84$) versus 2465 for samples collected in 2010 ($D = 5.2$), a dif-

ference of 2.9%, indicating that little to no sample degradation occurred.

3.3. Porosity measurements

Porosity measurements for this study represent a subset of a much larger data set and follow a clear exponential trend with increasing shock level (see Pontefract *et al.*, 2014). Values for the crystalline lithologies typically range from between 0% (for unshocked) and up to 70% for highly shocked samples. For the samples used in this study (see Supplementary Tables S1 and S2), unshocked lithology porosities range from 0% to 4% of rock volume, whereas the high shock samples range from 34% to 62% porosity. Porosity values typically show a high degree of variance within each shock level demarcation (*e.g.*, Singleton *et al.*, 2011), and a Pearson correlation analysis of porosity with diversity (inverse Simpson) shows a high degree of correlation, $R^2 = 0.79$. When plotted against each other, a simple linear regression gives an R^2 of 0.62 (Fig. 6).

4. Discussion

This study represents the first comprehensive assessment of a microbial community within impact-generated lithologies

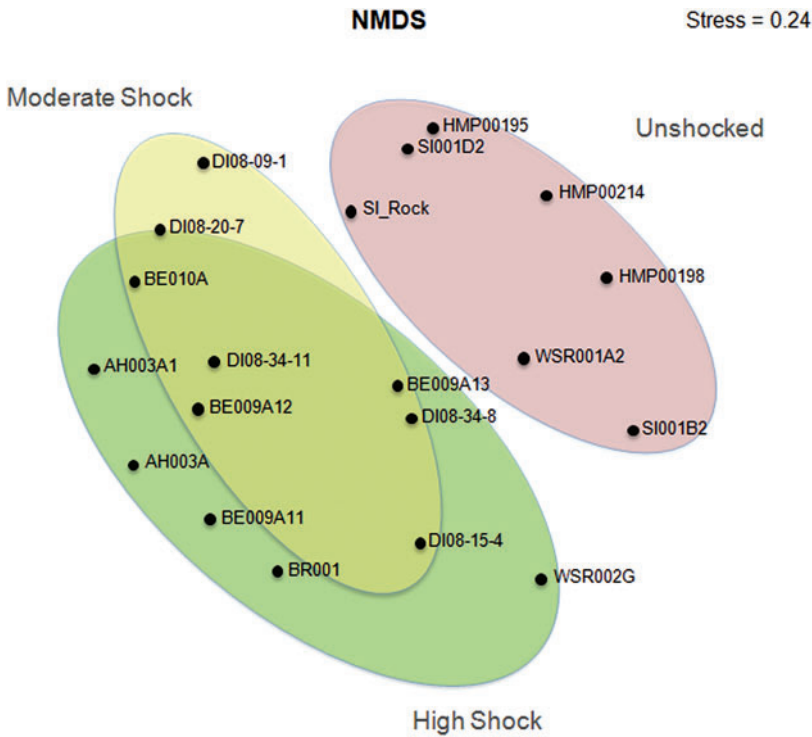


FIG. 5. Nonmetric multidimensional scaling (NMDS) plot for bacterial 16S rRNA from unshocked, moderately shocked, and highly shocked populations (AMOVA, $n=20$, $p<0.01$ for division in a pairwise comparison between unshocked and highly shocked populations, and $p<0.005$ for pairwise between unshocked and moderate shock). High shock samples do appear to show some grouping distinct from the unshocked communities but plot very closely with moderate shock samples, though the centers are different. (Color graphics available at www.liebertonline.com/ast)

and represents one of the few culture-independent studies of land-based high-Arctic microbial communities (e.g., Nernberg *et al.*, 2005; Wilhelm *et al.*, 2011; Deslippe *et al.*, 2012; Ziolkowski *et al.*, 2013; Rhind *et al.*, 2014).

Similar to Arctic permafrost communities, the Actinobacteria were the largest contributors to the endolithic community at all three shock levels, though very few OTUs were shared between groups; only 1.2% of Actinobacteria sequences were common to all three shock levels, generally belonging to the order Actinomycetales (77%) and Acidimicrobiales (23%). The unshocked lithologies contained a community structure close to that of an Arctic soil study based in Alaska (Deslippe *et al.*, 2012), having abundant Proteobacteria (specifically within the Alphaproteobacteria and Gammaproteobacteria—both of which contain N_2 -fixing species) and low (<3%) levels of the Firmicutes, Bacteroidetes, and Chloroflexi. Interestingly, the Gemmatimonadetes (Gram-negative aerobic bacteria), which are generally quite prevalent in Arctic soils, were found only within the high shock level samples. The higher shocked samples had microbial compositions similar to that of Fike *et al.* (2003) and Pellerin *et al.* (2009)—though their study did not identify any phototrophic contribution—and the permafrost layer communities described by Steven *et al.* (2007) and Wilhelm *et al.* (2011). Within endolithic communities, it has been suggested that nitrogen fixation is generally rare (Friedmann, 1982). We do not find this to be the case in our study, although the N_2 -fixing species, such as *Rhizobium*, were found only within sequence reads from shocked lithologies and were also accompanied by an increase in the phototrophic communities, several species of which are diazotrophs. Presumably, some nitrogen input for the community was also likely provided through atmospheric fixation and deposition (Walker and Pace, 2007).

4.1. The phototrophic community

The most profound effects of shock on the microbial community were seen within the phototrophic organisms, spanning four phyla within the bacteria. The Chloroflexi (green nonsulfur bacteria) were the dominant phototroph in each shock level (2%, 9%, and 16%, respectively), over the Cyanophyta, which, even in the high shock level samples, only reached an abundance of 6%. In addition, there was a very small addition to the phototrophic community in the Rhodospirillaceae, a photoorganoheterotroph also from the green nonsulfur bacteria. Two previous studies mention the presence of Chloroflexi within soils or endolithic communities (Deslippe *et al.*, 2012; Rhind *et al.*, 2014). This is in contrast, however, to other literature on Antarctic and Arctic endolithic communities that have consistently found cyanobacteria to be the dominant phylotype—up to 56% (e.g., de la Torre *et al.*, 2003; Pointing *et al.*, 2009). In a study by Ziolkowski *et al.* (2013), who examined microbial community composition in gypsum crusts on Axel Heiberg, the same primer sets were used as in the present study, yet cyanobacteria were found to be the dominant organism in the system. This study shows that, even if bias exists, this primer set can satisfactorily resolve cyanobacterial taxa within samples. A few studies have indicated anomalously high numbers of Chloroflexi sequences in their data sets (e.g., Costello and Schmidt, 2006; Wilhelm *et al.*, 2011), but the reason behind the occurrence of such high abundances in these locations remains unclear. Within the shocked gneiss community, the cyanobacteria present consisted of Chroococcales, Nostocales, Oscillatoriales, few of which were cultured in the lab. Cyanobacteria in endolithic communities from Antarctica appear to be much more diverse, containing 112 distinct species, many of

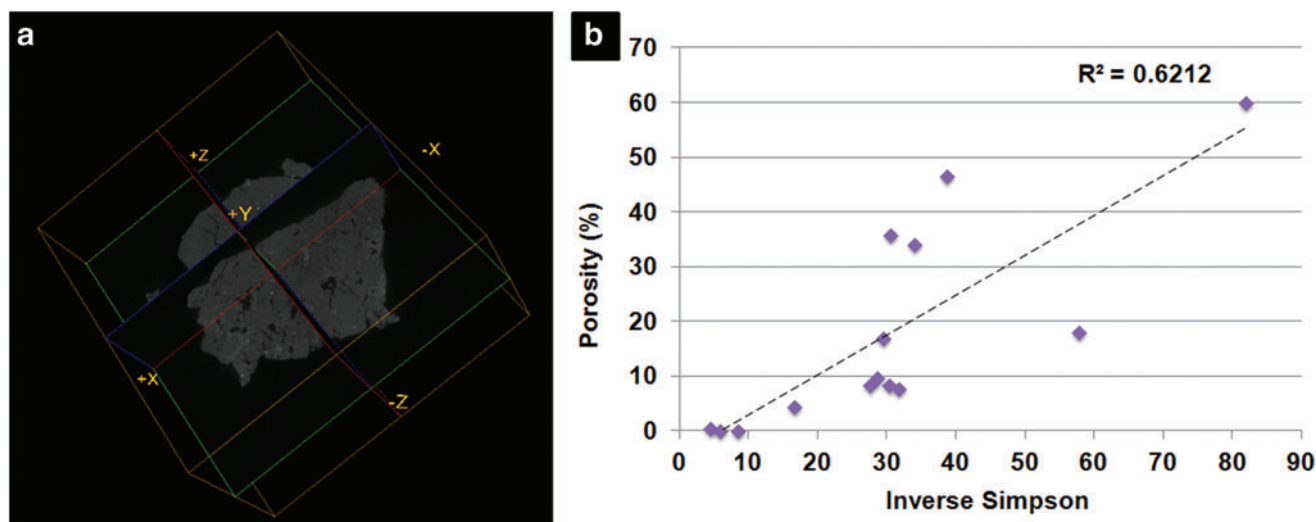


FIG. 6. (a) X-ray CT reconstruction of a highly shocked gneiss (AH003A1) with abundant, visible porosity. (b) Graph showing linear regression of calculated porosity values versus inverse Simpson values. Regression yields and R^2 of 0.62 and Pearson correlation, an R^2 of 0.79. (Color graphics available at www.liebertonline.com/ast)

which are unknown (Pointing *et al.*, 2009), compared to 62 found in the shocked gneisses. This is unusual considering the fact that the Arctic environment is considerably less extreme than that of the Antarctic, receiving much larger amounts of precipitation throughout the year and experiencing warmer temperatures, resulting in less stringent selective pressures (Omelson *et al.*, 2006). In addition, the sandstones of Antarctica have a porosity of between 2.3% and 13.0% (Cockell *et al.*, 2003), compared to the shocked gneisses, which can reach porosities of over 60% (Singleton *et al.*, 2011). It is clear, however, that the level of porosity does affect the colonization of shocked gneisses by phototrophs (both Cyanobacteria and Chloroflexi), or it is a main factor, evidenced both by the marked increase in bacterial diversity and species richness with increasing shock (Table 1) and by the presence of eukaryotic algae and large filamentous cyanobacteria, such as Oscillatoriales. In this instance, the increased porosity would allow for up to 100% transmission of light in some areas (Pontefract *et al.*, 2014) and provide sufficient space previously unavailable for such relatively large organisms.

Neither the algal nor fungal species within these samples appeared to be strong contributors to the overall community, existing at very low abundances, which is in keeping with previous microscopic observations by Cockell *et al.* (2003) and Omelson *et al.* (2006), though work on a larger sample size must be completed. As a whole, heterotrophic bacteria dominated the community, with the phototrophs supplying, at most, 12% of the sequence reads. This suggests that the organisms must, in addition to using carbon sourced from the primary producers, rely on an exogenous form of carbon input from the environment, likely along with other nutrients such as phosphorus and nitrogen. Without knowing the composition of the active community, it is difficult to surmise the metabolic diversity of these Arctic endolithic communities, and an investigation of the active community should be completed. The presence of so many bacterial species in such a harsh environment, as well as a plausible reliance on external sources of nutrients, indicates that few

species might occupy metabolic niches, with the remainder of the population potentially using similar metabolic pathways (Fierer and Lennon, 2011). The dominant presence of Actinobacteria within all the samples does show that a significant amount of carbon turnover is possible within these communities, made more likely by the wetter conditions experienced in the Arctic.

4.2. Impact-generated diversity

Overall, a significant change in microbial community composition with increasing shock pressure in the gneisses was observed, revealing the presence of distinct populations, likely based on the level of created porosity and light transmission within the rock. AMOVA was able to show that there is a statistical difference in community composition between the unshocked and shocked populations; however, due to the high variability inherent in the samples, the moderately and highly shocked communities showed no significant difference from each other. This is likewise reflected in the NMDS plot (Fig. 5), which, aside from showing a more distinct difference between unshocked and shocked populations, is not able to visually resolve any further patterns related to host-rock shock metamorphism. The high stress level (0.24) also indicates that the 3-D plot is not an optimum representation of the data. That porosity is highly variable within each shock level and that it correlates with species diversity accounts for a large amount of the variation seen in the NMDS plot, as well as in the standard deviations for the diversity indices in the high shock level communities. Other factors that affect diversity may have to do with placement along flat-lying or inclined areas within the crater, which would influence drainage, the amount of sun exposure experienced, and the amount of airborne material received. Geographical location did not appear to be a determining factor, as some samples collected from opposite regions within the crater plot together, while samples collected near each other along the same escarpment do not. Future work in this area requires a detailed ecological study

of the crater region so as to determine the effects of variable light incidence and precipitation on shocked lithologies and how these variables may interact to influence community composition.

4.3. Astrobiological implications

A wide range of studies spanning polar and nonpolar desert endolithic communities has shown that such populations are highly specialized and, in many cases, ideally adapted to surviving such harsh conditions (Friedmann *et al.*, 1993; de la Torre *et al.*, 2003; Wierzchos *et al.*, 2006; Walker and Pace, 2007; Omelon, 2008). As a result of this, these organisms tend to be remarkably persistent over long periods of geological time (Pointing *et al.*, 2009). What does this mean for potential life elsewhere in our solar system? On Mars for example—which is essentially a polar desert, albeit an extreme one—impact cratering and the generation of shocked lithologies as well as transient hydrothermal systems (Osinski *et al.*, 2013) is an important geological process. If life existed on Mars in the early Noachian, it is plausible that, as conditions began to deteriorate and Mars transitioned into a cold, dry planet, life may have survived in the abundant endolithic environments afforded by impact craters. Early work by Newsom (1980) posited the presence of widespread deposits of impact melt on Mars, which was confirmed in a recent study by Cannon and Mustard (2015), in which extensive deposits of glass-rich impactites were revealed. Not only do surficial brecciated deposits provide attractive targets for life-detection missions, but endolithic environments could likely have formed deep within the fractured subsurface of these craters (Boston *et al.*, 1992; Ascaso and Wierzchos, 2002; Cockell *et al.*, 2012). Future infilling of these deposits may then have preserved these organisms, making these brecciated, impact-melt lithologies an attractive target for future life-detection missions as well as for Mars sample return.

5. Concluding Remarks

This culture-independent study improves upon previous culture work on impact-generated lithologies and provides a clearer understanding of how microbial populations scale within different shock regimes. We found a dominant heterotrophic community at all shock levels, with a relatively small phototrophic community, largely dominated by the green nonsulfur bacteria. Altogether, the impact-created endolithic environment is a beneficial habitat within polar deserts, which has important implications for the potential for viable habitats on other terrestrial bodies in our solar system—especially in instances where impact cratering may be one of the only active geological processes.

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References

- Ascaso, C. and Wierzchos, J. (2002) New approaches to the study of Antarctic lithobiontic microorganisms and their inorganic traces, and their application in the detection of life in martian rocks. *Int J Microbiol* 5:215–222.
- Boston, P.J., Ivanov, M.V., and McKay, C.P. (1992) On the possibility of chemosynthetic ecosystems in subsurface habitats on Mars. *Icarus* 95:300–308.
- Cannon, K.M. and Mustard, J.F. (2015) Preserved glass-rich impactites on Mars. *Geology* 43:635–638.
- Castenholz, R.W. and Waterbury, J.B. (1989) Cyanobacteria. In *Bergey's Manual of Systematic Bacteriology*, Vol. 3, edited by J.T. Staley, M.P. Bryant, N. Pfennig, and J.G. Holt, Williams and Wilkins, Baltimore, MD, pp 1780–1799.
- Cockell, C.S. (2004) Impact-shocked rocks—insights into Archean and extraterrestrial microbial habitats (and sites for prebiotic chemistry?). *Adv Space Res* 33:1231–1235.
- Cockell, C.S. and Stokes, M.D. (2006) Hypolithic colonization of opaque rocks in the Arctic and Antarctic polar desert. *Arctic, Antarctic, and Alpine Research* 38:335–342.
- Cockell, C.S., Lee, P., Osinski, G.R., Horneck, G., and Broady, P. (2002) Impact-induced microbial endolithic habitats. *Meteorit Planet Sci* 37:1287–1298.
- Cockell, C.S., McKay, C.P., and Omelon, C.R. (2003) Polar endoliths—an anti-correlation of climatic extremes and microbial diversity. *International Journal of Astrobiology* 1: 305–310.
- Cockell, C.S., Lee, P., Broady, P., Lim, D.S.S., Osinski, G.R., Parnell, J., Koeberl, C., Pesonen, L., and Salminen, J. (2005) Effects of asteroid and comet impacts on habitats for lithophytic organisms—a synthesis. *Meteorit Planet Sci* 40:1901–1914.
- Cockell, C.S., Voytek, M.A., Gronstal, A.L., Finster, K., Kirshtein, J.D., Kallmeyer, J., Kelly, L., and Powars, D.S. (2012) Impact disruption and recovery of the deep subsurface biosphere. *Astrobiology* 12:231–246.
- Costello, E.K. and Schmidt, S.K. (2006) Microbial diversity in alpine tundra wet meadow soil: novel Chloroflexi from a cold, water-saturated environment. *Environ Microbiol* 8: 1471–1486.
- de la Torre, J.R., Goebel, B.M., Friedmann, E.I., and Pace, N.R. (2003) Microbial diversity of cryptoendolithic communities from the McMurdo Dry Valleys, Antarctica. *Appl Environ Microbiol* 69:3858–3867.
- Deslippe, J.R., Hartmann, M., Simard, S.W., and Mohn, W.W. (2012) Long-term warming alters the composition of Arctic soil microbial communities. *FEMS Microbiol Ecol* 82:303–315.
- Diels, L. (1914) Die Algen-Vegetation der Südtiroler Dolomittriffe. *Ber Dtsch Bot Ges* 32:502–526.
- Dykstra, M.J. and Reuss, L.E. (2003) *Biological Electron Microscopy: Theory, Techniques and Troubleshooting*, 2nd ed., Plenum Press, New York, pp 1–31, 86, 101.
- Fan, L., McElroy, K., and Thomas, T. (2012) Reconstruction of ribosomal RNA genes from metagenomic data. *PLoS One* 7, doi:10.1371/journal.pone.0039948.

- Fierer, N. and Lennon, J.T. (2011) The generation and maintenance of diversity in microbial communities. *Am J Bot* 98: 439–448.
- Fike, D.A., Cockell, C.S., Pearce, D., and Lee, P. (2003) Heterotrophic microbial colonization of the interior of impact-shocked rocks from Haughton impact structure, Devon Island, Nunavut, Canadian High Arctic. *International Journal of Astrobiology* 1:311–323.
- Friedmann, E.I. (1980) Endolithic microbial life in hot and cold deserts. *Orig Life* 10:223–235.
- Friedmann, E.I. (1982) Endolithic microorganisms in the Antarctic cold desert. *Science* 215:1045–1053.
- Friedmann, E.I. and Kibler, A.P. (1980) Nitrogen economy of endolithic microbial communities in hot and cold deserts. *Microb Ecol* 6:95–108.
- Friedmann, E.I. and Ocampo, R. (1976) Endolithic blue-green algae in the Dry Valleys: primary producers in the Antarctic desert ecosystem. *Science* 193:1247–1249.
- Friedmann, E.I., Kappen, L., Meyer, M.A., and Nienow, J.A. (1993) Long-term productivity in the cryptoendolithic microbial community of Ross Desert, Antarctica. *Microb Ecol* 25:51–69.
- Good, I.J. (1953) The population frequencies of species and the estimation of the population parameters. *Biometrika* 40:237–264.
- Hume, M.E., Hernandez, C.A., Barbosa, N.A., Sakomura, N.K., Dowd, S.E., and Oviedo-Rondón, E.O. (2012) Molecular identification and characterization of ileal and cecal fungus communities in broilers given probiotics, specific essential oil blends and under mixed *Eimeria* infection. *Foodborne Pathog Dis* 9:853–860.
- Jungblut, A.D., Lovejoy, C., and Vincent, W.F. (2010) Global distribution of cyanobacterial ecotypes in the cold biosphere. *ISME J* 4:191–202.
- Kieffer, S.W., Phahey, P.P., and Christie, J.M. (1976) Shock processes in porous quartzite: transmission electron microscope observations and theory. *Contributions to Mineralogy and Petrology* 59:41–93.
- Kring, D.A. and Cohen, B.A. (2002) Cataclysmic bombardment throughout the inner Solar System 3.9–4.0 Ga. *J Geophys Res* 107, doi:10.1029/2001JE001529.
- McCausland, P.J.A., Brown, P.G., and Holdsworth, D.W. (2010) Rapid, reliable acquisition of meteorite volumes and internal features by laboratory X-ray micro-CT scanning [abstract 2584]. In *41st Lunar and Planetary Science Conference Abstracts*, Lunar and Planetary Institute, Houston.
- Millar, C.D. and Lambert, D.M. (2013) Towards a million-year-old genome. *Nature* 499:34–35.
- Nemergut, D.R., Costello, E.K., Meyer, A.F., and Pescador, M.Y. (2005) Structure and function of alpine and Arctic soil communities. *Res Microbiol* 156:775–784.
- Newsom, H.E. (1980) Hydrothermal alteration of impact melt sheets with implications for Mars. *Icarus* 44:207–216.
- Omelson, C.R. (2008) Endolithic microbial communities in polar desert habitats. *Geomicrobiol J* 25:404–414.
- Omelson, C.R., Pollard, W.H., and Ferris, F.G. (2006) Chemical and ultrastructural characterization of high Arctic cryptoendolithic habitats. *Geomicrobiol J* 23:189–200.
- Osinski, G.R., Lee, P., Parnell, J., Spray, J.G., and Baron, M. (2005) A case study of impact-induced hydrothermal activity: the Haughton impact structure, Devon Island, Canadian High Arctic. *Meteorit Planet Sci* 40:1859–1877.
- Osinski, G.R., Tornabene, L.L., Banerjee, N.R., Cockell, C.S., Flemming, R., Izawa, M.R.M., McCutcheon, J., Parnell, J., Preston, L.J., Pickersgill, A.E., Pontefract, A., Sapers, H.M., and Southam, G. (2013) Impact-generated hydrothermal systems on Earth and Mars. *Icarus* 224:347–363.
- Pellerin, A., Lacelle, D., Fortin, D., Clark, I.D., and Lauriol, B. (2009) Microbial diversity in endostromatolites (*cf.* Fissure Calcretes) and in the surrounding permafrost landscape, Haughton impact structure region, Devon Island, Canada. *Astrobiology* 9:807–822.
- Pointing, S.B., Chan, Y., Lacap, D.C., Lau, M.C.Y., Jurgen, J.A., and Farrell, R.L. (2009) Highly specialized microbial diversity in hyper-arid polar desert. *Proc Natl Acad Sci USA* 106:19964–19969.
- Pontefract, A., Osinski, G.R., Cockell, C.S., Moore, C.A., Moores, J.E., and Southam, G. (2014) Impact-generated endolithic habitat within crystalline rocks of the Haughton impact structure, Devon Island, Canada. *Astrobiology* 14:522–533.
- Raymond, J., Siefert, J.L., Staples, C.R., and Blankenship, R.E. (2004) The natural history of nitrogen fixation. *Mol Biol Evol* 21:541–554.
- Rhind, T., Ronholm, J., Berg, B., Mann, P., Applin, D., Stromberg, J., Sharma, R., Whyte, L.G., and Cloutis, E.A. (2014) Gypsum-hosted endolithic communities of the Lake St. Martin impact structure, Manitoba, Canada: spectroscopic detectability and implications for Mars. *International Journal of Astrobiology* 13:366–377.
- Sapers, H.M., Osinski, G.R., Banerjee, N.R., and Preston, L.J. (2014) Enigmatic tubular features in impact glass. *Geology* 42:471–474.
- Schloss, P.D., Gevers, D., and Westcott, S.L. (2009) Reducing the effects of PCR amplification and sequencing artifacts on 16S rRNA-based studies. *PLoS One* 6, doi:10.1371/journal.pone.0027310.
- Schultz, P.H. and Mustard, J.F. (2004) Impact melts and glasses on Mars. *J Geophys Res* 109, doi:10.1029/2002JE002025.
- Sherwood, A.R. and Presting, G.G. (2007) Universal primers amplify 23S rDNA plastid marker in eukaryotic algae and cyanobacteria. *J Phycol* 43:605–608.
- Singleton, A.C., Osinski, G.R., McCausland, P.J.A., and Moser, D.E. (2011) Shock-induced changes in density and porosity in shock-metamorphosed crystalline rocks, Haughton impact structure, Canada. *Meteorit Planet Sci* 46:1774–1786.
- Steven, B., Briggs, G., McKay, C.P., Pollard, W.H., Greer, C.W., and Whyte, L.G. (2007) Characterization of the microbial diversity in a permafrost sample from the Canadian High Arctic using culture-dependent and culture-independent methods. *FEMS Microbiol Ecol* 59:513–523.
- Tamari, S. (2004) Optimum design of the constant-volume gas pycnometer for determining the volume of solid particles. *Meas Sci Technol* 15:549–558.
- Walker, J.J. and Pace, N.R. (2007) Endolithic microbial ecosystems. *Annu Rev Microbiol* 61:331–347.
- Wehr, J. (2003) *Freshwater Algae of North America: Ecology and Classification*, Academic Press, Boston, pp 51–191.
- Wierzchos, J. and Ascaso, C. (2001) Life, decay and fossilisation of endolithic microorganisms from the Ross Desert, Antarctica. *Polar Biol* 24:863–868.
- Wierzchos, J., Ascaso, C., and McKay, C.P. (2006) Endolithic cyanobacteria in halite rocks from the hyperarid core of the Atacama Desert. *Astrobiology* 6:415–422.
- Wierzchos, J., Cámara, B., de los Rios, A., Davila, A.F., Sánchez Almazo, I.M., Artieda, O., Wierzchos, K., Gómez-Silva, B., McKay, C.P., and Ascaso, C. (2011) Microbial colonization of Ca-sulfate crusts in the hyperarid core of the

- Atacama Desert: implications for the search for life on Mars. *Geobiology* 9:44–60.
- Wilhelm, R.C., Niederberger, T.D., Greer, C., and Whyte, L.G. (2011) Microbial diversity of active layer and permafrost in acidic wetland from the Canadian High Arctic. *Can J Microbiol* 57:303–315.
- Wilkison, S.L., McCoy, T.J., McCamant, J.E., Robinson, M.S., and Britt, D.T. (2003) Porosity and density of ordinary chondrites: clues to the formation of friable and porous ordinary chondrites. *Meteorit Planet Sci* 38:1533–1546.
- Yamada, T., Sekiguchi, Y., Hanada, S., Imachi, H., Ohashi, A., Harada, H., and Kamagata, Y. (2006) *Anaerolinea thermolimos* sp. nov., *Levilinea saccharolytica* gen. nov., sp. nov. and *Leptolinea tardivitalis* gen. nov., sp. nov., novel filamentous anaerobes, and description of the new classes *Anaelolineae* classis nov. and *Caldilineae* classis nov. in the bacterial phylum Chloroflexi. *Int J Syst Evol Microbiol* 56:1331–1340.
- Ziolkowski, L.A., Mykytczuk, N.C.S., Omelon, C.R., Johnson, H., Whyte, L.G., and Slater, G.F. (2013) Arctic gypsum endoliths: a biogeological characterization of a viable and active microbial community. *Biogeosciences* 10:2269–2304.

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Abbreviations Used

AMOVA = analysis of molecular variance
 EDX = energy-dispersive X-ray spectroscopy
 EPS = exopolymeric substance
 NMDS = nonmetric multidimensional scaling
 OTUs = operational taxonomic units
 SEM-BSE = back-scattered electron microscopy