A CHARACTERIZATION OF MICROBIAL DIVERSITY IN THE WINTER WONDERLAND ICE CAVE, UINTA MOUNTAINS, UTAH, USA

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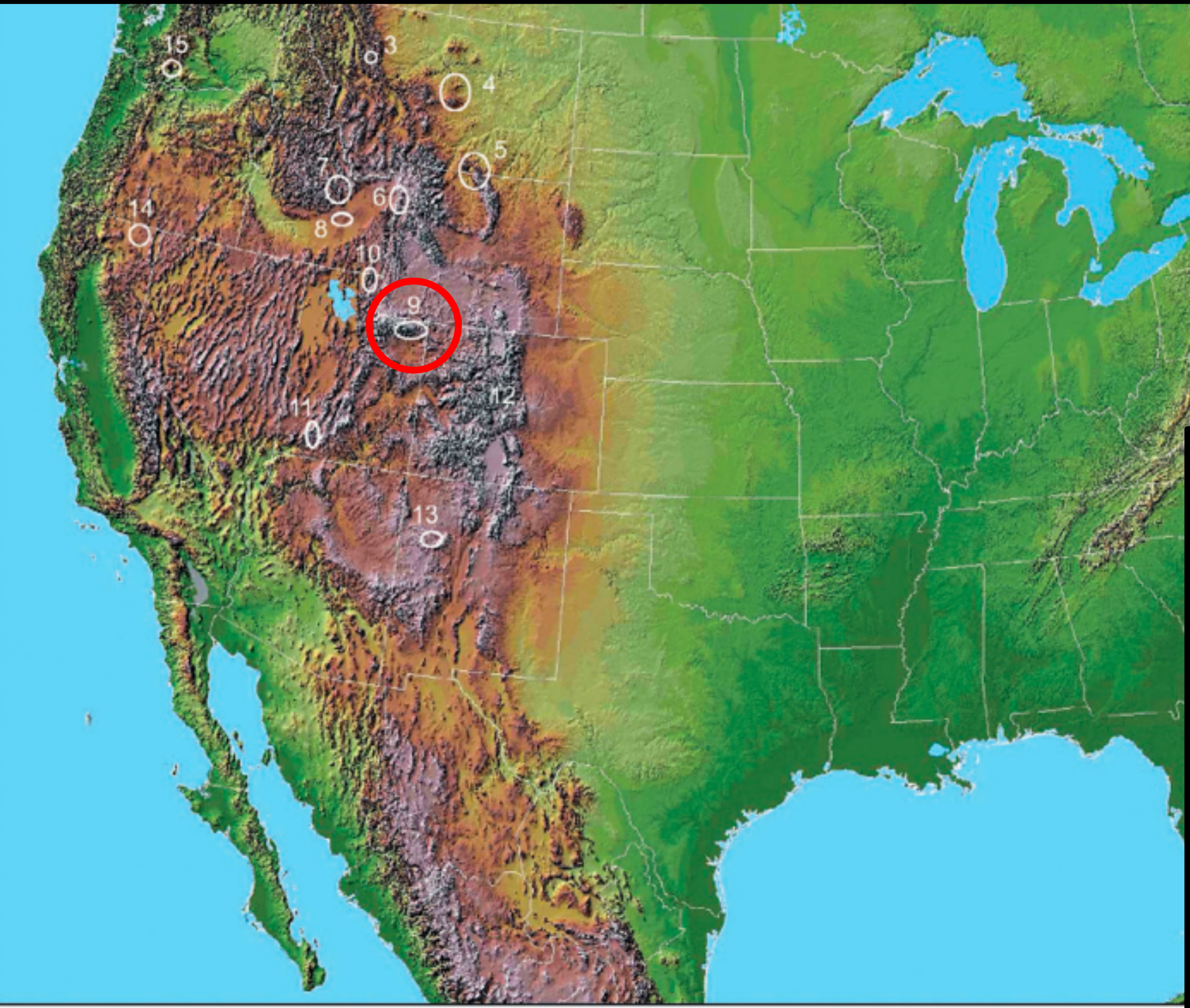
Location of the Winter Wonderland Ice Cave

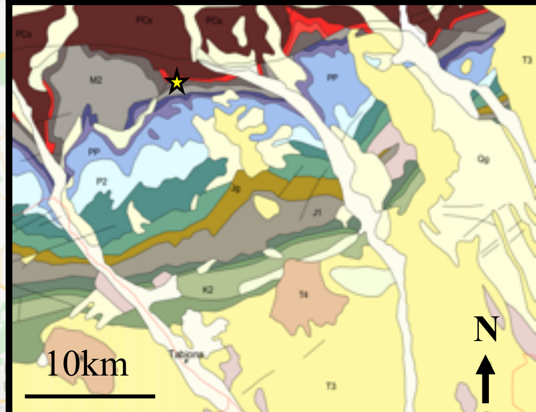
The Winter Wonderland (WW) ice cave is located within the E-W trending Uinta Mountains in northeastern Utah, USA. This cave formed in the vadose zone within Mississippian Madison Limestone (in dark gray below). The entrance is 3140 m a.s.l. and was discovered by the U.S. Forest Service in 2014. Water enters the cave, percolating through the above bedrock, and creates a layered ice mass containing organic matter dating back several centuries. As the water freezes, mineral precipitates called cryogenic cave carbonates crystallize and collect on the top of the ice.



Known ice caves in the continental USA

1000 km

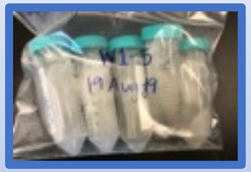
Adapted from Higham et al., 2018 and Seixas, 2020, unpublished thesis



SAMPLES

Three sample types were collected using sterile techniques

1) Water (n=5)



2) Ice (n=1)

3) Cryogenic cave carbonates (CCCs) (n=13)
Form as mineral precipitates on top of the ice

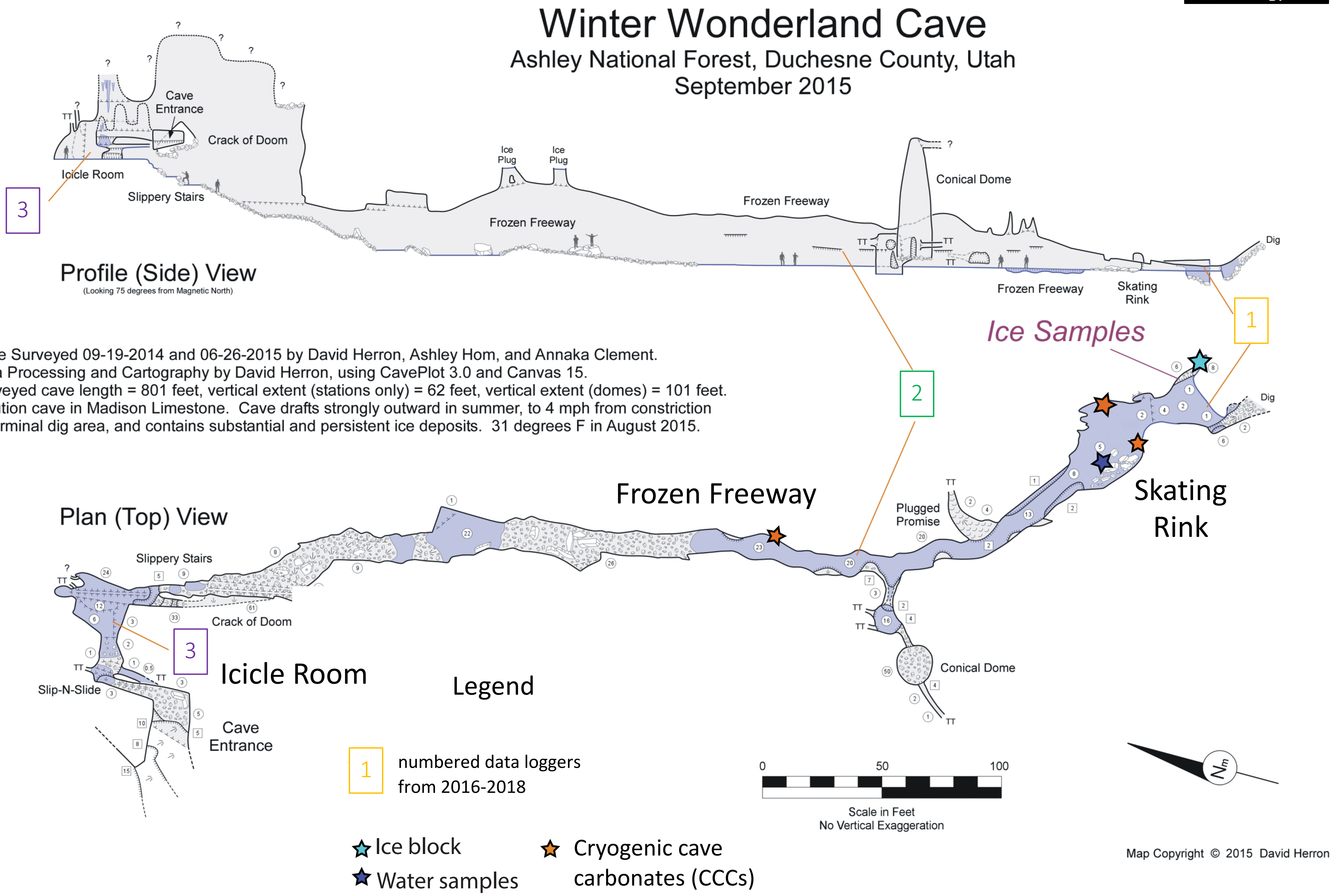
METHODS

1) Physical analysis to better understand conditions of formations for each sample type

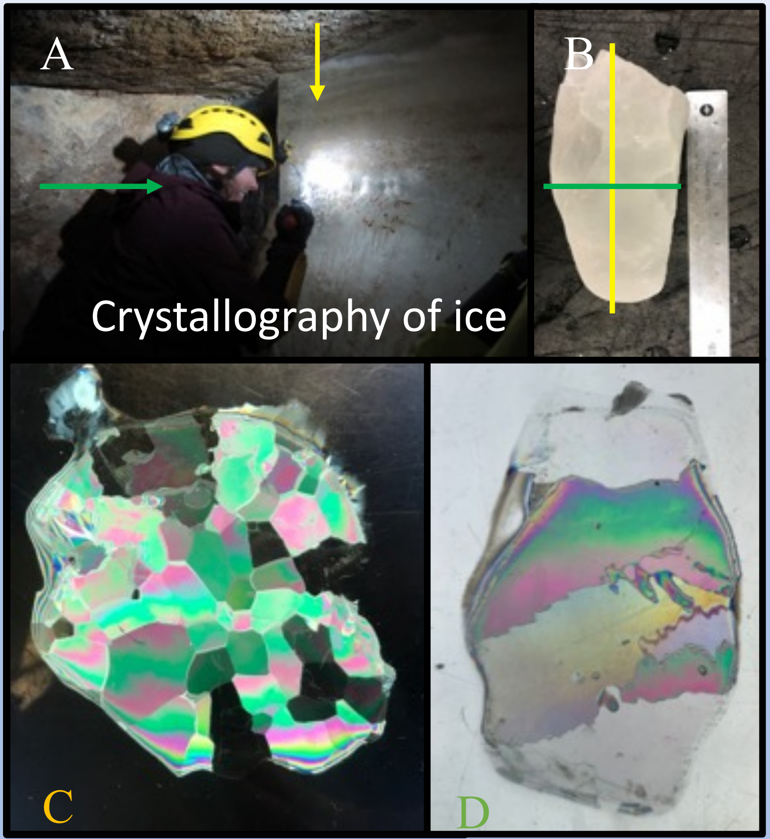
- -Temperature data loggers
- -Stable isotopes ($\delta^{18}O$ and δD)
- -Crystallography
- -SEM imagery and elemental analysis

2) Microbial analyses to understand community composition

- -Fluorescence microscopy
- -Flow cytometry
- -Illumina MiSeq Sequencing

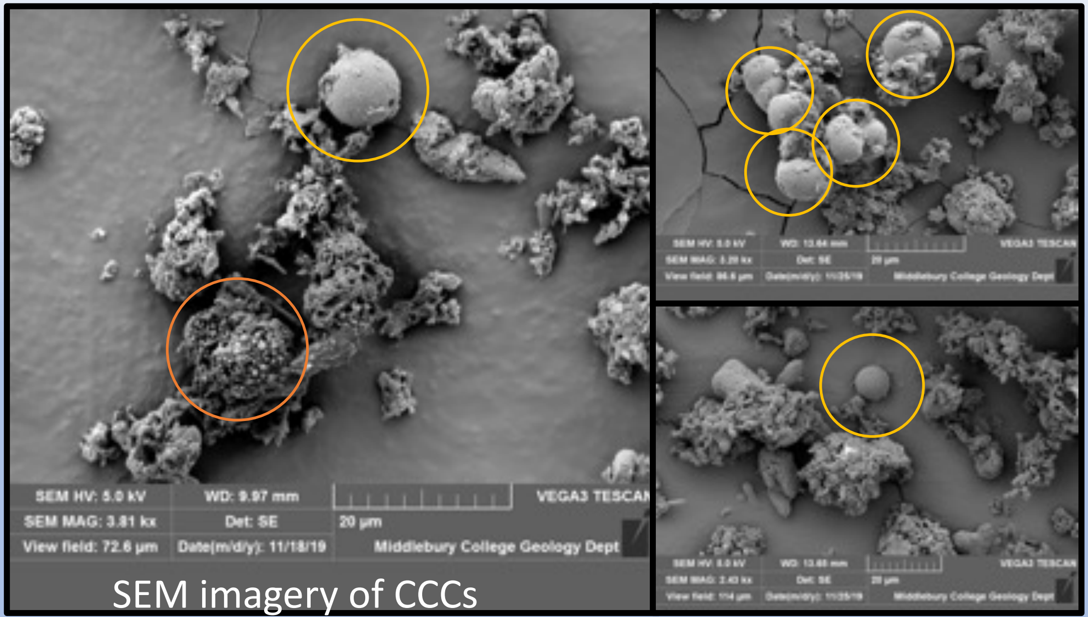


RESULTS

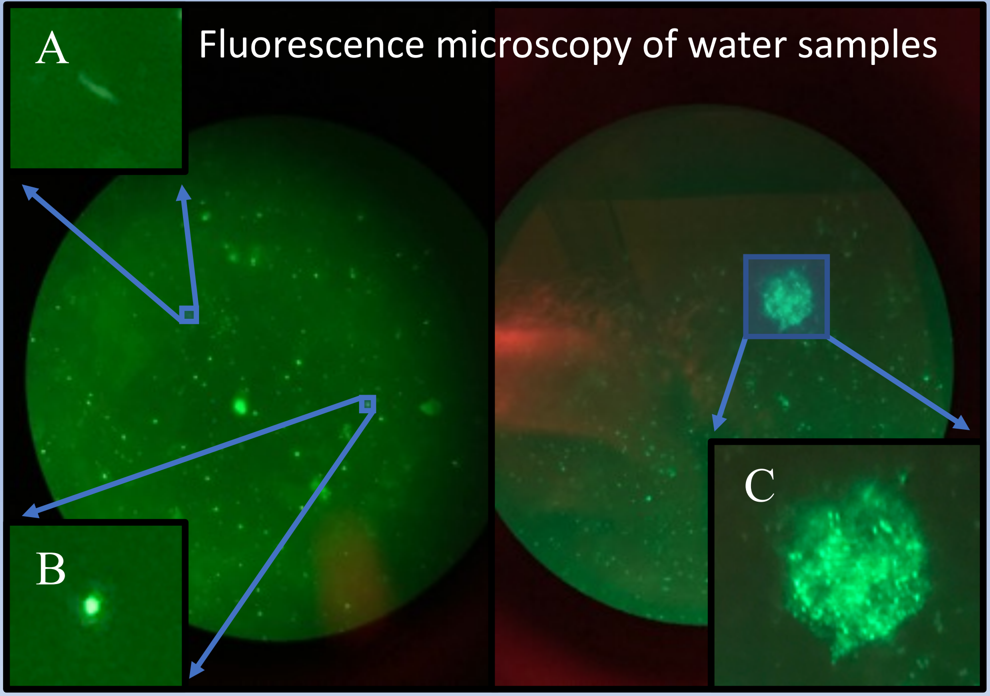


Crystallographic study of the ice block shows vertically elongated grains, which do not line up with the horizontal crystallographic c-axis, suggesting an environment of formation with low stress.

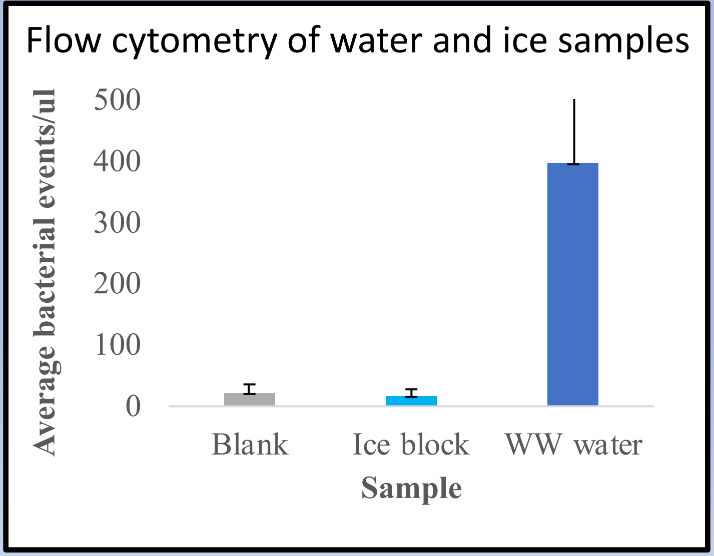
Flow cytometry using SYBR Green 1 of water samples using a 0.22um filter. Samples to the right were filtered using 3mL. Cocci and rods, as well as sediments and aggregates can be seen.



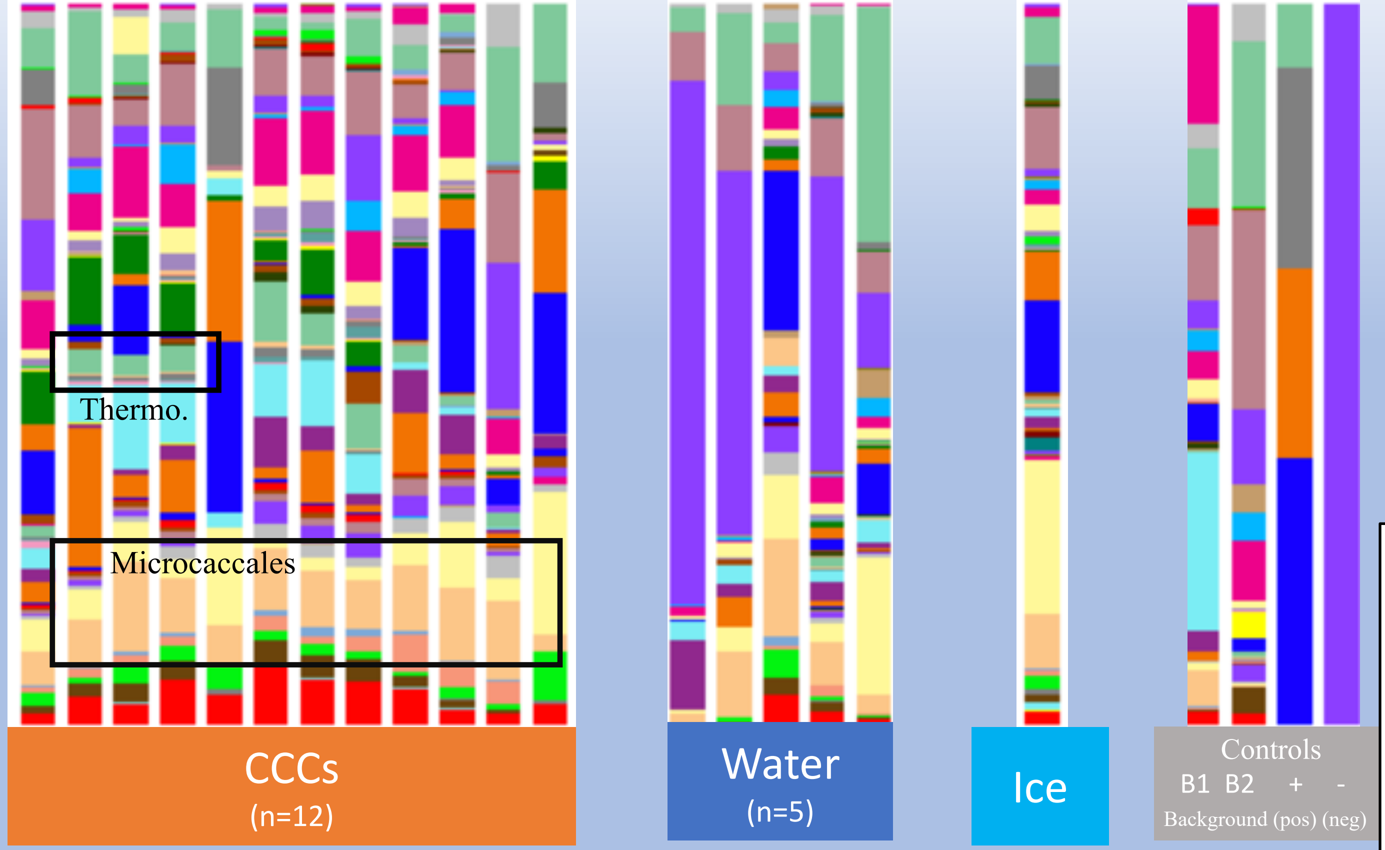
CCC samples were dried for 24 hours and then prepared for SEM imagery using a gold-palladium coating. Analysis showed the presence of uniform, spherical shapes of ~20um in diameter. When the outer layer of carbonate minerals was blasted with the laser, smaller regular spheres were found, ~2um in diameter. Hypotheses for the larger objects are eukaryotic cells, aggregates of smaller prokaryotic cells within a polysaccharide membrane, or the biomineralization of a calcareous shell or outer membrane.



Flow cytometry determined that the water samples were an order of magnitude more concentrated in microbial cells than the ice sample.



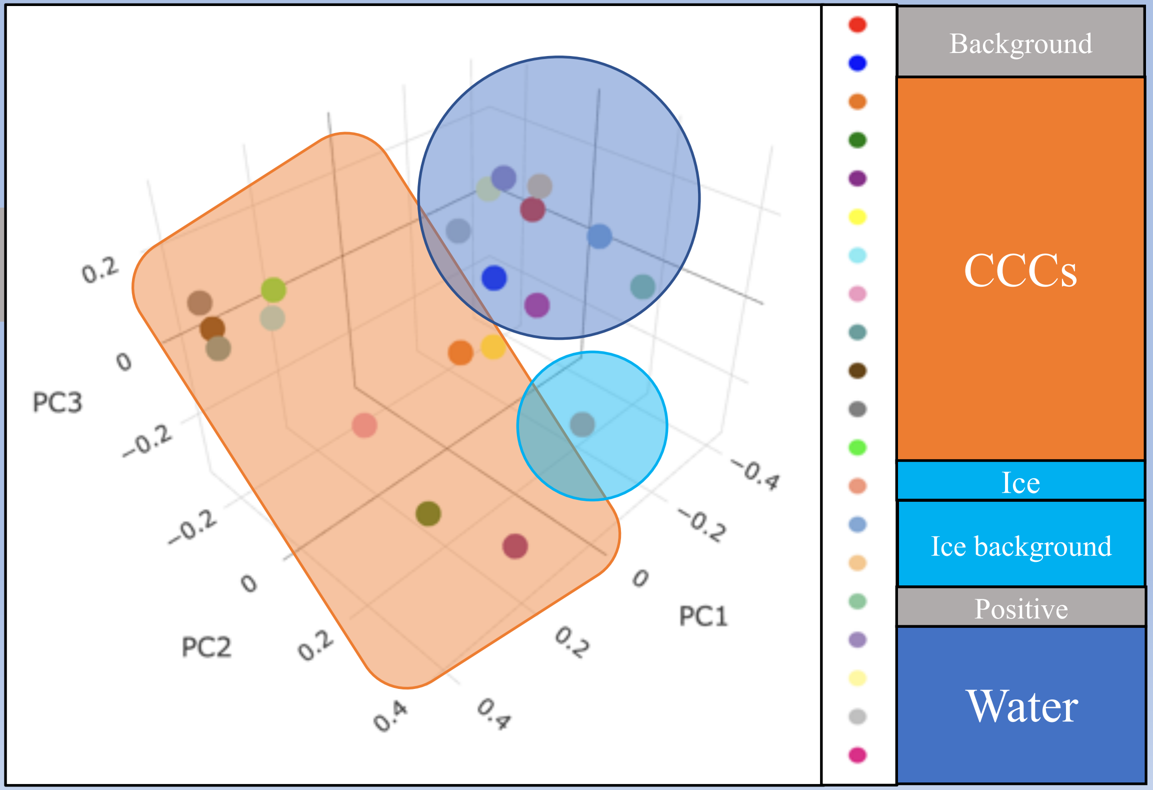
SEQUENCING RESULTS



Sequencing revealed the presence of many soil associated bacteria and archaea, suggesting that soil in the epikarst above the cave is the source of microbes found in WW.

Taxa abundance at the Order level as represented by a bar graph.. Each column represents a different sample. Sample types have been grouped. Each color in the columns notate a difference order of microorganisms and their relative abundance in the sample. Although there are similarities across sample type, the CCCs show regular and high abundance of Thermomicrobia (Chloroflexei) in light green and Micrococcales (Antinobacteria) in tan, which are photosynthesizing soil bacteria, suggesting that during precipitation the CCCs attract the soil bacteria in the water.

The Bray-Curtis beta-diversity is represented in a principle component analysis plot below. Beta diversity shows broad dissimilarities between communities, in this case base on taxa data. The clumping of samples with similar sample types shows that the water samples are more similar to each other than to the CCCs or the ice sample. Similarly, the CCCs are more similar to each other than to the water or ice. The positive control and the backgrounds are most like the water samples, supporting the hypothesis that the water samples are the youngest in the cave development and hold the most diversity and abundance of cells, similar to the dirty meltwater used as a background control.



SUMMARY

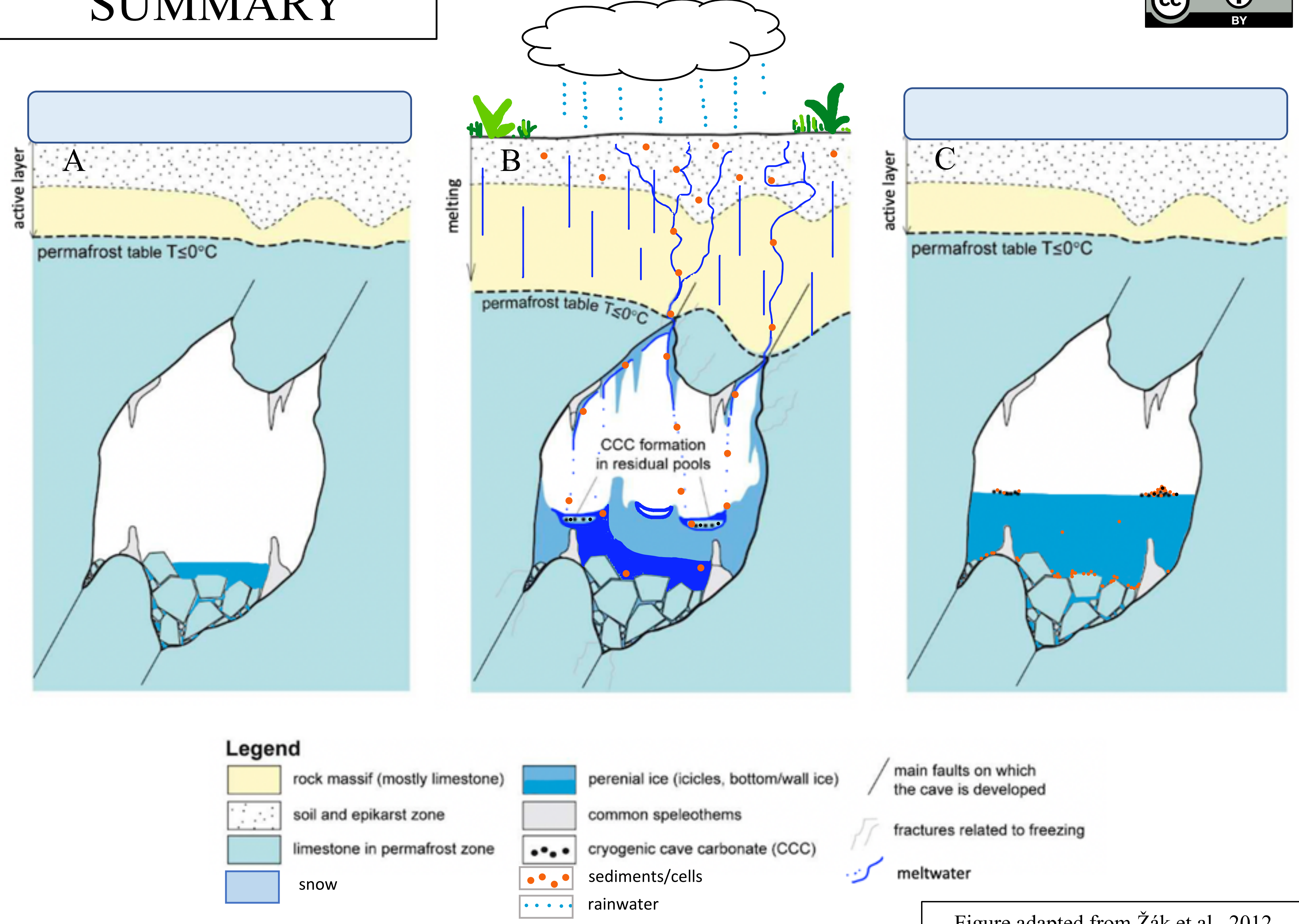


Figure adapted from Žák et al., 2012

Review of all results suggest a seasonal cycle in the Winter Wonderland ice cave, like the one modelled here. In the warmer months, the permafrost table lowers, allowing water from the soil above to percolated through fractures in the bedrock, carrying minerals, sediments, and microorganisms with it.

This water collects in small pools in the cave and then begins to freeze, as the cave is still < 0°C. The microbes in the water begin to attach to one another, sediments, and the mineralizing carbonates, forming aggregates, which fall to the floor of the pool or precipitate out with the cryogenic cave carbonates (CCCs), thus decreasing the concentration of microbial cells in the ice.

Further work needs to be done in order to understand if the microbes in the CCCs are psychrotolerant and able to actively metabolize within the conditions of the Winter Wonderland ice cave. Additionally, organisms in the CCCs may be able to construct calcareous structures similar to dinoflagellates (seen in SEM images).

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