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Microbiological observations in the caves of Grottedal, Kronprins Christian Land, northeast Greenland

Hazel A BARTON [1], **George J BRELEY** [2] and **M Paul SMITH** [3]

[1] Departments of Biology and Geosciences, University of Akron, Akron, OH, USA.
E-mail: **bartonh@uakron.edu**

[2] Integrated Bioscience, University of Akron, Akron, OH, USA.
E-mail: **gjb55@zips.uakron.edu**

[3] Oxford University Museum of Natural History, University of Oxford, Oxford, OX1 3PW, UK.
E-mail: **paul.smith@oum.ox.ac.uk**

Abstract: The caves of Grottedal, Kronprins Christian Land, northeast Greenland were examined for microbial activity during the 2019 Northeast Greenland Caves Project. Given the freezing temperatures, desiccating conditions, and poor organic content of these caves, little microbial activity was expected. Nonetheless, field observation demonstrated a surprising level of microbial activity, dominated by photosynthetic species in near-entrance zones. This included the presence of extremophilic green algae (chlorophytes), along with cyanobacteria that formed photokarren. Other microbial activity that was observed indirectly included microbialites and iron-oxide deposits, which might indicate microbial contributions to speleogenesis when the region was warmer and cave development was ongoing. The Grottedal area and caves have several environmental features in common with Mars: a polar desert under desiccating, low-light conditions. Nonetheless, observations at Grottedal indicate that significant microbial activity, which can generate long-term geochemical and geomorphological signatures, is possible. Such data suggest that the Grottedal caves could provide an important testbed for future astrobiological investigations and instrument development.

Keywords: Grottedal caves; microbialites; microbial iron-oxidation; low temperature photosynthesis; photokarren; Northeast Greenland National Park.

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Introduction

During the Greenland Caves Project 2019 expedition (GCP 2019), several caves were examined within limestones of the Odins Fjord and Samuelsen Høj formations (Llandovery, Silurian) of Grottedal, Kronprins Christian Land, northeast Greenland (Smith, *et al.*, 2004; Smith and Rasmussen, 2020). While there is no continuous meteorological record for Grottedal, its continental position suggests warmer summer temperatures than nearby Station Nord (81°43′N, 17°47′W) and Kap Moltke (82°09′N, 29°57′W), which have recorded mean annual surface temperatures of −17.6°C and −15.7°C, respectively (Bay, 1997; IAEA, 2015). In 1960, daily temperatures at Centrumsø were recorded from −20.0°C to +5.0°C in May, −6°C to +13.0°C in June, with above freezing temperatures throughout July; however seasonal cooling began again in late July (Krinsley, 1960; Needleman, 1960). During the GCP 2019, all of the caves examined had entrance air temperatures ranging from +4.2°C to +5.6°C, which was influenced by the surface temperatures (which reached +16°C); however, a short distance inside the caves the temperatures dropped to below freezing, ranging from −3°C to −17.1°C (Barton, *et al.*, 2020).

Whereas no specific data on permafrost in Grottedal have been recorded, the below-freezing temperatures and extensive ice within the caves indicate that they are within the continuous permafrost zone (Mavlyudov, 2018). Photosynthesis is not possible during the darkness of the arctic winter between late October (last sunrise October 19, 2018 in Grottedal; ESRL 2020) and February (first sunrise February 22, 2019), whereas the arctic summer provides sunlight for 24 hours a day between mid-April (last sunset April 12, 2019) and late August (first sunset August 31, 2019). Based on floral and vegetative characteristics, this area of Greenland is considered a herb-barren, arctic polar desert; vegetation is patchy (<2% ground cover) and generally dominated by cryptobiotic soils/crusts (up to 75% of surface cover; Bay, 1997).

During the GCP 2019 a broad inventory of microbial activity within the caves was carried out. Identified features included microbialites and other mineral deposits (iron oxides and calcite); however, given the cold conditions and limited organic carbon (due to the lack of surface vegetation), most of the extant microbial activity appears to be driven photosynthetically in the warmer, near-entrance zones.

Microbialites

What appeared to be a fossilized microbial biofilm (microbialite) was identified in Crystal Palace Cave (Chafetz and Folk, 1984; Melim *et al.,* 2016). This material had a mesh-like appearance (Fig.1a), that upon closer inspection included filaments, threads and U-loops (Fig.1b). Such deposits are more commonly observed in caves as pendulous pool fingers, which form in static water (Melim *et al.,* 2016). In Crystal Palace, the microbialite resembled the type more commonly associated with moving water, which is commonly an indication of energy input into the system from a spring or in-feeder that promotes microbial growth (Angert, *et al.,* 1998; Barton *et al.*, 2004; Barton and Luiszer, 2005). Sources of energy that can drive such growth in a subsurface geological setting include sulphide/sulphate, methane, or oil and gas deposits (Barton and Northup, 2007; Ehrlich and Newman, 2008). Whereas there are no oil and gas deposits in this area (Rasmussen and Smith, 2001), it is possible that evaporites are preserved within the peritidal tops of the parasequences that make up the Odins Fjord Formation, and could have provided groundwater sulphide/sulphate (Smith *et al.*, 2004). Pseudomorphed gypsum evaporites were recorded by Smith *et al.* (2004) in a 30m interval in the middle of the formation from Centrumsø to the southern edge of Kronprins Christian Land.

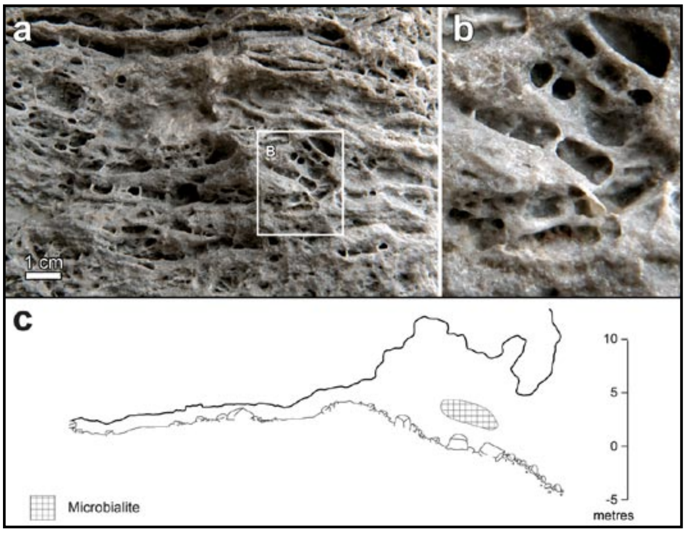

Figure 1 (above): *Fossilized microbialite within Crystal Palace Cave.* ***(a)*** *The wall in Crystal Palace contains a meshwork of calcified material that on closer inspection* ***(b)*** *appeared to be biogenic in origin, including filaments, threads, and u-loops.* ***(c)*** *Profile of Crystal Palace indicating the location of the observed microbialites.*

Alternatively, the Odins Fjord Formation also has high organic carbon levels within the subtidal parasequence bases (Smith *et al*., 2004), sufficient to produce a distinctive bituminous smell when hammered. Bitumen is a semi-solid, sulphur-rich organic deposit comprising a mix of complex polycyclic aromatic and saturated straight-chain hydrocarbons (Ohshiro and Izumi, 1999). Microorganisms can break down environmental bitumen as an energy source for growth, which liberates soluble organic carbon, along with sulphide, thiosulphate and sulphate (Brunner *et al*., 1987; Ohshiro and Izumi, 1999). The presence of either evaporites or bitumen has the potential to increase the level of reduced-sulphur compounds in groundwater, which might be sufficient to drive levels of microbial activity needed to create the fossilized biofilm observed in Crystal Palace (Angert *et al*., 1998). These data suggest that a biological component might have promoted the formation or enlargement of these caves (Hill, 1990; Barton, 2013).

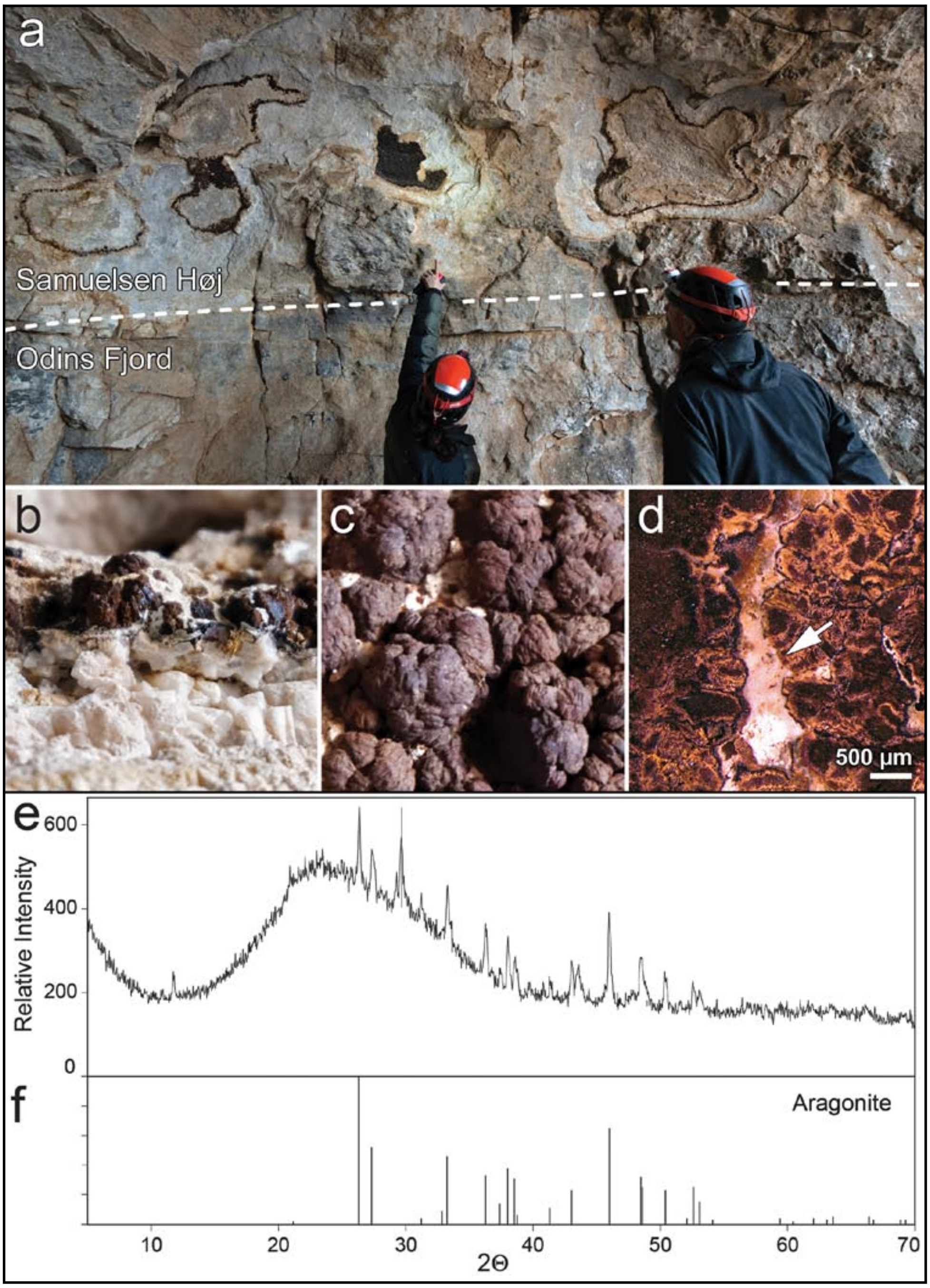

Figure 2 (right): *Iron oxides coating surfaces in Cairn Cave.* ***(a)*** *Patches of brown material were observed on exposed limestone above what appears to be the contact between the Samuelsen Høj and Odins Ford Formations (the apparent contact is highlighted by the dashed line; cavers for scale). Photographs of the crust-like iron deposits from the side* ***(b)*** *and* ***(c)*** *above.* ***(d)*** *Thin-sections demonstrate the botryoidal, laminated structure of the iron oxides, with intrusive aragonite (arrow).* ***(e)*** *XRD profile of the white material associated with the iron oxides, along with* ***(f)*** *the reference XRD peaks for aragonite.*

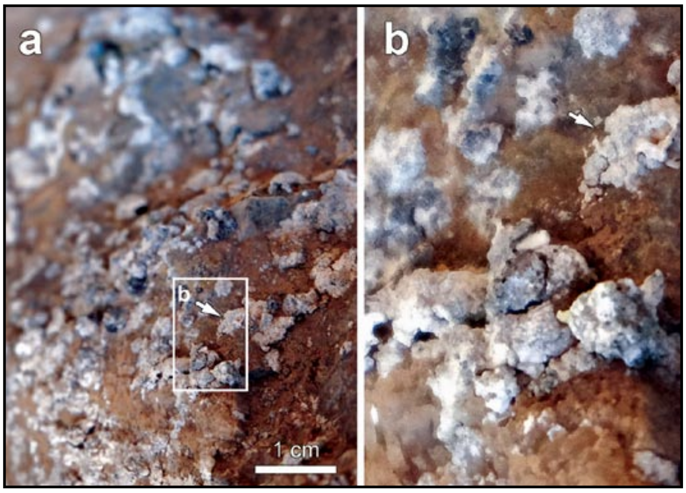

Figure 3: *Representative microbial calcification in Crystal Palace Cave.* ***(a)*** *Photograph of microbial colonies are covered with calcite on the cave wall, including* ***(b)*** *a close-up with an individual colony highlighted (white arrow) showing the calcite mineralization.*

Iron oxides

Cairn Climb Cave is developed at the boundary between the Odins Fjord and Samuelsen Høj formations (Smith and Rasmussen, 2020), where brown, crust-like deposits were identified along the wall above the boundary and lining the roof (Fig.2a). These deposits were observed on an area of exposed limestone that was not covered by a thin patina seen on other surfaces in the cave, suggesting that they might have been exposed by recent weathering (Fig.2a). Thin-sections and EDAX analyses revealed that these brown deposits contained a botryoidal iron oxide, with Ca^{2+}-rich intrusions (Fig.2d). Between this iron oxide layer and the wall rock, there appeared to be a zone of host calcite/aragonite re-crystallization (Fig.2b). Iron oxidation is generally associated with the microbial autotrophic or mixotrophic oxidation of Fe(II) to Fe(III), which creates poorly crystalline iron oxides (Weber *et al*., 2006; Ehrlich and Newman, 2008), whereas atmospheric auto-oxidation of Fe(II) by oxygen produces crystalline goethite and lepidocrocite. Based on XRD and EDAX analyses (not shown) the brown deposits comprise a poorly crystalline iron oxide with associated aragonite (presumably from the host-rock re-crystallization; Figs 2b–2f). Poorly crystalline iron oxides produced by microbial activity are quite common in caves, although they are not usually associated with low pH (Northup *et al.*, 2003; Weber *et al.*, 2006; Barton and Northup, 2007). On the other hand, Fe(III)-reduction, which is driven by Fe(II) in groundwater, reduces pH dramatically under anaerobic conditions (<pH 4.0). If such activity were occurring behind the cave walls, it would produce the observed Fe(III) oxides, and possibly lead to carbonate dissolution (Weber *et al.*, 2006; Parker *et al*., 2018). Additional work, including examining the iron oxides for the presence of microfossils, is needed to provide additional evidence of a biogenic origin for these deposits and the potential role of groundwater Fe(II).

No similar iron oxides were observed in other caves during GCP 2019. This might be because they are obscured by hoar frost, or (based on an apparent recent exposure in Cairn Climb) because they are susceptible to rapid weathering due to extreme environmental conditions and probable frost-fracturing in the caves.

Calcite

Microbial calcite deposition in caves is common, driven by the toxicity of Ca^{2+} ions released by microbial metabolic activity on limestone surfaces (Banks *et al.*, 2010). Microbes in caves overcome this toxicity by driving calcification, which traps excess environmental Ca^{2+} in an insoluble mineral form (Banks *et al.*, 2010). It was unclear whether such activity could be occurring in the caves of Greenland, due to the very cold conditions, which would reduce metabolic activity dramatically, and the absence of organic carbon derived from surface vegetation. Nonetheless, microbially-mediated calcite deposits were identified in all of the caves examined (Fig.3), though they were generally limited to the entrance zones. This suggests that either autochthonous carbon derived from photosynthesis drives such activity, or that temperatures limit metabolic activity to the entrance zone that warms above freezing (Barton, *et al*., 2020). The microbial burden within the cave sediments deeper into the cave, where below-freezing, aphotic, conditions prevail, is currently being examined, to determine whether this microbial activity is limited to the entrance zone.

Photosynthesis

Photosynthetic algae

Several of the caves contained a green material, attached to organic debris and sediment clasts (Fig.4a). Given that this material was found only in the twilight zone, under freezing conditions, where photosynthesis is unlikely, the possibility of the green material being a mineral, such as apatite, was first ruled out via XRD analyses (Barton, *et al*., 2020). Microscopy of a sample attached to bird detritus and clasts in Lemming Cave (ambient temperature −7.9°C) revealed single cells, ~5µm in diameter (Fig.4b), which adsorbed light at a wavelength of 570 – 650nm, suggesting algae and the presence of green chlorophylls (chlorophyll *a* and chlorophyll *b*; Rowan, 1989; Hawes, 1990). These photosynthetic pigments could be detected embedded in the mineral matrix of the clasts (Fig.4c), indicating a close association of these cells with the mineral substrate. The observed algal cells were non-flagellated, suggesting that they are a species of chlorophyte (van Vuuren *et al*., 2006). A thick cell, micro-colonies (up to 28 cells), and absence of an obvious pyrenoid indicates that they are a member of the genus *Oocystella,* which are commonly associated with a freshwater chemistry (as would be expected in these caves; van Vuuren *et al*., 2006). Species of *Oocystella* have not been described previously in the Arctic, but the genus does represent the dominant endemic species in a Mongolian lake that freezes solid from November to May (Tsujimura *et al*., 2003).

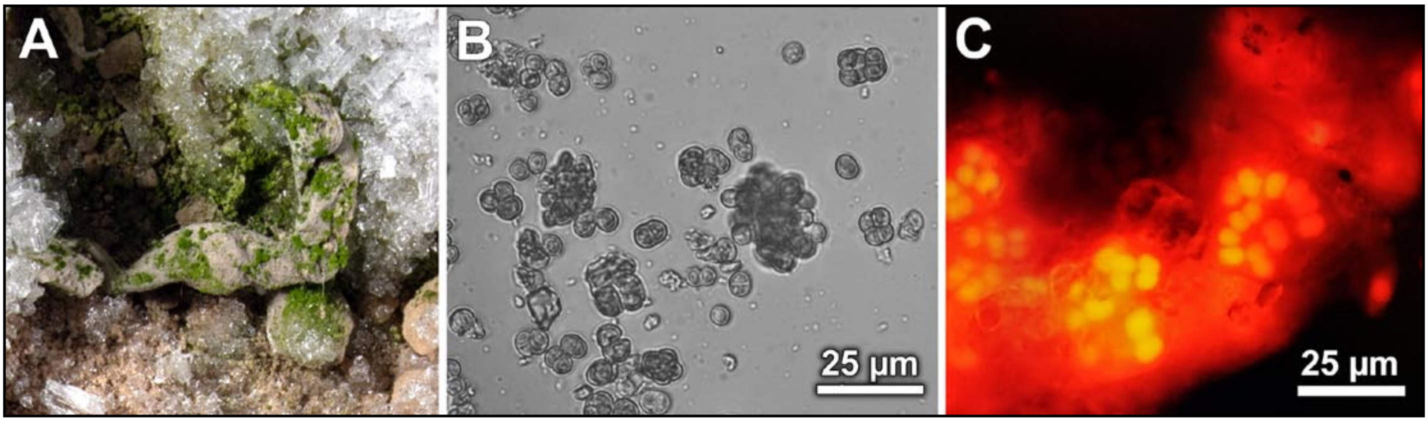

Figure 4: (a) *Green material associated with a bird pellet in Lemming Cave.* ***(b)*** *Examination of this material under the microscope revealed the presence of single, non-flagellated cells ~5µm in diameter.* ***(c)*** *Excitation with UV wavelengths from 570–650nm caused fluorescence of cells in and on clasts, which is indicative of the presence of chlorophylls a and b.*

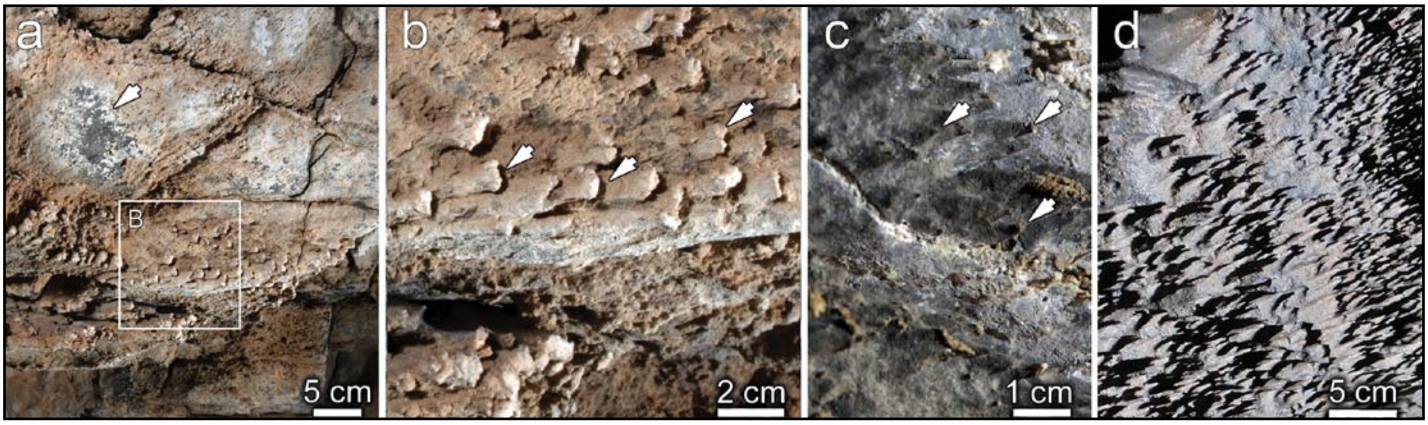

***Figure 5:** Phytokarst was identified in almost all caves examined during the GCP 2019. **(a)** Phytokarst and a close-up **(b)** of the phytokarst in U-Shaped Cave **(b)** and Grotte des Quatres **(c)**, with a comparison to phytokarst in Clearwater Cave, Borneo **(d)**. In all cases, sunlight is entering the cave from the right.*

Chlorophytes are distributed throughout the Arctic and Antarctic, where their ability to form cysts and spores allows them to survive under the extreme conditions of a polar winter (Remias *et al*., 2009; Remias *et al*., 2012). Their growth has been shown to be stimulated by phosphorous and nitrogen enrichment from bird guano (Hawes, 1983; Hawes, 1990; Bonilla *et al*., 2005; Remias *et al*., 2009), further supporting the association of this material with bird detritus in these caves, which have been used historically by birds of prey (Moseley *et al*., 2019). Photosynthesis in the chlorophytes has been described down to −7.0°C, where the production of extensive cryoprotectants and antifreeze proteins maintains activity (Kvíderová *et al*., 2013; Hájek *et al*., 2016). Additional molecular work needs to be carried out to identify the algal species present conclusively, along with the characterization of the photosynthetic pigments, to help understand the type of photosynthesis occurring under these low light conditions (Behrendt *et al*., 2019).

Photokarren

Small (5–20mm) spires of limestone, orientated toward the entrance, were identified within several caves (Figs 5a–5c). Such features, known as photokarren, are formed by the photosynthetic activity of cyanobacteria dissolving the host rock, with the resultant formation of sunlight-orientated spires (Folk *et al*., 1973; Viles 2006; Lundberg *et al*., 2010). Photokarren have predominantly been described in tropical regions, suggesting that warm and humid conditions favour their development (Brook and Waltham, 1978; Brook and Waltham, 1979; Lundberg *et al*., 2010; Barton and Breley, 2019), although the discovery of photokarren under marine conditions in Ireland suggests that humidity might be the dominant driver (Simms, 1990). Nonetheless, Oberender and Plan (2015) described small niches of photokarren in a shelter cave in the Austrian Alps. This high-altitude cave, Untere Trainsenbacher Höhle, is not an ice cave, but its entrance, formed at the contact between two limestone lithologies, is influenced by frost-weathering, similar to conditions observed in some of the Grottedal caves (Oberender and Plan, 2015).

Wherever photokarren have been described, they occur under low light conditions (Brook and Waltham, 1978; Simms, 1990; Viles, 2006; Oberender and Plan, 2015; Limbert *et al*., 2016; Barton and Breley, 2019); nevertheless, significant cyanobacterial growth is observed. This cyanobacterial growth is associated with intense carbonate boring activity, which might be an attempt by the bacteria to obtain scarce nutrients (N, P, Fe) from the host rock (Cockell and Herrera, 2008; Barton and Breley, 2019). It is unclear what chemistry drives this boring activity, but we previously demonstrated that cyanobacteria growing in caves under such low-light conditions are enhanced in red-shifted (700 – 800 nm) photosynthesis, which influences microbial community structure and metabolic activity (Behrendt *et al.*, 2019). Alternatively, Garcia-Pichel (2006) suggests that the photosynthetic dark cycle leads to heterotrophic respiration and CO_2 release in these cyanobacteria, producing the carbonic acid that drives dissolution. Such activity might be enhanced during the long polar winter, although this would be difficult to reconcile with the freezing temperatures. Alternatively, the higher levels of condensation that occur in these cave entrances (Barton *et al*., 2020) might lead to locally high humidity, which is more reflective of the conditions where photokarren are otherwise observed (Brook and Waltham, 1978; Simms, 1990; Viles, 2006; Lundberg *et al.*, 2010; Barton and Breley, 2019). Whatever the mechanism, the observations of photokarren under these highly unusual cave conditions may provide critical clues about the processes that lead to the development of this rare and unusual cave feature (Viles, 2006).

Conclusions

The caves examined during the GCP 2019 expedition demonstrated more evidence of microbial activity than had been anticipated, especially regarding the presence of photosynthetic algae and cyanobacteria (Figs 4 and 5). Whereas the observed cave conditions seemed inherently too extreme to allow photosynthesis, it appeared that photosynthesis was indeed the dominant microbial activity observed from the entrance into the twilight zone. While normally low light conditions, freezing temperatures, desiccating conditions, and poor organic content would limit photosynthesis, the extremophilic nature of the observed algae promoted significant growth, albeit only in the presence of bird detritus, which likely provides the N and P that are essential for growth. The presence of cyanobacteria with associated photokarren was likewise unexpected. Photokarren have previously been suggested as a possible astrobiological target, given their ability to preserve evidence of cyanobacterial activity within the geologic record (Corenblit *et al*., 2019); however, this is the first time that such geomicrobial activity has been detected and described under conditions that appear similar to those on Mars.

Other microbial activity was observed indirectly, providing clues to extinct microbial activity that might have occurred when this region was warmer, groundwater movement occurred, and the caves were not within the permafrost zone (Figs 1 and 2). Observations of iron oxides and microbialites in the caves could both provide clues to the involvement of microorganisms in cave development (Hill, 1981). Based on these observations, a model is suggested, wherein the reduced porosity of some horizons within the Odins Fjord Formation acts as an aquitard to groundwater movement, focusing some of the dissolutional activity and cave development at the Samuelsen Høj/Odins Fjord formational boundary. Examples of caves developed at this horizon include Cairn Climb, Crystal Palace, Skylight and Triangle caves on the southern side of Grottedal. At the aquitard, Fe-rich waters concentrated along the top of the Odins Fjord Formation could potentially be utilized for microbial growth and Fe(II)-reduction, as suggested by the observed iron oxides in Cairn Climb Cave (Fig.2). Microbial desulphurization of organic material within the Odins Fjord Formation could also have led to the release of sulphides, thiosulphates and organic carbon. This energy- and sulphide-rich water would then generate sulphuric acid through microbial oxidation, increasing the acidity of the water at the Samuelsen Høj/Odins Fjord contact, enhancing dissolution (Hill, 1981; Hill, 1990; Engel *et al*., 2004; Barton, 2013). If such processes had been involved in speleogenesis, this would be indicated by geomorphology within the cave, including localized dissolution, although the extensive frost-fracturing and evident condensation corrosion would likely obscure such morphological features (Hill, 1990; Barton, 2013; Barton *et al*., 2020). Nonetheless, fossilized biofilm in Crystal Palace suggests that extensive microbial activity has indeed occurred close to the Samuelsen Høj/Odins Fjord formational boundary (Fig.1). Additional analyses are needed to test this hypothesis, including extensive chemical analyses of the boundary zone lithologies and associated groundwater, the ability of the limestones of the Odins Fjord Formation to promote microbial growth, and a more extensive examination of the microfossils found associated with karst in Crystal Palace.

Although carbonates are scarce on Mars and most cave features identified on the planet to date are in basalt, the Grottedal caves do display a number of environmental features similar to those on Mars: a polar desert under cold, low light and desiccating conditions. Given that caves are considered the best location to preserve signatures of past (and even present) microbial activity on Mars (Boston *et al.,* 2001), the type of microbiological activity observed during expedition investigations within the Grottedal caves suggests that this site could represent an important analogue environment for studying the geochemical and geomorphological signatures of life under such extreme conditions, and an important testbed for future astrobiological investigations and instrument development.

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