

## Bacterial communities changes in cryoconite on an alpine glacier

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### Abstract

Cryoconite holes are small pits present on the glacier surface containing a fine-grained sediment on the bottom. They are a biologically active and protected microhabitat in the glacial ecosystem. In this study it has been studied the temporal variation of bacterial communities in cryoconite holes on the Forni Glacier (Stelvio National Park, Italian Alps). The results showed a temporal trend characterized by a predominant autotrophic Cyanobacteria population in the first part of the ablation season, followed by an increasing in abundance of heterotrophic Sphingobacteriales populations. Also some hole feature explained a fraction of the variation of bacterial communities, but less than temporal variation.

### Keywords

Cryoconite, Glaciers, Alps, Bacteria communities, Temporal variation

### Introduction

Glaciers represent a real ecosystem, in fact, even if they are characterized by extreme conditions, they host viable microorganisms (BOETIUS et al., 2015). Cryoconite holes are peculiar structure of glaciers surface that host a metabolically active microbial community. They are the consequence of the deposition of a wind-born sediment (cryoconite) on the glacier surface composed by both abiotic and biotic matter. This sediment decreases the albedo effect melting the underlying ice and forming a small pit full of melting water with cryoconite on the bottom: the cryoconite hole (WHARTON et al., 1985).

These structures have been found in different places like Arctic, Antarctic and also on temperate mountain glaciers (COOK et al., 2016; EDWARDS et al., 2013). Cryoconite holes are of big interest and different studies evidenced the influence of many environmental factors on their bacterial communities: sediment thickness (TELLING et al., 2012), hole area (COOK et al., 2010) and hydrology (EDWARDS et al., 2011). Few studies instead investigated the temporal variation of bacterial communities, and they were conducted on Arctic glaciers (STIBAL et al., 2015). At the best of our knowledge these studies have never been conducted on a temperate mountain glacier (COOK et al., 2016). In this study the aim was to investigate temporal variation of bacterial communities during the ablation season in relation with environmental conditions of cryoconite holes on Forni Glacier.

### Methods

On Forni glacier (46°12'30" N, 10°13'50" E; Italian Alps, Fig.1) 20 samples of cryoconite were aseptically collected on July, August and September for a total of 60 samples. Oxygen concentration, pH and temperature were measured in each hole during the sampling with a portable oximeter/pH meter (HACH LANGE HQ40D, Loveland, CO), and maximum depth recorded by a ruler. The area was estimated using the picture of each hole with a reference ruler by an automatic method (Hodson et al., 2010). Organic matter content of cryoconite was measured with the loss-on-ignition method by heating the samples at 400°C overnight (ASTM, 2000). DNA extraction was performed using the FastDNA Spin for Soil Kit (MP Biomedicals, Solon, OH). DNA processing, PCR amplifications and Operational Taxonomic Unit (OUT) definitions were conducted according to DAGHIO et al. (2016). The number of OTUs obtained and selected to compare all the samples was considered an index of alpha diversity. Statistical analyses were performed with the LMERTTEST, MASS, MICE, MULTCOMP, MULTTEST, PACKFOR and VEGAN packages in R 3.2.2 (R Core Team, 2013).

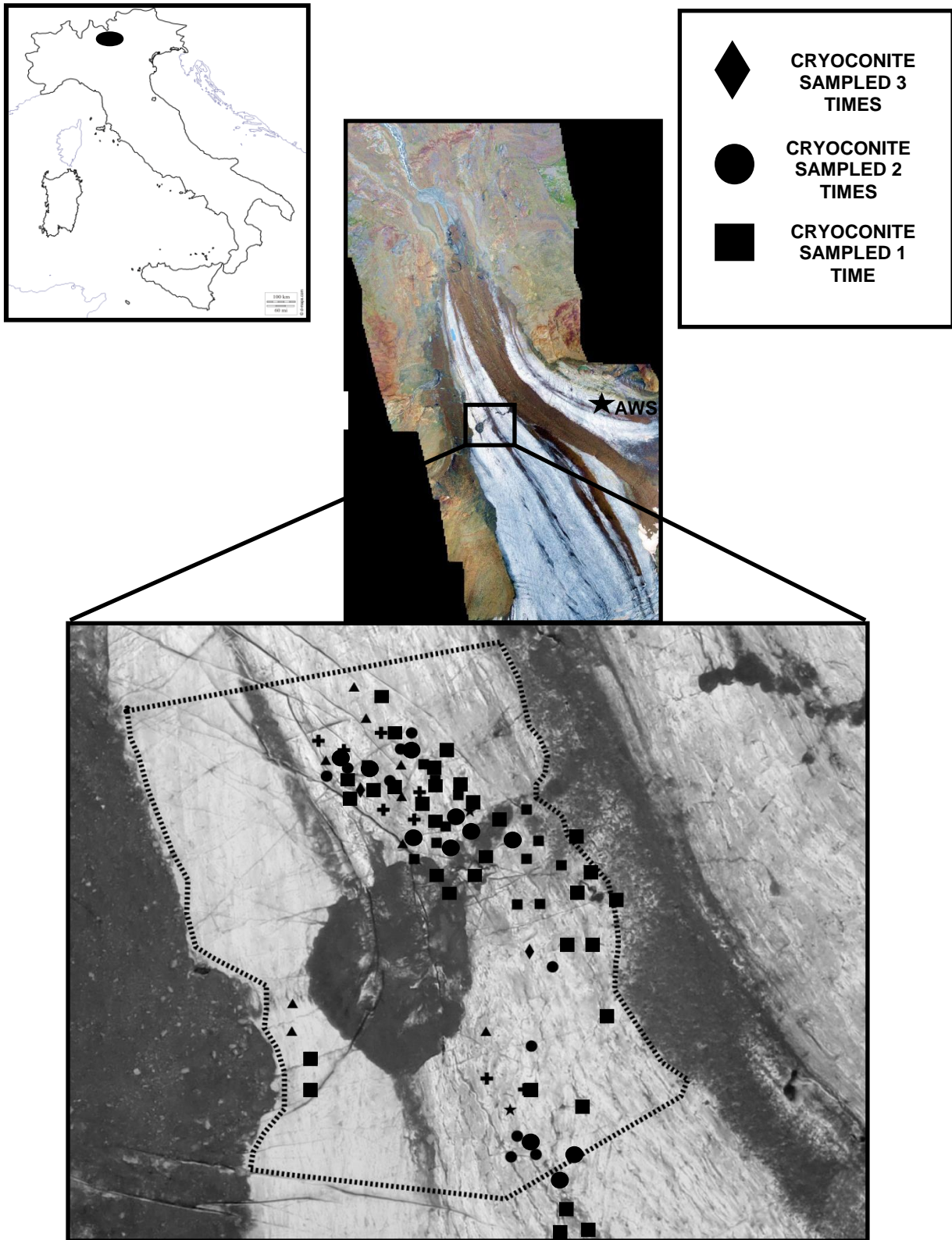


Figure 1: Forni Glacier

## Results

60 samples have been collected, and 596 OTUs were registered. The most abundant orders found were Sphingobacteriales, Pseudomonadales, Rhodospirillales, Burkholderiales and Clostridiales, that together with Cyanobacteria and an unclassified order belonging to Actinobacteria represented more than the 80% of bacteria in cryoconite. Redundancy analysis (RDA) showed that the structure of the communities changed in relation with months (Pseudo- $F_{2,57}=11.565$ ,  $p=0.005$  in all classes) and that such variation accounted for 26.4% of total variance in bacterial communities.

Bacterial communities also changed according to environmental conditions, hole depth and area, amount of organic matter in the cryoconite and oxygen concentration (Pseudo- $F_{2,57}=3.431$ ,  $p=0.001$ ), which overall explained 14.2% of variance. Generalized linear mixed models and Post-hoc tests were performed and indicated that the abundance of Cyanobacteria and Clostridiales was higher in July than in August and September (Fig. 2). While the opposite was seen for Sphingobacteriales and Burkholderiales. Depth of cryoconite holes and oxygen concentration changed significantly between months ( $F_{2,52}\geq 8.794$ ,  $pFDR\leq 0.002$ ).

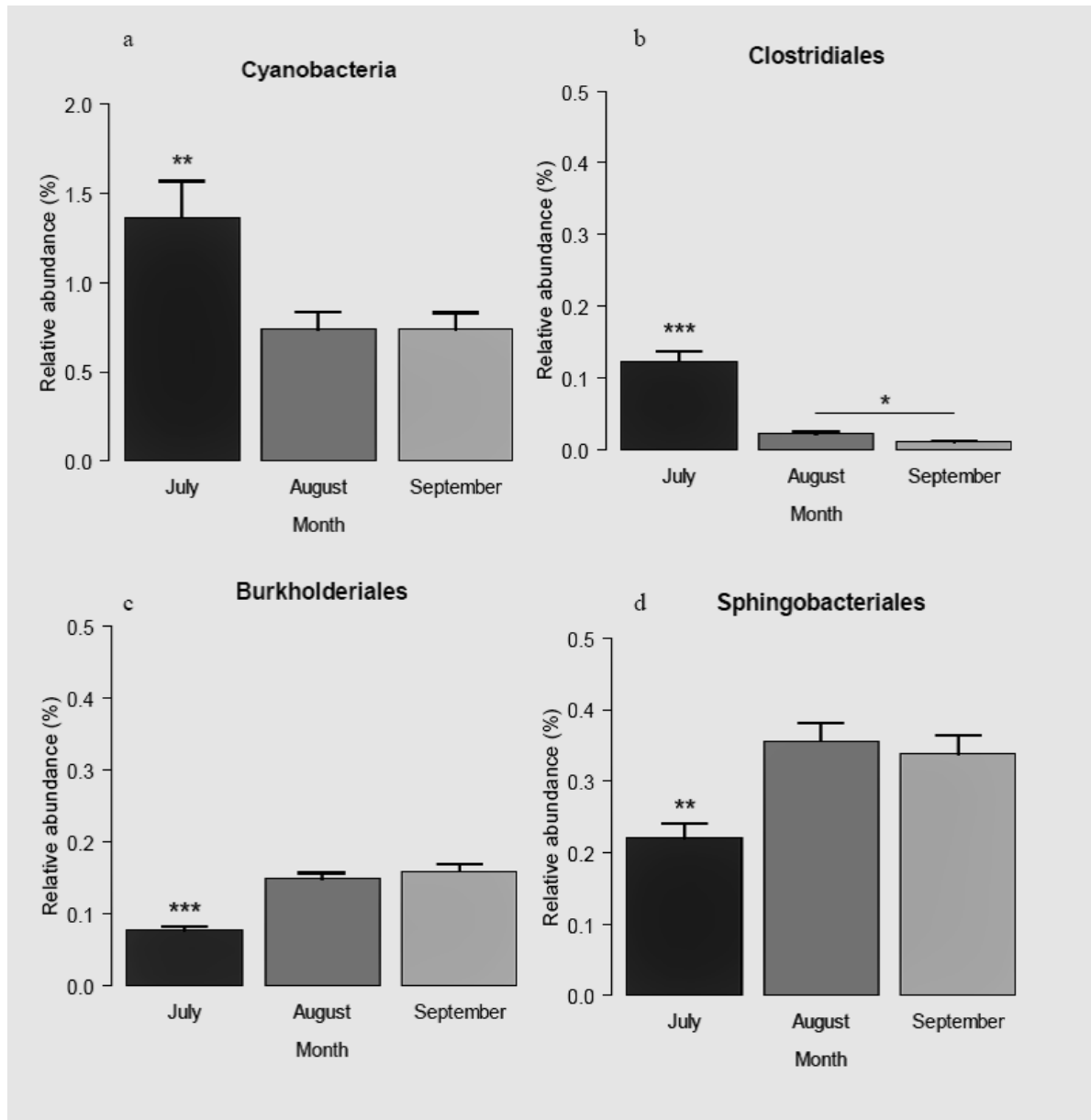


Figure 2: Relative abundance of Cyanobacteria, Clostridiales, Burkholderiales and Sphingobacteriales during the ablation season.

## Discussion

Bacteria communities in cryoconite holes changed among months, in particular the relative abundance of photosynthetic bacteria decreased, while heterotrophic populations increased later in the ablation season. This trend is consistent with previous studies (SIGLER et al., 2002). Also the effect of environmental conditions changed the structure of bacterial communities, in particular hole depth and oxygen concentration which in turn changed along the melting season as already reported (GOKUL et al., 2016). Variation partitioning analyses, however, indicated that variation among months per se was the most relevant effect. Since cryoconite holes on Forni glacier seem ephemeral structures it's more probable that variations of bacterial communities are driven by temporal changes of ecological conditions rather than by an ecological succession within the holes.

## Conclusion

This study showed that bacteria communities in cryoconite holes on Forni Glacier exhibit a temporal variation during the ablation season with an initial colonization of cryoconite by autotroph bacteria, followed by a heterotroph bacteria predominance. Temporal variation could act both directly on cryoconite communities and indirectly changing the hole features, but temporal variation seems to be more important than local environmental conditions in modifying bacterial communities. In this study other variables have been considered but didn't show any significant contribute (i. e. organic matter and hole area).

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