

**Păun VI<sup>1</sup>, Icaza G<sup>2</sup>, Lavin P<sup>2</sup>, Marin C<sup>3</sup>, Ițcuș C<sup>2</sup>, Mondini A<sup>1</sup>, Hillebrand-Voiculescu A<sup>3</sup>, Haidău C<sup>3</sup>, Bădăluța C<sup>1</sup>, Perșoiu A<sup>1</sup>, Iancu L<sup>1</sup>, Dorador C<sup>2</sup>, Purcărea C<sup>1</sup>**

<sup>1</sup>Institute of Biology, Bucharest, Romania, <sup>2</sup>University of Antofagasta, Antofagasta, Chile, <sup>3</sup>”Emil Racovita” Institute of Speleology, Bucharest, Romania  
E-mail: ioana.paun@ibiol.ro, cristina.purcarea@ibiol.ro

## INTRODUCTION

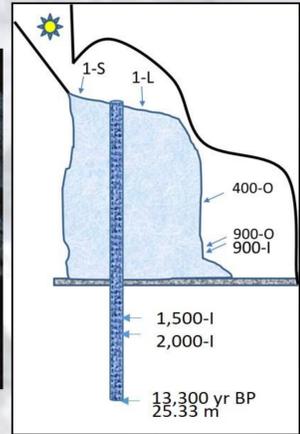
Over the last decades, the diversity and functional characteristics of bacterial communities found in cold environments (e.g. glaciers, polar ice-sheets and soil, permafrost, frozen lakes) have been intensively studied, but little is known so far on the diversity and activity of such communities from perennial ice found in ice caves.

Our study, focused on cave ice microbiome, unraveled the total and active bacterial community structure in the ~13,000 years old ice core from the perennial Scarisoara ice block, based on 16S rRNA gene Illumina sequencing, in relation with the age and geochemical composition of ice strata.

Vertical ice coring was carried out in the Great Hall area, reaching a record depth of 25.3 m.

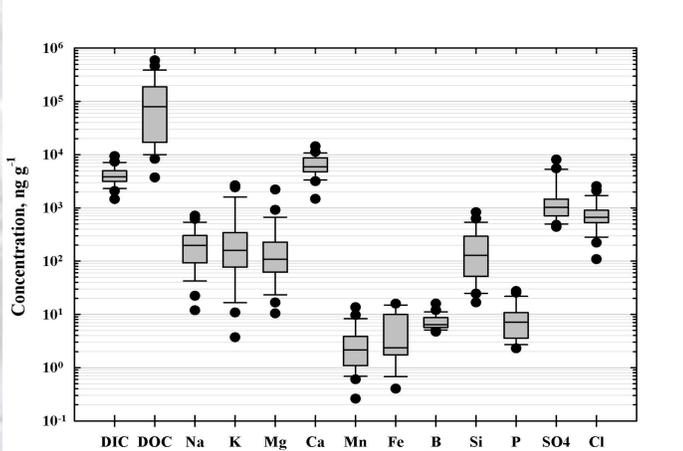
The chronology of the ice core was based on 26 <sup>14</sup>C AMS radiocarbon ages, and the depth-age model was constructed using a Bayesian model for the 0-22.5 m depth and linear extrapolation up to 25.33 m

**Samples: 15 gDNA samples, in triplicate and 15 cDNA samples starting from 100 years old up to 13,000 years old ice samples.**



## RESULTS

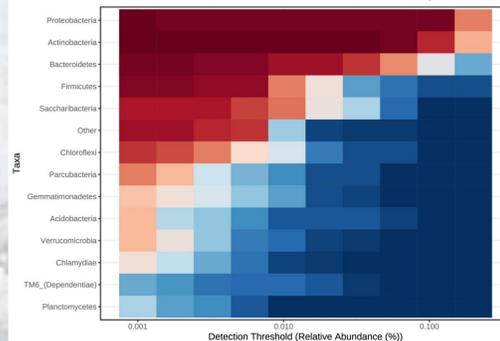
### Ice block geochemistry



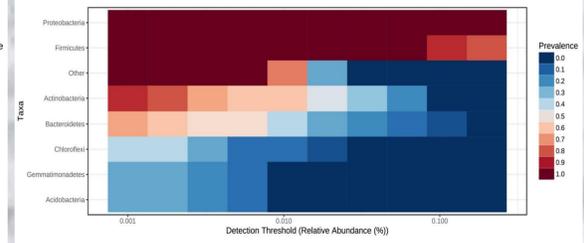
- ❖ Na, K, Mg, SO<sub>4</sub> and Cl were the major constituents throughout the ice core, while Mn, Fe, B and P were present in lower concentrations.
- ❖ The relatively high DOC/DIC values found along the Scarisoara ice core were similar to those found in other ice caves.
- ❖ Geochemical profile showed a non-homogenous temporal distribution, with higher values occurring in ice layers from the last millennium, in addition to a spike in all elements' content during the 4,500-5,000 cal BP period.
- ❖ Slightly increased concentrations of DOC, Si, Ca, P and Na were also observed in ice strata formed 7,000 years ago, followed by a somewhat increasing concentration profile of these elements in older ice deposits.

### Bacterial community structure of the ice block

#### Total bacterial community



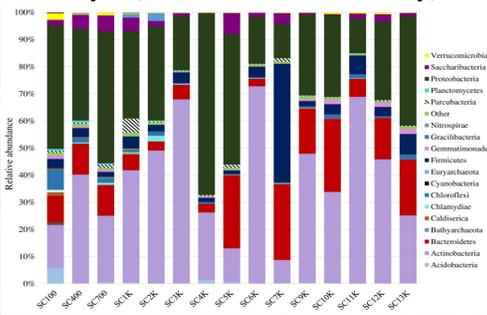
#### Active bacterial community



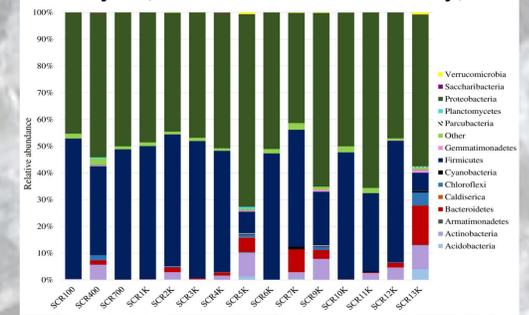
- ❖ Actinobacteria and Proteobacteria dominated the total bacterial community structure, while Bacteroidetes and Firmicutes had a high representation. The active prokaryotic community structure showed the dominance of Proteobacteria and Firmicutes, with Actinobacteria and Bacteroidetes occupying only a small fraction of this type of community from the perennial cave ice block.
- ❖ The environmental cave ice samples consisted of 42 phyla, 101 classes, and 554 genera, while the active community identified in the 13,000 years old cave ice strata contained 28 phyla, 67 classes and 334 genera.
- ❖ Alpha-, Beta-, Gamma-, Actinobacteria and Sphingobacteriia classes belonging to the total bacterial community, were present in various proportions throughout ice block strata.
- ❖ In the active community, Clostridia was the major bacterial class across the ice block, but relatively equal proportions of Gammaproteobacteria and Betaproteobacteria characterized most of the ice core strata.

### Taxonomic distribution of the prokaryotic community

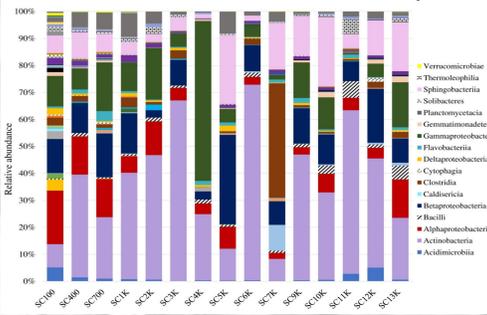
#### Phyla (total bacterial community)



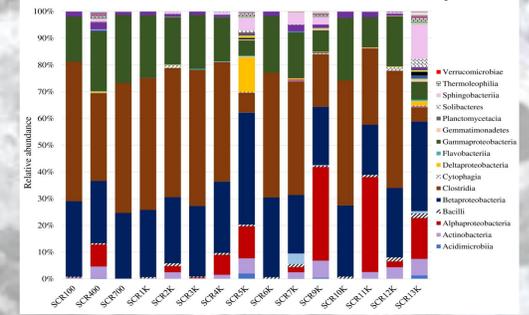
#### Phyla (active bacterial community)



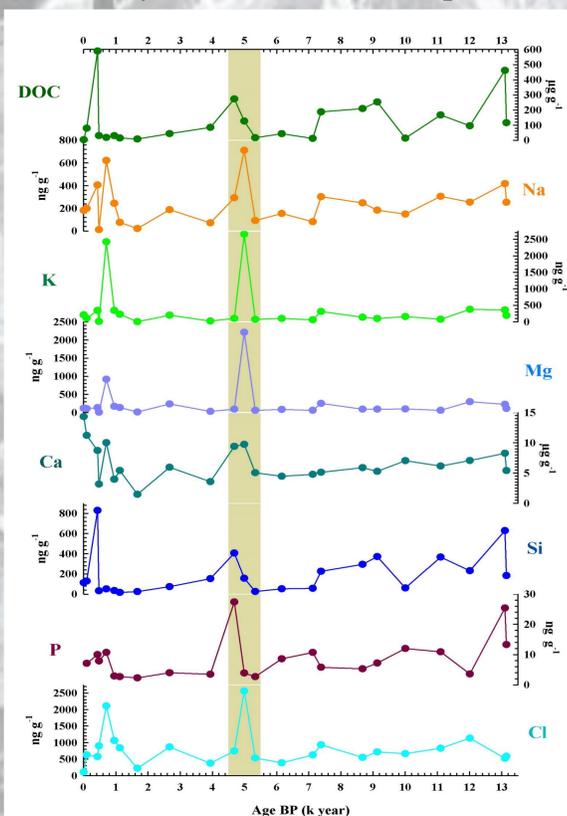
#### Class (total bacterial community)



#### Class (active bacterial community)



### 13,000 years old ice chemical profile



## CONCLUSIONS

This survey of the bacterial community from the 13,000 years old perennial ice of Scarisoara Ice Cave, a pioneering such study in ice caves, revealed a highly diverse population and the presence of an active microbiome in ice strata formed since the Late Glacial Period. A complex active bacterial community, composed of both autotrophs and heterotrophs, was found in the perennial ice block. This type of icy habitat is thus capable of supporting various metabolic processes, which in return shapes a unique microbiome. Carbon content appeared to be a major factor in determining the microbial community structure variability found in each strata, with an important role in depositional and post-depositional processes.

This first investigation of microbial communities entrapped in perennial cave ice since the Late Glacial through Holocene may help to untangle the glacial microbiome response to climate variations.

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### Prokaryotic composition at genera level

