



## Microbial Diversity in Cryoconite Holes and Dispersed Cryoconite Revealed Through Culture-Dependent and Culture-Independent Approaches

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Organic and inorganic materials from atmospheric, aeolian, and subglacial origins on glacier surfaces are colonized by microorganisms, which produce extracellular polymeric substances to bind them into cohesive aggregates known as cryoconite. Dispersed across the glacier surface, these biologically active particulates may form cylindrical cryoconite holes through localized melting driven by their dark coloration, which strongly reduces surface albedo. Dispersed cryoconite serves as both a precursor and a transitional stage in the lifecycle of cryoconite holes, which can collapse and reform multiple times during the melting season. While cryoconite holes provide a stable environment that shields microbial communities from environmental extremes, dispersed cryoconite is exposed to intense solar irradiance, freezing temperatures, and desiccation. This contrast in environmental conditions experienced by the same material is hypothesized to significantly impact the microbial dynamics and ecological functioning of cryoconite.

In this study, we investigated the cryoconite microbiome from both cryoconite holes and dispersed cryoconite collected approximately 1 km from the margin of the Greenland Ice Sheet. By comparing the microbial communities in these two environments, we aimed to understand differences in their composition and diversity. Amplicon sequencing targeting the V3–V4 region of the 16S rRNA gene was applied to capture bacterial diversity directly from raw samples and cultured communities grown under various conditions, providing insights into both the overall bacterial composition and the subset of microbes that can be cultured.

We found that species diversity and evenness were significantly higher in dispersed cryoconite than in cryoconite holes, whereas species richness remained unaffected. The microbial composition also differed, with cryoconite holes exhibiting higher relative abundances of Proteobacteria and Actinobacteria, and a lower abundance of Cyanobacteria compared to dispersed cryoconite. Differential abundance analysis revealed significant enrichment of certain taxa in each environment, including several Cyanobacteria-associated taxa that were nearly absent in cryoconite holes but abundant in dispersed cryoconite. This contrast suggests that these

Cyanobacteria may have developed advanced stress-adaptation strategies that may give them an advantage over other microorganisms in dispersed cryoconite, whereas their near absence in cryoconite holes could be driven by higher predation or other ecological pressures.

In summary, these findings underscore differences in microbial diversity between cryoconite holes and dispersed cryoconite, indicating that distinct environmental pressures may shape their microbial communities. By highlighting key differences in community composition, this work lays a foundation for future research into the broader microbial dynamics and ecological functions of cryoconite in polar environments. Further research is needed to elucidate the specific roles and succession dynamics of key taxa, such as Cyanobacteria.