Niche Partitioning of Microbial Communities at an Ancient Vitrified Hillfort: Implications for Vitrified Radioactive Waste Disposal


Pacific Northwest National Laboratory, Richland, WA, USA; School of Biological Sciences, Washington State University, Richland, WA, USA; Department of Biology, Eastern Washington University, Cheney, WA, USA; Chemical Process and Nuclear Measurements Group, National Institute of Standards and Technology, Gaithersburg, MD, USA; Department of Civil, Environmental and Natural Resources Engineering, Luleå University of Technology, Luleå, Sweden; School of Mechanical and Materials Engineering, Washington State University, Pullman, WA, USA; Arkeologerna, Geoarchaeological Laboratory, National Historical Museums (SHMM), Uppsala, Sweden; US Department of Energy, Office of River Protection, Richland, WA, USA

ABSTRACT
Because microbes cannot be eliminated from radioactive waste disposal facilities, the consequences of bio-colonization must be understood. At a pre-Viking era vitrified hillfort, Broborg, Sweden, anthropogenic glass has been subjected to bio-colonization for over 1,500 years. Broborg is used as a habitat analogue for disposed radioactive waste glass to inform how microbial processes might influence long-term glass durability. Electron microscopy and DNA sequencing of surficial material from the Broborg vitrified wall, adjacent soil, and general topsoil show that the ancient glass supports a niche microbial community of bacteria, fungi, and protists potentially involved in glass alteration. Communities associated with the vitrified wall are distinct and less diverse than soil communities. The vitrified niche of the wall and adjacent soil are dominated by lichens, lichen-associated microbes, and other epilithic, endolithic, and epigeic organisms. These organisms exhibit potential bio-corrosive properties, including silicate dissolution, extraction of essential elements, and secretion of geochemically reactive organic acids, that could be detrimental to glass durability. Long-term biofilms can also possess a homeostatic function that could limit glass alteration. This study documents potential impacts that microbial colonization and niche partitioning can have on glass alteration, and subsequent release of radionuclides from a disposal facility for vitrified radioactive waste.

Introduction
Nuclear power represents the most efficient path to eliminate carbon emissions from energy production (IAEA 2016), but disposal of the by-product, radioactive waste, is a major challenge to widespread implementation. Legacy radioactive waste must be immobilized in a solid form that isolates the radionuclides from the environment for a specified time period. This can be achieved through vitrification, in which glass-forming oxides are added to the waste, heated to form molten glass, and cooled to produce a solid wasteform in which the radionuclides are part of the glass and can only be released upon structural degradation of the glass (EPA 2002). To ensure that radioactive elements, which can have half-lives of tens to thousands to millions of years, remain below regulatory limits once the glass is placed in a disposal facility, it is critical to understand the aqueous corrosion behavior of the glass wasteform under variable environmental conditions (temperature, pH, Eh, and microbial activity, etc.). However, obtaining experimental data to create models that estimate radioactive release from glass wasteforms over thousands of years in contact with the disposal facility’s near-field environment, and its associated microbiome, is challenging. Hence, well-documented archeological glass artifacts provide a fortuitous means of observing glass wasteform-related alteration over millennia, under natural environmental conditions, and including interaction with microbes (Gin et al. 2017).

The glasses found in Iron Age vitrified stone hillforts of northern Europe, dating mostly from 400 to 850 CE, with some from as early as 450 BCE (Childe and Thorneycroft 1938), can potentially be suitable alteration analogues for the study of radioactive waste glass corrosion, because they are of known composition and are from known alteration environments (Sjöblom et al. 2016; Weaver et al. 2016, 2018). Archeological glass artifacts from Broborg, a pre-Viking vitrified hillfort site in southeastern Sweden (≈400 CE) are...
being studied by an international, multi-institutional team of archaeologists, geochemists, microbial ecologists, and glass scientists (Kresten and Ambrosiani 1992; Kresten et al. 1993, 1996, 2003), to enable more accurate prediction of the long-term corrosion behavior of glass (Sjöblom et al. 2016). The granitic stone walls at Broborg were fortified, in-situ, with a glassy material derived from melted amphibolite rock acting as mortar. The melting of predominately two rock types produced different glass chemistries at Broborg that have been shown to be suitable analogues for vitrified nuclear waste glass (Weaver et al. 2016, 2018). The vitrified material in the hillfort wall has been in contact with soil and subsoil and associated microbiomes for ≈1,500 years, offering the opportunity to empirically study glass alteration in an environment analogous to that expected for near-surface waste glass disposal, such as the Integrated Disposal Facility (IDF) at the Hanford Site, Washington state, USA, over a time-scale of thousands of years (Weaver et al. 2016, 2018). In previous analyses of Broborg glasses to assess their potential as alteration analogues for the study of radioactive waste glass corrosion, evidence of microbial populations interacting with the glass was provided by electron microscopy (Weaver et al. 2018). Combined microscopic and spectroscopic analysis revealed morphological and chemical alterations of the substrate consistent with microbial activity, suggesting that microbes may influence glass corrosion behavior. However, no attempts to identify the relevant microbial species were made (Weaver et al. 2018). Thus, the examination of the long-term fate of Broborg glass, with emphasis on the associated microbiome, was warranted, to identify and better understand the role microbes may play in glass corrosion.

Microbes are involved in the weathering of rock and mineral-based substrates (silicates, phosphates, carbonates, sulfides, oxides) and have the ability to influence glass alteration through biochemical and biophysical processes (Gadd and Dyer 2017; Junier and Joseph 2017; Mellor 1923; Weaver et al. In review). A study of basalt weathering by the model fungus Aspergillus sp. FS-4, showed that geologic material with a higher glass percentage was more prone to microbial weathering (Hu et al. 2020). Although the role of microbes in geological disposal systems for the isolation of radioactive waste is recognized, only limited short-term laboratory tests have been conducted in attempts to assess potential microbial effects on the alteration of vitrified radioactive waste over thousands of years (Pierce EM et al. 2004; Weaver et al. 2018, In review). Microbial influence on glass alteration was recognized in the 2017 performance assessment to determine the impact of radioactive waste disposal at the IDF on the nearby population and environment (DOE 2017). However, due to limited data, it was not possible to determine how sensitive the performance of low activity waste glass would be to microbial influence. Attempts made in the early 2000s to obtain data on biocorrosion potential were limited by an experimental design that did not account for abiotic effects (Pierce EM et al. 2004).

This work represents a first of a kind study, using Broborg as a habitat analogue for vitrified radioactive waste in a near-surface disposal facility. Although vitrified high-activity radioactive wastes are to be stored in the deep subsurface, here we focus on near surface disposal of low-activity radioactive waste, where conceptual models of facility performance assume direct contact between vitrified waste and near surface soil, as waste containers corrode, and surface barriers degrade over time (DOE 2017). High levels of radioactivity associated with high-activity waste could significantly affect the diversity of microbes colonizing the vitrified waste, but for vitrified low-activity waste, the effect of radioactivity on the microbial community will be limited, allowing the glass at Broborg, exposed to low levels of background ionizing radiation, to be considered a suitable analogue. The acute exposure of an intruder into the IDF is limited to a dose equivalent of 5 millisievert (mSv) (DOE 2017), and there is extensive evidence for radiation-resistance in bacteria and fungi at absorbed doses many times higher (15,000 gray (Gy), with 1 Gy equal to 1 Sv for the most penetrating gamma rays) (Møller and Mousseau 2013). It has also been demonstrated that microbes indigenous to Hanford vadose sediments, including Rhodococcus, Nocardia, and Deinococcus radiodurans, are effective at surviving the acute doses of ionizing radiation (approaching 20 kGy) associated with radioactive waste (Fredrickson et al. 2004).

Here, we take advantage of a recent surficial excavation at Broborg to study the microbial colonization and niche partitioning of anthropogenically vitrified material. Studies have shown that microbes colonize rock surfaces according to rock mineralogy and chemistry, and the organism’s metabolic requirements and tolerances, suggesting that the vitrified niche could support a unique microbial consortium that expresses specific metabolic functions to take advantage of, or protect itself from, the unique aspects of the vitrified material (Jones and Bennett 2014). The goal of this work was to examine the microbial community on and in the vicinity of the vitrified material at Broborg to assess the contribution of both biotic and abiotic factors to glass alteration. Samples of vitrified material, soil, and surficial swabs of the vitrified wall were collected aseptically from the uppermost layer at Broborg (referred to as ‘topsoil’ in archeological terms) for DNA analysis. The chemistry and mineralogy of the samples were assessed, and the vitrified material was examined by scanning electron microscopy (SEM) for microbial colonization. Extracted DNA from the soil and from the vitrified material was sequenced using 16S rRNA gene primers for prokaryotes, 18S rRNA gene primers for eukaryotes, and internal transcribed spacer (ITS) primers specifically for fungi. The resulting community analyses were examined in relation to sample proximity to the vitrified wall, soil chemistry and mineralogy, vegetation cover, and in the context of the microbial colonization of stone, glass, and analogous materials. The potential role of niche partitioning in these communities in long-term glass alteration or stabilization at Broborg is discussed in the context of vitrified radioactive waste disposal.

Materials and methods

Site location

The Broborg hillfort study site (Figures S1 and S2) is located ≈20 km SE of Uppsala, Sweden (Husby-Långhundra Parish,
Uppland; 662818N, 162066E), along an elongated valley which was a part of the Långhundra Waterway. The fort was built on a relatively large hill, ≈45 m above sea level, on the northern side of the valley. The hillfort, which includes an outer, un-vitrified wall of large granitoid boulders, and an inner, vitrified wall, was constructed in the early 400s CE and is believed to have been in use from 400 CE to 575 CE (Englund et al. 2018; Fagerlund 2009; Kresten et al. 1993; Weaver et al. 2018) (Figure S1(A)). Previous excavations were conducted in 1982, 1983, and 1985 to study the site’s geologic and archeological characteristics (Fagerlund 2009; Kresten et al. 1993; Kresten and Ambrosiani 1992; Sjöblom et al. 2016; Weaver et al. 2018). It is likely that the thin layer of topsoil (up to 15 cm) was removed from some or all the vitrified inner wall during these excavations (and replaced afterwards) (Fagerlund 2009). The inner wall consists of a vitrified layer (20–80 cm thick) at the top of the wall in which amphibolite was melted to form a dark, porous melt that cemented together the under-lying heat affected gneissic granitoid boulders (Kresten et al. 1993; Weaver et al. 2018). Within the vitrified material are two distinct glass types: an iron-rich, dark glass from the molten amphibolite and an alkali-rich, clear glass from contact melting with the gneissic granitoid rock (Weaver et al. 2018). The fort is surrounded by forest that form part of the Boreal coniferous belt (Figure S1(B)). The area inside and outside the fort has likely been recurrently forested and deforested since its abandonment, as evidenced by samples from other locations in the Uppland area. There is some present-day tree and shrub cover, including oak and juniper (Figure S1(B,C)). Structural geological measurements indicate that the hill on which the fort was constructed consists of granitoid bedrock, with cross-sectional diabase seams overlain with glacial moraine deposits (Englund et al. 2018), including a large number of boulders (Figure S2). In the near vicinity of the hill, intensive agriculture is currently practiced and likely has been for hundreds of years.

**Field sample collection**

Surficial soil, vitrified material, and other samples were collected June 7 to June 8, and October 4 to October 11, 2017. The dark topsoil layer, archaeologically defined as the uppermost horizons of the soil profile, contained high levels of organic material and the root systems of surface vegetation, interspersed with fire-damaged granitoid rock (Darvill 2008). The topsoil was primarily covered by grasses, forbs, and mosses (Figure 1; Figures S1(B,C) and S3) and reached a maximum depth of ≈15 cm, although it was thinner above the upper vitrified part of the inner wall, with vitrified material exposed at the surface in places (Figure S1(C)). The extent of vitrification was irregular, and the depth varied from ≈0.2 m along the outer edges to ≈0.8 m in the center of the wall (Englund et al. 2018). A few fragments of vitrified material present in the topsoil were interpreted as having fallen from the vitrified part of the wall, possible through degradation of the underlying fire-damaged granitoid rock.

![Figure 1. Sampling locations in relation to the vitrified wall.](image)

The locations of sampling points with respect to the vitrified wall are shown in Figure 1. Samples were collected counter-clockwise around the wall, starting from the fort entrance, to the east, at twelve sampling locations. The position of each site was recorded using a real-time kinematic (RTK) positioning system (Trimble) based on data derived from satellite-based Global Positioning System (GPS). To evaluate the biogeochemical properties of the niche environment created by the vitrified material and the influence on microbial community partitioning, samples were collected that included: (1) soil samples in the vitrified wall niche environment (≈100 g); and (2) topsoil samples (≈100 g) at a distance of 10–15 m from vitrified wall (both in the center of the hillfort and between the inner and outer walls), representing the general environment (Figure 1). At each of the twelve locations, two separate samples were collected for chemical and biological analysis, for both the vitrified niche and general topsoil. Biological soil samples were collected aseptically and were immediately transferred to sterile bags (Whirl-Pak®). Subsequent soil samples were collected non-aseptically for soil chemistry analysis [pH/Eh, moisture content, organic matter (OM) content, geochemistry, mineralogy, etc.]. Samples were collected by lifting off the surface vegetation and the uppermost rooting zone with a
sterile scoop and collecting the soil beneath to a depth of approximately ≈10 cm (replacing the surficial layer after sampling). Although separate soil samples for biological and chemical analysis were collected from the same area (within 10 cm of each other), the chemical and biological soil samples were given separate, exact GPS coordinates for tracking. All bagged soil samples were stored in the dark on ice in a cool box to maintain the temperature at ≈4°C and prevent deterioration of biological material prior to analysis. Samples were shipped within 5 days of collection, with instructions for limiting exposure of samples to high temperatures and X-rays during shipping, and the packages included temperature tracking devices (3 M MoniterMark TTI, 9860B) to confirm that the temperature did not exceed 10°C during shipment. Upon receipt, samples were stored at 4°C in the dark pending analysis. DNA extraction from the biological samples was completed within 4 weeks to 8 weeks of sample collection. Prior to shipping, the chemical soil samples were analyzed, within 2 days of collection, using Environmental Protection Agency (EPA) Method 9045 D for Soil and Waste. Prior to shipping, the chemical soil samples were analyzed, within 2 days of collection, using Environmental Protection Agency (EPA) Method 9045 D for Soil and Waste pH (see SI, Section 1.1). Photographs of the twelve sampling locations before and after sampling, annotated with genus-

Soil and vitrified material characterization

All methods were performed in accordance with United States Department of Agriculture (USDA) permit requirements (Animal and Plant Health Inspection Service). Moisture, organic matter (OM) content and chemistry of the soil samples were determined as described in the SI, Section 1.2. Vaporized hydrogen peroxide (VHP) sterilization of the vitrified material was carried out as described in the SI, Section 1.3, to release the samples from the USDA permit prior to electron microscopy analysis. The mineralogy of soil and vitrified material was determined by X-ray diffraction (XRD, see SI, Section 1.4). Surface characteristics were analyzed by SEM (see SI, Section 1.5).

Microbial cell densities and DNA extractions

Cell densities were estimated by staining with 4’,6-diamidino-2-phenylindole (DAPI) and fluorescence microscopy (see SI section 1.2 for additional details). DNA extractions were performed using the MO BIO PowerSoil® DNA Isolation Kit. Extractions followed the protocol from the manufacturer (MO BIO protocol), with the following exceptions. As required by USDA permit, the steps utilizing the PowerBead Tubes were completed in a HEPA-filtered biological safety cabinet (class II A), and once out of the biological safety cabinet, the sealed samples were processed on the benchtop. To concentrate the DNA, 50.0 μL of the elution buffer was added, instead of 100.0 μL. DNA concentration was measured on a NanoDrop 8000 (Thermo Scientific).

Microbiome characterization

Amplicon sequencing

Polymerase chain reaction (PCR) amplification of the V4 region of the 16S rRNA genes, the 18S rRNA genes, and the ITS region was performed using the protocol developed by the Earth Microbiome Project (Caporaso et al. 2012, 2018; Walters et al. 2016), except that the twelve base barcode sequence was included in the forward primer. Amplicons were sequenced on an Illumina MiSeq using the 500-cycle MiSeq Reagent Kit v2 according to manufacturer’s instructions (Illumina, San Diego, CA) (Illumina 2011). Bacterial vs. fungal load was determined by qPCR of the 16S rRNA genes and the ITS region with small adjustments to the protocol used by Fierer et al. (Fierer et al. 2005). Briefly, qPCR assays were optimized with SsoAdvanced Universal Inhibitor-Tolerant SYBR Green Supermix (Bio-Rad, Hercules, CA, USA). The 25 μL reactions contained 12.5 μL of SsoAdvanced MasterMix, 10 μL molecular biology grade water, 250.0 nmol/L of each forward and reverse primer, and 2.0 μL of template. Cycling conditions were 2 min at 98°C followed by 35 cycles of 98°C for 15 sec, 58°C for 60 sec for bacteria and 55°C for 60 sec for fungi. Melt curves were generated for each target and melting temperature, respectively. No-template controls were also included.

Amplicon analysis

The Hundo protocol was used to process 16S rRNA gene, 18S rRNA gene, and ITS region amplicons (Brown et al. 2018). In brief, sequences were trimmed and filtered of adapters and contaminants using BBduk2 of the BBTools package. VSEARCH (Rognes et al. 2016) was used to (i) merge; (ii) filter to an expected error rate of one de-replicating reads back to these clusters. BLAST+ (Camacho et al. 2009) was used to align OTU sequences to the database curated by CREST (Lanzén et al. 2012) (SILVA v128 for 16S and 18S rRNA genes, UNITE v7 for ITS region) and taxonomy was
assigned based on the CREST LCA method. Multiple sequence alignment was performed with MAFFT (Katoh and Standley 2013) and a phylogenetic tree was constructed using FastTree2 (Price et al. 2010).

Diversity analysis
Downstream analyses were performed using R (R Core Team, 2020), using the phyloseq (McMurdie and Holmes 2013) and vegan packages (Oksanen et al. 2019). To preserve the maximum consistency, samples with under 5,000 reads were removed, and remaining samples were randomly subsampled without replacement to an even depth. The Simpson’s, Inverse Simpson’s, Chao1, and unique OTUs observed were calculated as diversity metrics. Alpha diversity was characterized using the vegan package. Beta diversity was measured with Weighted UniFrac (Lozupone et al. 2011) distances between samples, and Permutational Multivariate Analysis of Variance Using Distance Matrices (the ‘adonis’ function in vegan) was used to measure changes associated with subtype. Differential abundance testing was performed using the DESeq2 package (Love et al. 2014) to identify significantly enriched taxa in the vitrified wall over the general topsoil (adjusted p-value < 0.0001).

Data repository, reproducible analyses and uncertainties
Sequencing data and processing scripts are available on the Open Science Framework (osf.io) for 16S rRNA gene, 18S rRNA gene, and 1TS amplicons as part of this project: https://osf.io/q3g7a/. There are three major uncertainties associated with amplicon data: (i) detection of relic DNA; (ii) extraction and sequencing errors due to biases in the amplification and sequencing process; and (iii) bioanalytical uncertainty due to errors in assigning sequences to taxa.

Statistical and geospatial analyses
Correlations between chemical and microbial properties and between biological taxa were calculated using two-tailed Pearson Product-Moment Correlation tests (significance level at 0.05), a statistical measure of linear correlations between two variables based on their covariation (ranging from −1 to 1). Observed values were interpolated across the entire sampling domain using kriging estimation to estimate chemical and microbial distributions (Ettema and Wardle 2002; Robertson 1987). Kriging predicts the value at a given point in space as a function of data in the neighborhood of the point. Kriging parameters were derived from maximum likelihood estimation with the function ‘likfit’ in the ‘geoR’ package, and predicted values were generated with the ‘krige.conv’ function in ‘geoR’ (Ribeiro and Diggle 2001). Predicted values were plotted using the function ‘image.plot’ in the ‘fields’ package (Furrer et al. 2012).

Results and discussion
The vitrified material that has been in the topsoil (near-surface) environment at Broborg for the last ≈1,500 years provides an ecological niche that supports a diverse microbial community. SEM images from the vitrified niche show evidence of a variety of fungi (yeast, filamentous saprotrophs, lichens), testate amoebae, eukaryotic algae (including diatoms), and prokaryotes (Actinomycetes) (Figure 2). The biogeochemical factors that shape this niche, and the partitioning of microbial communities within the niche, are
described in the subsequent sections, along with the implications for radioactive waste glass disposal.

**Biogeochemistry of the vitrified niche**

Soil chemistry (pH, Eh, % H2O, OM) and microbial biomass (cell density, Fungi-to-Bacteria ratio, F:B) results are summarized in Table 1, and are interpolated in space over the site, via autocorrelation (kriging), in Figure S4(B). Soil pH from the 12 samples collected ranged from 6.1 to 6.6. Soil Eh, measured at the same time as pH, ranged from +180 mV to +250 mV. Soil OM, measured as loss on ignition, varied from 3.3%, from soil on the surface of vitrified material, to 37–38%, for soil collected at the northeastern area of the site (Figure 1). Gravimetric soil moisture generally was greater in soil samples with greater OM and ranged from 13% to 52%. Microbial cell density in the soil samples, as determined by nucleic acid staining (DAPI) of soil extracts followed by epifluorescent microscopy, ranged from 5 × 10^7 to 3 × 10^8 cells per gram dry soil, or nearly an order of magnitude over all sampling points. In 11 of the 12 soil samples, F:B ratios, as determined by DNA extraction followed by qPCR of the ITS region (fungi) and 16S rRNA genes (bacteria) sequences, ranged approximately an order of magnitude, from 0.0044 F:B to 0.028 F:B. However, one sample from the vitrified niche (#2) had nearly a two order of magnitude higher F:B (1.8), with the high ratio driven by a high fungal biomass value. The four topsoil samples representing the general environment had a narrower F:B range than the six samples from the niche environment, 0.0054 F:B to 0.022 F:B (topsoil) vs. 0.0044 F:B to 0.028 F:B (vitrified niche). Except for the one outlier at 1.8, the F:B values were in the lower range of values seen in Swedish boreal forests, 0.02 F:B to 0.4 F:B (Högberg et al. 2007).

Data from soil elemental analysis (ICP-OES) are given in Table S1 and are shown geospatially in Figure 3(B) and Figure S4(C–E), with statistical correlations (Pearson’s, p < 0.05) shown in Figure S5. Kriging of major oxides and minor elements present in the soils show that biologically important elements P, Mn, Fe, and S have similar spatial patterns that overlap with the spatial patterns for soil OM, soil moisture, and microbial cell density. In contrast, Na (an indication of proximity to vitrified wall niche) was negatively correlated with OM (r = −0.597), moisture (r = −0.611), P (r = −0.777), Mn (r = −0.666), and S (r = −0.666), among other factors. These results suggest that OM and higher soil microbial biomass accrue in areas distal to the wall, where soil depths (to rock and vitrified wall) are likely to be greater. Other elements largely present as oxides in feldspar, and its weathering product kaolinite (e.g. CaO and Al2O3), showed no spatial patterns amenable to kriging. Kriging of oxides that were found in high abundance in Broborg glass (Weaver et al. 2018) showed SiO2 and Na2O to be higher in vitrified niche soils than in the general topsoil (Table S1; Figure 3; Figure S4(C,D)).

XRD analysis of the soil mineralogy is provided in Table S2, and geospatial interpolation of these data is shown in Figure 3(B) and Figure S4(F), with statistical correlations given in Figure S5. Although the amorphous content of the soils collected from around the hillfort was relatively high (up to 52%), some of the highest values were for the soil samples away from the vitrified wall, suggesting that the amorphous material is not related to the glassy component of the wall. The building materials for the hillfort were granitic gneiss and amphibolite, and vitrification of the walls has been ascribed to incongruent melting of amphibolite rock. The incongruent melting involves (1) dehydration and melting of the amphiboles, the primary mineral in the amphibolite rock, to form pyroxene; and (2) precipitation of spinels (e.g. magnetite, Fe3O4) in the glass upon cooling (Weaver et al. 2018). Thus, weathered material derived from the melted (vitrified) amphibolite would likely contain pyroxene and magnetite. Consistent with this analysis, hot spots for amphibole minerals were all associated with soil away from the vitrified wall, and the one hot spot for soil pyroxene was associated with soil sampled at the vitrified wall (Figure 3). Amphibole correlated negatively with Na (r = −0.715) and correlated positively with Cr (r = 0.818) and V (r = 0.706), among other factors (moisture, OM, Ca, Cu, Mg, Mn, P, S, Ti, Zn). For the granite-based glass, the starting material would have contained magnetite (Kresten et al. 1993; Kresten and Ambrosiani 1992). Maghemite (γ-Fe2O3) and hematite (α-Fe2O3) which can be thermal oxidation products of magnetite, were found primarily in soil associated with

---

**Table 1.** Selected chemical and microbiological properties of vitrified niche soil and general topsoil.

<table>
<thead>
<tr>
<th>Sample #</th>
<th>Location¹</th>
<th>pH</th>
<th>Eh (mV)</th>
<th>% H₂O</th>
<th>% OM (LOI)²</th>
<th>Cells/g soil</th>
<th>F:B</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Vitrified niche</td>
<td>6.11</td>
<td>+240.0</td>
<td>21.97</td>
<td>9.65</td>
<td>4.8 × 10^-7</td>
<td>0.0262</td>
</tr>
<tr>
<td>2</td>
<td>Vitrified niche</td>
<td>6.20</td>
<td>+202.7</td>
<td>29.96</td>
<td>19.8</td>
<td>1.7 × 10^-8</td>
<td>1.8</td>
</tr>
<tr>
<td>3</td>
<td>General topsoil</td>
<td>6.27</td>
<td>+207.6</td>
<td>51.08</td>
<td>37.5</td>
<td>2.0 × 10^-8</td>
<td>0.00537</td>
</tr>
<tr>
<td>4</td>
<td>Vitrified niche</td>
<td>6.39</td>
<td>+202.7</td>
<td>31.13</td>
<td>21.33</td>
<td>1.2 × 10^-8</td>
<td>0.00283</td>
</tr>
<tr>
<td>5</td>
<td>Vitrified niche</td>
<td>6.43</td>
<td>+179.5</td>
<td>40.30</td>
<td>17.74</td>
<td>1.8 × 10^-8</td>
<td>0.00984</td>
</tr>
<tr>
<td>6</td>
<td>General topsoil</td>
<td>6.53</td>
<td>+197.3</td>
<td>34.52</td>
<td>15.78</td>
<td>1.5 × 10^-8</td>
<td>0.0215</td>
</tr>
<tr>
<td>7</td>
<td>Vitrified niche</td>
<td>6.54</td>
<td>+201.0</td>
<td>51.73</td>
<td>37.04</td>
<td>1.9 × 10^-8</td>
<td>0.0140</td>
</tr>
<tr>
<td>8</td>
<td>Vitrified niche</td>
<td>6.28</td>
<td>+209.8</td>
<td>21.42</td>
<td>8.39</td>
<td>8.2 × 10^-7</td>
<td>0.00448</td>
</tr>
<tr>
<td>9</td>
<td>Vitrified niche</td>
<td>6.51</td>
<td>+227.1</td>
<td>25.68</td>
<td>14.57</td>
<td>1.7 × 10^-6</td>
<td>0.0109</td>
</tr>
<tr>
<td>10</td>
<td>Vitrified niche</td>
<td>6.54</td>
<td>+236.1</td>
<td>12.62</td>
<td>3.33</td>
<td>0.0265</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>General topsoil</td>
<td>6.59</td>
<td>+231.4</td>
<td>42.30</td>
<td>32.58</td>
<td>2.7 × 10^-8</td>
<td>0.00955</td>
</tr>
<tr>
<td>12</td>
<td>General topsoil</td>
<td>6.56</td>
<td>+250.0</td>
<td>37.53</td>
<td>20.34</td>
<td>9.0 × 10^-7</td>
<td>0.00595</td>
</tr>
</tbody>
</table>

¹Vitrified niche soil samples were obtained at locations in contact with the vitrified wall. General topsoil samples were obtained from locations at 10–15 m from the vitrified wall.

²Percent organic matter (loss on ignition).
the vitrified wall and were generally absent in soils away from the vitrified wall (Figure 3; Table S2). Likewise, maghemite correlated negatively with amphibole ($r = -0.674$) and with Cr ($r = -0.823$) and V ($r = -0.719$), among other factors (P, Ti), and correlated positively with Na ($r = 0.755$). Similarly, hematite correlated positively with quartz ($r = 0.696$), feldspar ($r = 0.741$), and mica ($r = 0.590$). Spinels in the soil samples were closer to the maghemite end of the solid solution series between magnetite and maghemite, i.e. more oxidized, in contrast to spinels found in vitrified samples (Kresten et al. 1996), which were closer to the magnetite end, i.e. more reduced. Given the presence of other granitic minerals in the soil, it is likely that the maghemite was derived from physical weathering of the fire-cracked granitoid rock. However, the XRD identification of felsic minerals, including plagioclase feldspar (e.g. albite) and alkali feldspar (e.g. microcline), and the lack of kaolinite (the major weathering product of feldspar) in any of the soil samples suggest that limited chemical weathering took place.

**Spatial and statistical correlations among microbial community and vitrified niche biogeochemistry**

Spatial correlations (krig maps) for biological taxa (18S rRNA genes, ITS region, and 16S rRNA genes) are shown in Figure 3 (18S rRNA genes at the phylum-level for Ascomycota and Chlorophyta) and Figures S6–S8. As shown in Figure 3(B), Ascomycota, which contains the majority of lichen primary mycobionts (Lücking et al. 2017), was co-located spatially with the Chlorophyta phylum, which contains the majority of eukaryotic algal lichen photobionts. Members of the phylum Ascomycota, which are ubiquitous throughout terrestrial ecosystems, include unicellular and filamentous members with a breadth of trophic strategies,
including lichen mycobionts, saprotrophs, pathogens, endophytes, and ectomycorrhizal (EcM) fungi. However, their spatial co-location with the Chlorophyta suggests lichen abundance. These taxa were also spatially associated with soil minima for P and Mn (and S, soil moisture, soil OM, and cell density) and with soil maxima for Si, and the hot spots for these two phyla all were associated with vitrified niche soils, rather than the general topsoil (Figure 3; Figure S4(B)). In the 18S rRNA genes, taxa that were primarily associated with soil proximal to the vitrified wall included the Nematoda phylum (nematodes), the Eurotiomycetes fungal class (includes obligately and facultatively lichenized taxa, as well as unicellular taxa, including endolithic yeasts), the Microbotryomycetes fungal class (unicellular, including endolithic taxa), and the eukaryotic algal classes Trebouxiophyceae (green algae) and Chrysophyceae (golden algae) (Figure S6). Taxa in the 18S rRNA genes primarily associated with soil distal to the vitrified wall included members of the Cercozoa (testate amoeba) and Tracheophyta (vascular plants) phyla and the Magnoliophyta (flowering plants) class (Figure S6). In the soil ITS region, the Eurotiomycetes fungal class were spatially associated with the vitrified wall (Figure S7(D)), as in the 18S rRNA genes. Taxa in the ITS region spatially associated with soil distal to the vitrified wall included phyla Basidiomycota (includes both unicellular and filamentous taxa, the latter rarely lichenized), Glomeromycota (arbuscular mycorrhizal fungi, AMF), GS19 (soil-inhabiting fungi), Olpidiomyctea (e.g. plant pathogens), Rozellomyctea (e.g. unicellular parasites), and Entorrhizomyctea (e.g. root-associated endophytes), along with the Basidiomycota class Agaricomycetes (includes free-living and lichenized forms, plant pathogens, ectomychorrizae, and insect symbionts) and unclassified Basidiomycota (Figure S7(B–D)) (Naranjo-Ortiz and Gabaldon 2019). The 16S rRNA genes from soil, which contained few samples, as some samples did not amplify (see Figure S8(A)), were less amenable to kriging (Figure S8(B, C)). However, the Verrucomicrobia phylum and the Spartobacteria class within the phylum, which have been shown to be significant components of the lichen holobiome (Cernava et al. 2017), both were associated with the vitrified wall.

Statistically significant correlations (Pearson’s, \( p < 0.05 \)) among biological classes (18S rRNA genes, ITS region, and 16S rRNA genes) and soil properties are shown in Figure S9(A–D) and are discussed in further detail in the SI. The correlations between ITS region class and soil biogeochemical properties are shown in Figure S9(A). The Ascomycota class Eurotiomycetes, which includes lichen mycobionts, lichen-associated fungi, and endolithic yeasts, was positively correlated with the vitrification product maghemite (\( r = +0.679 \), ITS region) and with the weathering-resistant mineral feldspar (\( r = +0.607 \), 18S rRNA genes). Likewise, 18S rRNA genes (Figure S9(B)) indicate that the Microbotryomycetes, known to be endolithic yeasts, were positively correlated with mica (\( r = +0.690 \)) and quartz (\( r = +0.644 \)). The Basidiomycota class Tremellomycetes (e.g. yeasts, including bryophiles, saprotrophs, and endolithic and pigmented forms) was negatively correlated with the vitrification product pyroxene (\( r = -0.705 \), ITS region). The correlation analyses of either the ITS region or 18S rRNA genes with soil chemistry indicate that Agaricomycetes, most commonly free-living, but sometimes lichenized, were positively correlated with Fe, Ti, Zr, Sr, Zn, and V. Likewise, the highest values of Cr, Ti, Zr, and V were in soils distant from the vitrified wall and lowest values in soils adjacent to the vitrified wall (Figure S4; Table S3). As indicated in Figure S9(B), the Trebouxiophyceae, which are a primary eukaryotic phycobiont of lichens, were negatively correlated with Cr, Zr, and V. Likewise, the Chrysophyceae, which can be free-living but which can also be important members of biological soil crusts (BSCs) (Rippin et al. 2018), were positively correlated with Na (given as \( \text{Na}_2\text{O}, r = 0.714 \)), which was highest in vitrified niche soil samples and lowest in the general topsoil (Figure S4(D); Table S1). Fungibacteria (Figure S5) was positively correlated with feldspar (\( r = 0.905 \)), mica (\( r = 0.779 \)), and hematite (\( r = 0.736 \)) and was negatively correlated with Ti (\( r = -0.745 \)), V (\( r = -0.696 \)), and amorphous minerals (\( r = -0.853 \)), suggesting that high F:B are associated with soils with high numbers of lithic lichens (the outlier with a high F:B was excluded from this analysis). Likewise, F:B was positively correlated with Trebouxiophyceae (\( r = 0.689 \)). The only significant prokaryotic class correlations with geochemical data (Figure S9(C)) were a positive correlation of Spartobacteria with mica (\( r = 0.762 \)) and Actinobacteria with pH (\( r = 0.794 \)). Although prokaryotic classes showed many class-class correlations (Figure S9(C,D)), there were fewer significant correlation of prokaryotic classes with fungal classes. Among the latter (Figure S9(D)), Spartobacteria were positively correlated with Microbotryomycetes (\( r = 0.888 \), 18S rRNA genes) and with unclassified Ascomycota classes (\( r = 0.907 \), ITS region), suggesting a lithic niche for this prokaryotic class. Gammaproteobacteria correlated negatively with the commonly lichenized Sordariomycetes fungal class (\( r = -0.688 \), ITS region), consistent with a higher abundance of Pseudomonads in control soil than in test soil (Table S4). Clostridia were positively correlated with Sordariomycetes (\( r = 0.672 \), ITS) and Dothideomycetes (\( r = 0.672 \), 18S rRNA genes), which have been shown to be abundant in some BSCs (Zhang B et al. 2018), and were negatively correlated with the primarily saprotrophic Mortierellomycetes (\( r = -0.674 \), ITS), consistent with the hypothesis that Clostridia (and Bacteroidia, which were positively correlated with Clostridia, \( r = 0.965 \)) are associated with lichen-containing BSCs (see SI for further discussion).

**Partitioning of microbial communities between the vitrified niche and the general Broborg topsoil**

Here, the hillfort site microbial biogeography is discussed, with the microbial functions in the vitrified niche discussed in a separate section below. Principal coordinates analysis (PCoA) on weighted Unifrac distances was used to examine the association of the entire Broborg microbiome with sample type (Figure S13). Microbial communities directly associated with the vitrified wall were distinct from communities
in soil on the vitrified wall, and communities in soil on the vitrified wall were distinct from communities in the general topsoil, away from the vitrified wall (Figure S13). The eukaryotic (18S rDNA genes and ITS region) communities from the vitrified material and the soil on the vitrified wall are more distinguishable from those in the general topsoil away from the vitrified wall than the analogous bacterial (16S rDNA genes) communities (Figure S13). Overall, the general topsoil at Broborg hosts a more diverse community composition than the vitrified niche environment (Figures 4 and 5; Figure S14).

Phylogenetic analyses of the vitrified niche, associated soil, and general topsoil are shown in Figure 4 and Figure S12 and are discussed in further detail in the SI. At the phylum level, communities associated with vitrified wall were dominated by Ascomycota and Basidiomycota fungi (Figure S12(A,B)); Chlorophyta (eukaryotic green algae) (Figure 6(A) and S12A); and Proteobacteria (Figure 6(C) and Figure S12(C)). At the class level, the vitrified niche included fungi of the Ascomycota classes that are known lichen mycobionts (Eurotiomycetes, Sordariomycetes, Dothidiomycetes); Microbotryomycetes, which include melanized endophytic yeasts; Trebouxiophyceae; and Alphaproteobacteria and Gamma proteobacteria (Figure 4). The three most frequent classes of Ascomycota recovered from the vitrified niche were Eurotiomycetes, Sordariomycetes, and Dothidiomycetes (Figure 4(A,B)), all of which include fungi with diverse trophic strategies. Although members of each of these three classes can be primary lichen mycobionts, other members are frequently found as lichen-associated fungi, i.e. those that occur with lichens but are not the primary mycobiont. At the class level, the soil associated with the vitrified niche included Eurotiomycetes, Dothidiomycetes, Agaricomycetes, and Mortierellales fungi; Cryptophyceae (eukaryotic golden algae); and Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Clostridia, Bacteroidia, Spartobacteria, and Actinobacteria (Figure 4 and Figure S12(A–C)). In general, communities present in the vitrified niche consisted of microbes associated with the lichen holobionte (Cernava et al. 2017; Hardoim et al. 2015), with the adjacent soil containing communities associated with moss and lichen BSCs (see Table S5), including Cryptophyceae and distinctive prokaryotic communities (see discussion in SI) (Cernava et al. 2017; Hardoim et al. 2015). In contrast, communities from the general topsoil were dominated by plant rhizosphere organisms and saprotrophs (see Table 2 and Table S4).

The testate amoebae observed in SEM images (Figure 3(I,J) and Figure S10) are identified as Euglypha by their elongate ovoid tests, composed of overlapping plates (likely biogenic silica, see Figure S10), and the aperture with dentate mouth plates, and this genus was also detected in 18S rDNA genes (Table S4). These organisms are commonly associated with mosses, a major ground cover in the sampling sites, and in some cases can be mixotrophic (both phototrophic and heterotrophic).

Taxa that were enriched in the vitrified niche and associated soil as opposed to taxa in the general topsoil are shown in Figure 6. In the 18S rDNA genes, phyla with classes enriched in the vitrified niche included Ascomycota and Basidiomycota, Cercozoa (testate amoebae), Chlorophyta, and Tracheophyta (vascular plants) (Figure 6(A)). The Tracheophyta included Pinophyta (coniferous trees) and Magnoliophyta (flowering plants). Many Trebouxiophyceae (a common eukaryotic phycobiont of lichens) and the Chlorophyceae class of green algae, which also includes lichen phycobionts, were enriched in vitrified niche (Figure 6(A)). In the ITS region, classes enriched in the vitrified niche included members of Ascomycota, Basidiomycota, and Mortierellomycota phyla (Figure 6(B)). In the 16S rDNA genes, the vitrified niche was enriched in only four prokaryotic OTUs, from the Proteobacteria, Chloroflexi, Bacteroidetes, and Verrucomicrobia phyla (Figure 6(C)); the Sphingomonas genus; the C0119 order of the Ktedonobacteria (Actinomycetes-like filamentous morphology found to be abundant in polar desert soil in Antarctica (Ji et al. 2016)) class of Chloroflexi; the Chitinophagales order of the Bacteroidetes; and the DA101 family of the Chthoniobacteriales (Spartobacteria family, Verrucomicrobia phylum). In contrast, the 16S rDNA genes for the vitrified niche soils were enriched in a variety of taxa (Figure 4(C) and Figure S12(C,F)). Analysis of the Pseudomonas genus, which dominated the prokaryotic data in vitrified samples, showed a predominance of species of the P. fluorescens group (31 of 102 Pseudomonas hits, 20 of 76 Pseudomonas organisms), followed by organisms of P. syringae, P. chlororaphis, P. putida, and P. tolaasii groups (data not shown). The general topsoil was enriched in common eukaryotic phycobionts in lichens associated with BSCs, such as Cryptophyceae (golden algae, flagellates) (Figure 6(A)). The general topsoil was also enriched in one OTU of the marine Stramenopile (microeukaryote) MAST 12-C (Worden et al. 2015).

**Functionality of microbial community partitioned in the vitrified niche and implications for radioactive waste glass disposal**

The Broborg site may differ climatologically, pedologically, and geologically/hydrologically from sites where nuclear waste is to be disposed, such as the IDF at the Hanford Site (Hoitink et al. 2005; Wildung et al. 1975). However, analyzing the functionality of the microbial community that inhabits this vitrified niche offers insights into possible long-term biogeochemical corrosion/stabilization mechanisms that could affect vitrified materials over millennia. Below the topsoil, in the subsoil and vadose zone, which are most immediately relevant to radioactive waste glass disposal in the near surface IDF at the Hanford Site, the influence of the plant rhizosphere and the effects of filamentous fungi are likely to be less important, and the influence of oligotrophs that are able to metabolize in the absence of light and oxygen are likely to dominate. Nevertheless, eukaryotes, including fungi (in some cases, unicellular, non-filamentous yeast species), protists, nematodes, and other eukaryotes, including Arthropoda, have been observed in subsurface environments (Borgenie et al. 2015; Sohlberg et al. 2015).
Figure 4. Most abundant class-level taxa directly associated with the vitrified wall, in soil adjacent to the vitrified wall, and in the general topsoil: (A) Most abundant 18S rRNA genes at the class level; (B) Most abundant ITS region at the class level; (C) Most abundant 16S rRNA genes at the class level. Taxa labeled as ‘_7’ are either unclassified at that level during taxonomy assignment or unclassified in the database.
A summary of the organisms identified in the Broborg vitrified niche, along with potential corrosion mechanisms associated with them, or related genera, inferred from previous studies in the literature, is given in Table 2, and described in the text below.

**Eukaryotic communities**

The microbial community in the vitrified niche was enriched in Ascomycota, the phylum containing the majority of lichen primary mycobionts and also the most commonly identified fungal phylum on glass surfaces (Mellor 1924; Weaver et al. In review). Lichens, which have the capacity to
remain intact and growing on lithic substrates for thousands of years (Gellally 1982), are well-documented agents of both mineral weathering and glass corrosion (Banfield et al. 1999; Chen et al. 2000; Favero-Longo et al. 2015; Strachan et al. 2014; Verney-Carron et al. 2010). Lichens break down substrates by mechanical weathering through hyphal penetration and subsequent expansion and by chemical weathering, especially by the excretion of secondary metabolites. Secondary metabolites (Table S5) include organic acids, such as usnic acid, which functions as an ultraviolet absorber and

Figure 6. Taxa enriched in vitrified niche (top) or general topsoil (bottom): (A) 18S rRNA gene enrichments; (B) ITS region enrichments; (C) 16S rRNA gene enrichments. Taxa labeled as “?” are either unclassified at that level during taxonomy assignment or unclassified in the database.
antibiotic, and oxalic acid, which reacts with alkali (e.g. Na) and alkaline earth (e.g. Ca) metals (Me) to form Me oxalate crystals (Cocchietto et al. 2002; Weaver et al. In review). However, lichens can also exert a bioprotective influence (Kip and van Veen 2015), particularly on limestone substrates (Carter and Viles 2003, 2005; Concha-Lozano et al. 2012). Lichen taxa (primary mycobionts) in the 18S rRNA genes and ITS region are shown in Table S5, with relative abundance of lichen containing classes in the three sample types shown in Table 2. The fungal classes from these data that can include primary lichen mycobionts, along with facultatively lichenized and unicellular (yeast) forms (lichenized or free living), are Lecanoromycetes, Eurotiomycetes, Leotiomycetes, Sordariomycetes, Dothidiomycetes, and (rarely) Agaricomycetes. Members of these classes colonize anthropogenic glass (Mellor 1924; Weaver et al. In review); natural and anthropogenic architectural and sculptural stone (Coutinho et al. 2015; Reblova et al. 2016); and sandstone, quartzite, granite, and limestone tombstones (Brewer and Fierer 2018). Under natural settings, members of these classes form microbial communities on polar desert rocks (Choe et al. 2018) and other endolithic environments (Gorbushina 2007; Pokharel et al. 2017; Staley et al. 1982), including the subsurface (Sohlberg et al. 2015). The Ascomycota class Dothideomycetes, which includes melanized endolithic yeasts, showed greater than 10% relative abundance (ITS region) on the vitrified rocks. The Capnodiales order of the Dothideomycetes contained one genus, Cladosporium, that was present at 2.1% relative abundance in vitrified material, and 1.6 and 0.4% in test soil and control soil, respectively (Table S4). Organisms in this genus of Dothideomycetes are halotolerant, found on cave walls and building materials (Bensch et al. 2012), and produce acids as secondary metabolites (Chlebicki and Jakus 2019). The vitrified niche also contained an abundance of Microbotryomycetes and Tremellomycetes, which include unicellular endolithic forms (Rovati et al. 2013; Sohlberg et al. 2015) as well as, in the case of Tremellomycetes, obligately lichenized yeasts (Tuovinen et al. 2019).

Autotrophs, such as cyanobacteria, are often the pioneering inhabitants on rock/glass surfaces. At Broborg, Chlorophyta protists were more prevalent in the vitrified niche than in the soil. Chlorophyta of the Class Trebouxiophyceae were major components of the vitrified niche but were only minor components in the soil. These green algae, which can be either free-living or lichenized phototrophs (photobionts), are a primary eukaryotic lichen photobiont, and are common inhabitants of exposed rock (tombstone) surfaces (Brewer and Fierer 2018). A consequence for potential colonization of radioactive waste glass by cyanobacteria and algae is that they maintain a water film as a living environment (Heimann 2018). This water film is implicated in glass weathering, as the dissociation of a water molecule into a hydronium ion and a hydroxyl ion results in ion exchange with alkali/alkaline earth ions in the glass (Heimann 2018). Conversely, algal biofilms have the potential to limit water uptake, by preventing it from reaching the glass surface, as has been shown with stone cultural artifacts (Charola et al. 2011). The algal and cyanobacterial phototrophs also reduce HCO₃⁻ or CO₂ to organic...
Table 2. Selected organisms identified in the Broborg vitrified niche samples that have been implicated as possible agents of glass corrosion or rock weathering, either mechanistically or by virtue of ecological niche.

### A. Fungi

<table>
<thead>
<tr>
<th>Phylum</th>
<th>Class</th>
<th>Ecological niche and/or weathering/corrosion mechanisms(s)</th>
<th>Abundance in vitrified niche/general topsoil/vitrified niche-associated soil (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ascomycota</strong></td>
<td><strong>Lecanoromycetes</strong></td>
<td>Anthropogenic glass (Mellor 1924). Sandstone, quartzite, and limestone tombstones (Brewer and Fierer 2018; Gehrmann et al. 1988).</td>
<td>1.21/0.03/0.29 (18S) 2.35/0.17/1.63 (ITS)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ultramafic and non-ultramafic rocks (Rajakaruna et al. 2012). Marble sculpture (Hallmann et al. 2013). Granite and gneiss building facades (Gaylarde et al. 2017).</td>
<td>2.10/1.90/5.43 (18S) 3.47/2.06/3.34 (ITS)</td>
</tr>
<tr>
<td></td>
<td><strong>Eurotiomycetes</strong></td>
<td>Granite and limestone tombstones (Brewer and Fierer 2018). Sandstone and quartzite (Chen et al. 2000; Choe et al. 2018; Coleine et al. 2018).</td>
<td>2.10/1.90/5.43 (18S) 3.47/2.06/3.34 (ITS)</td>
</tr>
<tr>
<td></td>
<td><strong>Leotiomycetes</strong></td>
<td>Granite and limestone tombstones (Brewer and Fierer 2018). Alpine rocks, Austrian Alps (Muggia et al. 2016).</td>
<td>3.16/2.19/1.96 (18S) 10.2/8.59/11.7 (ITS)</td>
</tr>
<tr>
<td></td>
<td><strong>Sordariomycetes</strong></td>
<td>Alpine rocks, Austrian Alps (Muggia et al. 2016). High Arctic polar desert rocks (Choe et al. 2018). Isotopic fractionation of Mg (Pokharel et al. 2017).</td>
<td>0.11/0.83/0.99 (18S) 6.64/5.37/13.6 (ITS)</td>
</tr>
<tr>
<td></td>
<td><strong>Dothideomycetes</strong></td>
<td>Arctic and Antarctic endolithic communities (Choe et al. 2018; Coleine et al. 2018). Natural underground stone walls of Stockholm, Sweden, metro station (Reblova et al. 2016).</td>
<td>6.24/2.47/5.43 (18S) 17.5/5.31/15.4 (ITS)</td>
</tr>
<tr>
<td></td>
<td><strong>Archeorhizomycetes</strong></td>
<td>Rock weathering in rhizosphere (Pinto-Figueroa et al. 2019).</td>
<td>0.002/2.01/0.54 (18S) 0.015/0.33/0.09 (ITS)</td>
</tr>
<tr>
<td><strong>Basidiomycota</strong></td>
<td><strong>Microbotryomycetes</strong></td>
<td>Antarctic endolithic communities (Coleine et al. 2018). Deep groundwater of crystalline bedrock fracture zones in Finland (Sohlberg et al. 2015).</td>
<td>9.95/0.13/0.16 (18S) 3.22/0.32/0.39 (ITS)</td>
</tr>
<tr>
<td></td>
<td><strong>Tremellomycetes</strong></td>
<td>Antarctic rocks and endolithic lichens (Rovati et al. 2013). Worldwide cold rock-associated habitats (Selbmann et al. 2014). Secondary lichen mycobiont in wolf lichens (Tuovinen et al. 2019).</td>
<td>3.02/0.94/1.05 (18S) 12.3/3.88/3.64 (ITS)</td>
</tr>
<tr>
<td></td>
<td><strong>Agaricomycetes</strong></td>
<td>Arctic and Antarctic endolithic communities (Choe et al. 2018; Coleine et al. 2018).</td>
<td>1.77/11.4/5.46 (18S) 2.51/23.4/9.91 (ITS)</td>
</tr>
<tr>
<td></td>
<td><strong>Mortierellomycota</strong></td>
<td>Deep groundwater of crystalline bedrock fracture zones in Finland (Sohlberg et al. 2015).</td>
<td>5.30/38.7/27.4 (ITS)</td>
</tr>
<tr>
<td></td>
<td><strong>Mortierellomycetes</strong></td>
<td>Ice nucleation (Fröhlich-Nowoisky et al. 2015). Formation of Se and Te nanoparticles (Li et al. 2019).</td>
<td>5.30/38.7/27.4 (ITS)</td>
</tr>
<tr>
<td></td>
<td><strong>Mortierellales</strong></td>
<td>Lichen-dominated biological soil crusts on Colorado Plateau (Bates et al. 2010). Antarctic soil (Wentzel et al. 2019). Increased plant P uptake (Almarino et al. 2017).</td>
<td>0.57/5.08/5.17 (18S) 0.21/0.36/0.49 (ITS)</td>
</tr>
<tr>
<td><strong>Mucoromycota</strong></td>
<td>--</td>
<td>Antarctic cryptoendolithic fungal communities (Coleine et al. 2018).</td>
<td>--</td>
</tr>
</tbody>
</table>

### B. Phototrophs (eukaryotic algae, cyanobacteria, and diatoms)

<table>
<thead>
<tr>
<th>Phylum</th>
<th>Class</th>
<th>Ecological niche and/or weathering/corrosion mechanisms(s)</th>
<th>Abundance in vitrified niche/general topsoil/vitrified niche-associated soil (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chlorophyta</strong></td>
<td><strong>Trebouxiophyceae</strong></td>
<td>High Arctic polar desert rocks (Choe et al. 2018). Glass. Marble sculpture (Hallmann et al. 2013).</td>
<td>15.5/0.15/0.50 (18S)</td>
</tr>
<tr>
<td></td>
<td><strong>Chrysophyceae</strong></td>
<td>Subaqueous river pebble biofilm (Ng et al. 2016). Natural underground stone walls of Stockholm, Sweden, metro station (Nörbäck Ivansson et al. 2013). Si cycling (Konhauser 2016).</td>
<td>0.37/6.64/8.44 (18S)</td>
</tr>
<tr>
<td></td>
<td><strong>Cyanobacteria</strong></td>
<td>Quartz and anthropogenic glass (Brehm et al. 2005). Tombstones (Brewer and Fierer 2018).</td>
<td>0.09/0.06/0.08 (18S) 0.003/0.02/0.12 (ITS)</td>
</tr>
</tbody>
</table>
C. Prokaryotes (excluding cyanobacteria)

**C-1. Proteobacteria phylum**

<table>
<thead>
<tr>
<th>Class</th>
<th>Order</th>
<th>Ecological niche and/or weathering/corrosion mechanisms(s)</th>
<th>Abundance in vitrified niche/general topsoil/vitrified niche-associated soil (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Xanthomonadales</td>
<td>Architectural ceramic materials (Coutinho et al. 2015). Phosphate solubilization (Uroz et al. 2009). Biological soil crust (Couradeau et al. 2019).</td>
<td>3.94/4.00/2.81 (16S)</td>
</tr>
<tr>
<td></td>
<td>Rhizobiales</td>
<td>Traverline rock in High Arctic polar desert (Choe et al. 2018). Ferrihydrite in rhizosphere (Whitman et al. 2018).</td>
<td>1.40/1.44/0.68 (16S)</td>
</tr>
<tr>
<td></td>
<td>Caulobacteriales</td>
<td>Travertine rock in High Arctic polar desert (Choe et al. 2018). Ferrihydrite in rhizosphere (Whitman et al. 2018).</td>
<td>1.01/1.02/1.12 (16S)</td>
</tr>
<tr>
<td></td>
<td>Alphaproteobacteria</td>
<td>Implicated in oxidative weathering of granitic bedrock (Napieralski et al. 2019). Ferrihydrite and quartz in rhizosphere (Whitman et al. 2018). Fe and Si solubilization (Wang et al. 2018). Lichen holobiome (Cernava et al. 2017).</td>
<td>2.31/3.45/2.02 (16S)</td>
</tr>
<tr>
<td></td>
<td>Nitrosomonadales</td>
<td>Intertidal sand grains (Probanid et al. 2017). Weathered sulfide-bearing rock from copper-nickel deposits in Minnesota (Jones et al. 2017).</td>
<td>0.11/3.10/1.43 (16S)</td>
</tr>
<tr>
<td></td>
<td>TRA3-20</td>
<td>Weathered volcanic rock (Byloos et al. 2018). Weathered rock surfaces (release of Al, Fe, K, and Si from biotite and feldspar) (Cheng et al. 2017).</td>
<td>2.45/4.32/2.18 (16S)</td>
</tr>
<tr>
<td></td>
<td>Sphingomonadales</td>
<td>Lichen holobiome (Cernava et al. 2017). Fe and P solubilization (Uroz et al. 2009). Rock weathering, Al solubilization (Wang et al. 2017).</td>
<td>1.01/1.02/1.12 (16S)</td>
</tr>
<tr>
<td></td>
<td>Caulobacteriales</td>
<td>Travertine rock in High Arctic polar desert (Choe et al. 2018). Ferrihydrite in rhizosphere (Whitman et al. 2018).</td>
<td>1.40/1.44/0.68 (16S)</td>
</tr>
<tr>
<td></td>
<td>Rhizobiales</td>
<td>Implicated in oxidative weathering of granitic bedrock (Napieralski et al. 2019). Ferrihydrite and quartz in rhizosphere (Whitman et al. 2018). Fe and Si solubilization (Wang et al. 2018). Lichen holobiome (Cernava et al. 2017).</td>
<td>2.31/3.45/2.02 (16S)</td>
</tr>
<tr>
<td></td>
<td>Sphingomonadales</td>
<td>Intertidal sand grains (Probanid et al. 2017). Weathered sulfide-bearing rock from copper-nickel deposits in Minnesota (Jones et al. 2017).</td>
<td>0.11/3.10/1.43 (16S)</td>
</tr>
<tr>
<td></td>
<td>TRA3-20</td>
<td>Weathered volcanic rock (Byloos et al. 2018). Weathered rock surfaces (release of Al, Fe, K, and Si from biotite and feldspar) (Cheng et al. 2017).</td>
<td>2.45/4.32/2.18 (16S)</td>
</tr>
<tr>
<td></td>
<td>SC-1-84</td>
<td>Antarctic glacial moraine (Pershina et al. 2018).</td>
<td>0.24/0.93/0.59 (16S)</td>
</tr>
<tr>
<td>Deltaproteobacteria</td>
<td>Myxococcales</td>
<td>Lichen holobiome (Cernava et al. 2017). Biological soil crust (Angel and Conrad 2013).</td>
<td>0.41/3.93/2.27 (16S)</td>
</tr>
</tbody>
</table>

**C-2. Bacteroidetes, Firmicutes, Actinobacteria, and Verrucomicrobia phyla**

<table>
<thead>
<tr>
<th>Phylum</th>
<th>Class</th>
<th>Ecological niche and/or weathering/corrosion mechanisms(s)</th>
<th>Abundance in vitrified niche/general topsoil/vitrified niche-associated soil (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteroidetes</td>
<td>Bacteroidia</td>
<td>Biological soil crust (Coutard et al. 2019). Ferrithydrite and quartz in rhizosphere (Whitman et al. 2018). Solar panels in California (Porcar et al. 2018). Rocks in High Arctic polar desert (Choe et al. 2018).</td>
<td>0.003/0.00/13.6 (16S)</td>
</tr>
<tr>
<td></td>
<td>Sphingobacteria</td>
<td>Lichen holobiome (Cernava et al. 2017). Solar panels in Spain (Dorado-Morales et al. 2016). Architectural ceramic materials (Coutinho et al. 2015).</td>
<td>1.93/2.15/1.61 (16S)</td>
</tr>
<tr>
<td>Firmicutes</td>
<td>Clostridia</td>
<td>Recent volcanic flow (Byloos et al. 2018). Subsurface rocks (Jones and Bennett 2014). Antarctic rock surfaces (Koo et al. 2018).</td>
<td>0.06/0.12/17.7 (16S)</td>
</tr>
<tr>
<td></td>
<td>Bacilli</td>
<td>Medieval stained glass window (Marvasi et al. 2009). Altered mica schist surfaces and adjacent soil (Fe and Si solubilization) (Wang et al. 2018). Potassium-bearing rock (release of Al, Ca, Fe, and K).</td>
<td>1.31/1.91/2.18 (16S)</td>
</tr>
<tr>
<td>Actinobacteria</td>
<td>Actinobacteria</td>
<td>Mineral dissolution (Wang et al. 2018). Deteriorating stone church facades (Gaylarde et al. 2017).</td>
<td>6.18/6.48/5.37 (16S)</td>
</tr>
<tr>
<td></td>
<td>Thermoleophila</td>
<td>Weathered terrestrial volcanic glass (Kelly et al. 2010). Recent volcanic flow (Byloos et al. 2018). Sub-marine basaltic glass.</td>
<td>5.52/5.84/11.3 (16S)</td>
</tr>
<tr>
<td>Verrucomicrobia</td>
<td>SP-4</td>
<td>Lichen holobiome (Cernava et al. 2017).</td>
<td>0.025/0.56/3.97 (16S)</td>
</tr>
<tr>
<td></td>
<td>OPB35 soil group</td>
<td>-</td>
<td>0.124/1.90/1.12 (16S)</td>
</tr>
</tbody>
</table>
compounds via photosynthesis, which encourages colonization by a succession of different organisms, including fungi, amoebae, etc. Alternatively, cyanobacteria can mineralize CO₂ to CaCO₃ via calcification (Kamennaya et al. 2012), which induces a pH increase at the cell exterior and could significantly increase the localized glass dissolution rate, with a 1.0–1.5 order of magnitude increase in the dissolution rate observed for radioactive waste glasses as pH values changed from 8.0 to 12.0 (Pierce et al. 2008). Colonization by cyanobacteria and algae can occur beneath the surface of granitic rock but not basaltic rock, and is likely due to the light color and high quartz content allowing light to penetrate further into the granitic rock (Piervittori et al. 1996). This has implications for radioactive waste glass, as these microbes may be restricted to the surface or shallow cracks of the glass, depending on the chemical composition and its effect on light penetration. Members of the fungal order Pleosporales, the largest order of the class Dothideomycetes, were common in both the vitrified niche and adjacent soil. Pleosporales are known to inhabit exposed rock tombstones (Brewer and Fierer 2018) and to be a component of BSCs (Zhang et al. 2012). Such microcolonial fungi have been shown to be dominant organisms in subaerobic biofilms on rocks and to contribute to rock weathering (Gorbushina 2007). Filamentous fungi identified in the vitrified niche, including members of the Lecanoromycetes and Eurotiales classes, have the potential to weather glass physically, as a result of mechanical stress from the contraction and expansion of fungal filaments, through wetting and drying cycles (Burford et al. 2003). Additionally, melanization can contribute to mechanical strength to hyphae, increasing their ability to penetrate rock crevices (Gorbushina 2007).

The vitrified niche was enriched in Pinophyta (conifer trees), which may be significant, because EcM fungi associated with roots of pine trees in Sweden have been shown to dissolve mineral substrates, releasing biologically important elements such as Mg (Fahad et al. 2016). Likewise, the vitrified niche was also enriched in EcM fungi, possibly indicating nutrient mining by plant roots and associated mycorrhiza. The role of rhizosphere microbes, both prokaryotic and eukaryotic, in the weathering of minerals, including natural basalt glasses, is well established (Berner and Cochran 1998; Fahad et al. 2016; Whitman et al. 2018). The order Mortierellales (Mortierellales phylum in the 18S rRNA genes, Mortierellomycota phylum in the ITS region), which includes soil-inhabiting saprotrophic fungi, was detected in low to medium abundance in the vitrified material (Figure S12(D,E)). However, spores of these organisms are important in potential glass weathering through ice nucleation and water retention (Fröhlich-Nowoisky et al. 2015). Mortierellales can also extract P from the glass by solubilization, P mineralization, or hyphal P transfer to promote P uptake by plants (Almario et al. 2017).

Although testate amoebae found on vitrified material and in the topsoil do not necessarily interact directly with the glass, they presumably feed on the microbial biofilm growing on the glass surface, and thereby may indirectly drive the local chemical gradients by preferentially consuming one microbial species over another. Testate amoebae can also be used as a proxy for past changes in the hydrological status of the local alteration environment around the glass (Royles et al. 2013).

**Bacterial communities**

Proteobacteria were the dominant prokaryotes in the vitrified niche. Acidobacteria and Actinobacteria, which are common colonizers of glass surfaces (Weaver et al. In review) and tombstones (Brewer and Fierer 2018) and are present in rock coatings at a Swedish Lapland site (Marnocha and Dixon 2014), were abundant in the vitrified niche and soil. At the order level, the vitrified niche was dominated by Pseudomonadales. Members of this order are significant components of the lichen microbiome (Aschenbrenner et al. 2016; Cernava et al. 2017), and also have been implicated in mineral weathering (Sokolova 2011; Uroz et al. 2009). Other non-phototrophic prokaryotes that are significant components of the ‘lichen holobiome’ (Cernava et al. 2017) and were found in abundance in the vitrified niche include Chthoniobacterales (Cernava et al. 2017), Rhizobiales (Piervittori et al. 1996), and other actinobacteria and mycobacteria (Fahad et al. 2016).
(Aschenbrenner et al. 2016; Cernava et al. 2017), and "Burkholderia" (Cernava et al. 2017; Napieralski et al. 2019). Burkholderiales presence is of significance because Burkholderia cepacia (formerly Pseudomonas cepacia) has been identified as an organism representative of acid-producing bacteria that create conditions conducive to the degradation of low radioactivity waste forms such as concrete (Rogers RD et al. 1995). B. cepacia also extract P from phosphate ore (Goldstein et al. 1993), and are known as plant growth promoting microbes, due to their ability to solubilize Fe and P. This has significance, as the dark glass within the vitrified niche contains up to ≈1% P as P₂O₅ and up to ≈11% Fe as Fe₂O₃ (Krensten et al. 1996). Radioactive waste glass can also contain significant concentrations of these elements, but additional work is required to understand how available these elements would be to B. cepacia in a homogeneous glass, versus the multiphase archeological glass examined here (Müller, Buechele, et al. 2001; Müller, Drewello, et al. 2001). Within the Chthoniobacteriales order, the DA101 soil group family (Verrucomicrobia phylum, Spartobacteria class), which is a significant member of the lichen holobiome on tree bark (Cernava et al. 2017), showed high abundance in the vitrified niche. To our knowledge, this is the first documentation of the DA101 soil group as an endolithic organism.

In the Bacteroidetes phylum, members of the Chitinophagia class (Figures 4(C) and 6(C)) are members of microbial communities on rhizosphere minerals (Whitman et al. 2018), recent volcanic flows (Byloos et al. 2018), weathered rock surfaces (releasing of Al, Fe, K, and Si from biotite and feldspar) (Cheng et al. 2017), and deep subsurface fissure water. Microbes can accelerate silicate mineral dissolution through the production of organic acids, protons, and siderophores, and mineral-weathering bacteria can significantly lower the local pH (pH < 4) (Uroz et al. 2009; Weaver et al. In review). The ability of microbes to release Si from silicates can have implications for radioactive waste glass disposal, as breakage of the Si–O bond, via a hydrolysis reaction, is the initial rate-determining dissolution mechanism in glass weathering, prior to the solution reaching saturation with respect to Si (Heimann 2018). Clostridia (Firmicutes phylum), in addition to being components of BSCs, are members of communities on recent volcanic flows (Byloos et al. 2018), Antarctic rock surfaces (Koo et al. 2018), and subsurface rock surfaces (Jones and Bennett 2014). Members of the Bacilli class of Firmicutes colonize medieval stained glass windows (Marvasi et al. 2009) and are agents of mineral weathering in soil (Wang et al. 2018). Specifically, Bacillus strains produce geochemically reactive organic acids, protons and siderophores, and are highly effective Fe and Si solubilizers. Organic acids such as citric, succinic, and tartaric acid can enhance glass dissolution based on their ability to form chelate complexes at the glass surface (Perez et al. 2016). Members of the Verrucomicrobia phylum colonize weathered terrestrial volcanic glass (Kelly et al. 2010), with Verrucomicrobiae and Spartobacteria classes forming components of microbial communities on recent volcanic flows (Byloos et al. 2018), as well as being members of the lichen holobiome (Cernava et al. 2017). These microbial communities can weather silicate minerals and glasses to access essential nutrients, e.g. phosphorus, iron and potassium, bound within the silicate matrix (Rogers and Bennett 2004).

Conclusions

Predicting vitrified wasteform alteration and its impact on radionuclide mobility requires knowledge of the altering environment and how it constrains microbial growth. Examination of microbial communities established on the walls at Broborg, a ≈1,500-year-old hillfort, demonstrates that the vitrified niche, adjacent soil, and general topsoil support distinct microbial communities in close spatial proximity. The differences in taxa for each reflect the niche partitioning, with the vitrified niche supporting a less diverse microbial community of bacteria, fungi, and protists. The vitrified niche is colonized by lichens, endolithic yeasts, and other endolithic/epilithic organisms, including Pseudomonads. The soil proximal to the vitrified wall is enriched in chemical and mineralogical vitrification products, and in endolithic/epilithic organisms, including those associated with BSCs. In contrast, the general topsoil is chemically and mineralogically enriched in pre-vitrification material and in non-endolithic organisms. The results show that the vitrified niche plays a significant role in partitioning the structure of the microbial community by influencing the nutrient source, growth substrate, and growth conditions, e.g. pH and humidity. The vitrified niche microbial community contains taxa with bio-corrosive properties that could be detrimental to glass durability, including silicate mineral dissolution, extraction of essential elements, secretion of geochemically reactive organic acids, and dissolution induced by improved water retention. However, such stable long-term biofilms also can possess a homeostatic function (Charola et al. 2011) that could limit glass corrosion, as rapid changes in the environment are more destructive to glasses than any given constant environment. To the best of our knowledge, this represents the first study of a habitat analogue for disposed radioactive waste glass and demonstrates that a specialized microbial community has adapted to life in the vitrified niche. The identified microbes could impact glass dissolution rates through various biocorrosion/bio-stabilization processes, though the consequences for radioactive waste glass disposal require further investigation.

Acknowledgments

The authors give special thanks to Uppsala County Administrative Board for permission to conduct an archaeological field survey and to Fredrik Larsson and Torbjörn Jakobsson Holback (The Archaeologists, National Historical Museums) for conducting the excavation. A portion of the research was performed using the Environmental Molecular Sciences Laboratory (EMSL), a national scientific user facility sponsored by the DOE’s Office of Biological and Environmental Research located at Pacific Northwest National Laboratory under proposal number 49141. The authors acknowledge Kayla C. Johnson, Elsa A. Cordova, Kent E. Parker, and Charles T. Resch (PNNL) for performing analyses to determine the moisture, OM content, and chemistry of the...
soil samples. The authors also acknowledge James J. Neeway and Kitt Bagwell (PNNL) for their careful review of the manuscript and insightful discussions.

Disclosure statement

The authors declare no competing interests of a financial nature that, through their potential influence on behavior or content, or from perception of such potential influences, could undermine the objectivity, integrity or perceived value of this manuscript. Trade names and commercial products are identified in this paper to specify the experimental procedures in adequate detail. This identification does not imply recommendation or endorsement by the authors or by the National Institute of Standards and Technology, nor does it imply that the products identified are necessarily the best available for the purpose.

Funding

This work is partially supported by United States Department of Energy (US DOE) Office of Environmental Management, International Programs, and by the US DOE Waste Treatment and Immobilization Plant Project.

ORCID

Andrew E. Plymale http://orcid.org/0000-0003-3307-0155
Jacqueline R. Wells http://orcid.org/0000-0001-7043-278X
Carolyn I. Pearce http://orcid.org/0000-0003-3098-1615
Colin J. Brislawn http://orcid.org/0000-0002-9109-1950
Emily B. Graham http://orcid.org/0000-0002-4623-7076
Tanya E. Cheeke http://orcid.org/0000-0002-5335-6225
Jessica L. Allen http://orcid.org/0000-0002-6152-003X
Sarah J. Fansler http://orcid.org/0000-0003-4190-907X
Mark E. Bowden http://orcid.org/0000-0003-3812-3340
Danielle L. Saunders http://orcid.org/0000-0002-7943-3102
Vincent G. Danna http://orcid.org/0000-0002-2748-6181
Jamie L. Weaver http://orcid.org/0000-0002-6762-0568
Rolf Sjøblom http://orcid.org/0000-0003-2544-6087
Rick Paul http://orcid.org/0000-0002-6366-1901
John S. McCloy http://orcid.org/0000-0001-7476-7771
Erik Ogenhall http://orcid.org/0000-0003-1990-8572
Albert A. Kruger http://orcid.org/0000-0001-8468-0813

References


