Integrating metagenomics and physico-chemical methods to unveil bioweathering patterns on petroglyph sites in the Negev desert of Israel

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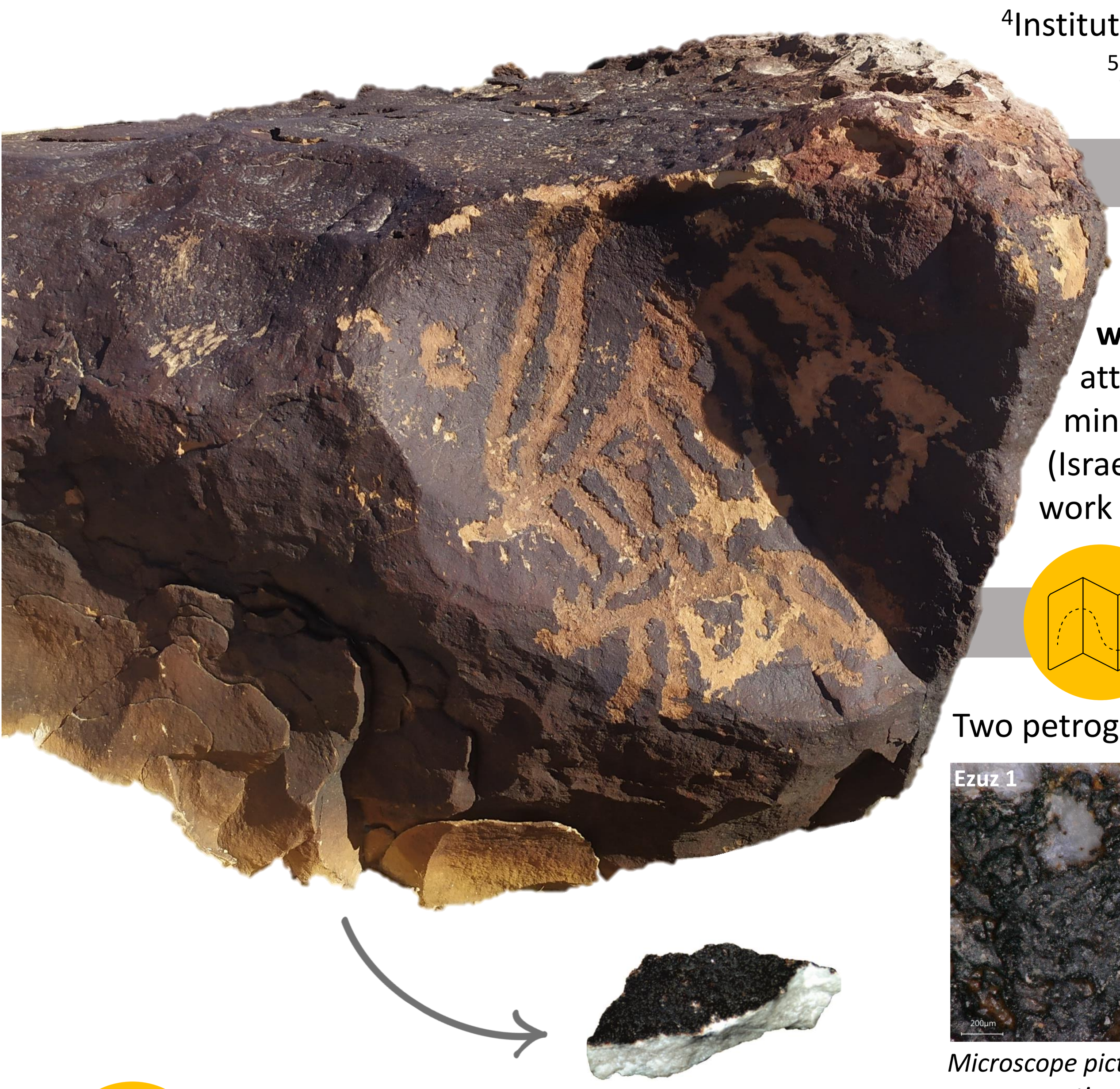
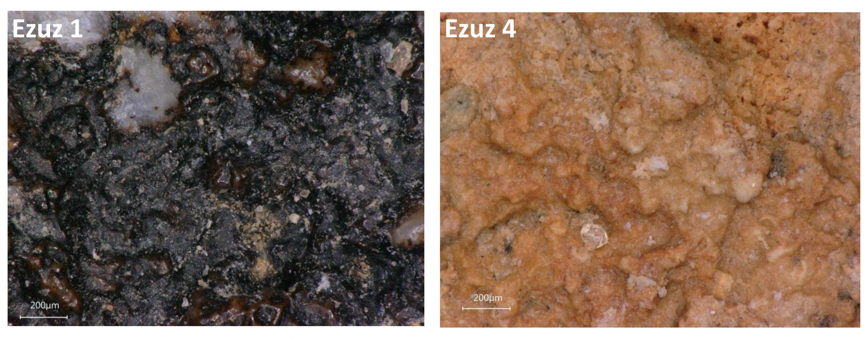


INTRODUCTION

Rock art, in the form of **petroglyphs** and pictograms, has an immense value, as it is considered one of the first forms of expression of ancient societies, and the prehistoric precursor to art. As part of the natural landscape, rock art is constantly exposed to natural and anthropogenic **weathering processes**. To protect and preserve this unique cultural heritage better knowledge of the degradation patterns is needed with special attention to **biodeterioration.** Indeed, the research focusing on the **role of biological agents** in the deterioration processes specific to rock art is still minimal. This contribution is part of a broader bilateral project between the Academy of Fine Arts Vienna (Austria) and the Ben Gurion University (Israel) that aims to study the **role of microorganisms in the deterioration processes of stone cultural heritage sites** in Austria and Israel. The present work presents the results obtained by culture independent microbiological methods (metagenomics) and physico-chemical analyses on the petroglyphs sites in the Negev desert of Israel.

PETROGLYPHS SITES AND SAMPLES

Two petroglyphs sites: AVDAT and EZUZ, central-west highlands of the Negev desert → 7 samples collected from similar rock types

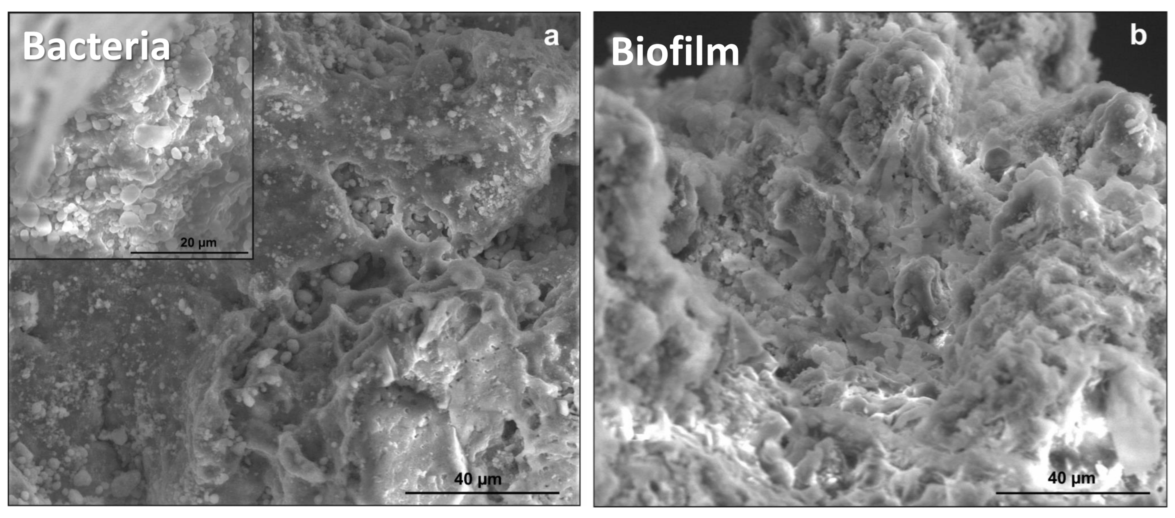


Microscope pictures of two samples showing the dark crust and the orange coating, 200x.

- **Silicified limestone** rock with dark coating (**desert varnish**) composed of clay minerals, iron (**Fe**) and manganese (**Mn**) oxides (Goldsmith,2014). A orange layer is present underneath the black one.
- Sample EZ4 presents only a orange thick crust.
- Different studies suggest a connection between the microbiome and the formation/deterioration of the desert varnish (Lingappa 2021, Nir 2021).

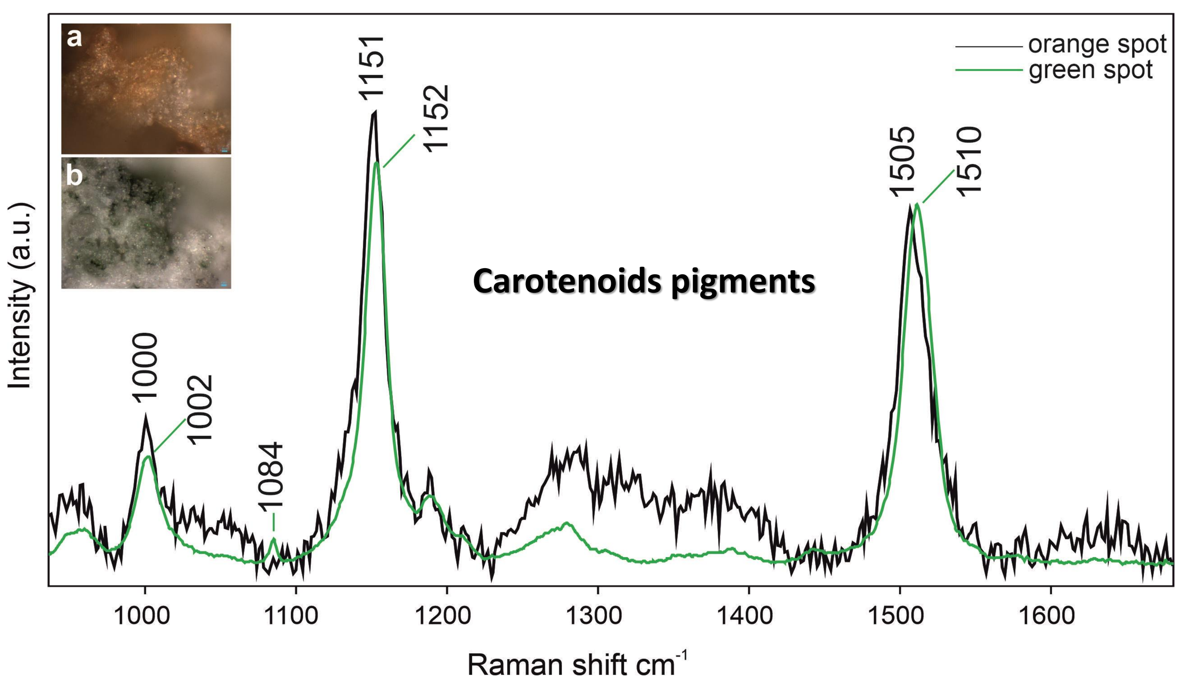
RESULTS

Biological colonization: SEM observation



SEM images in secondary electron mode showing putative bacteria in the microcavities of the stone (a) and a cyanobacterial biofilm (b) on the stone surface.

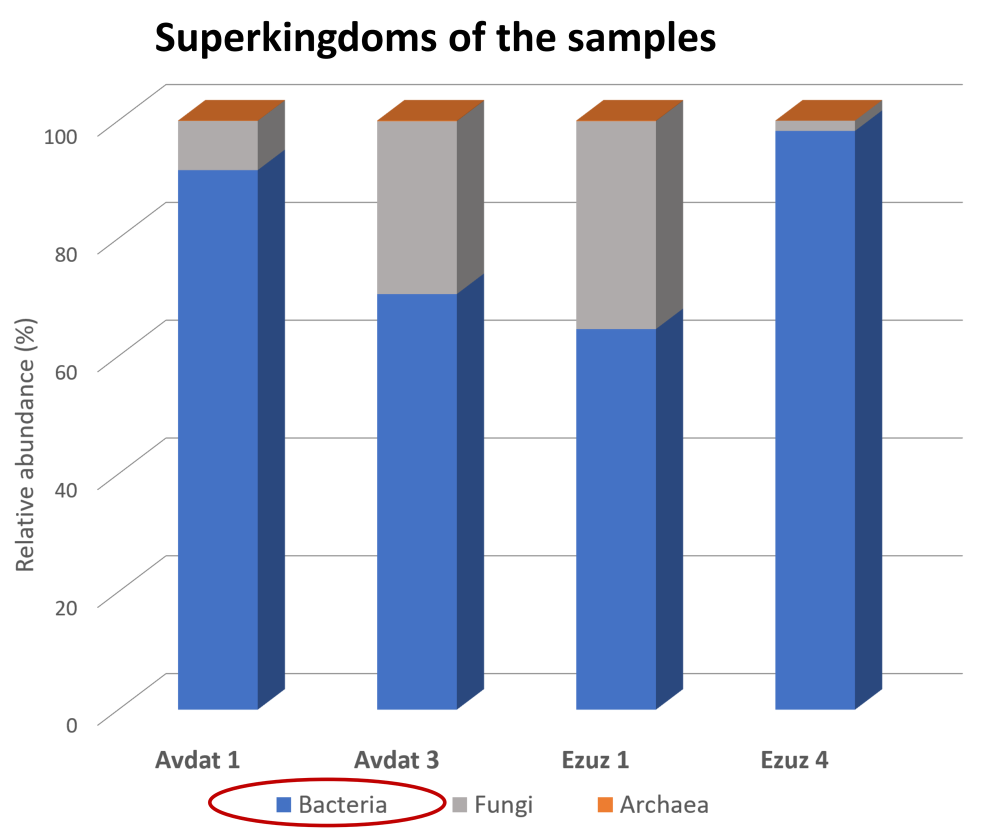
Biological colonization: Raman spectroscopy



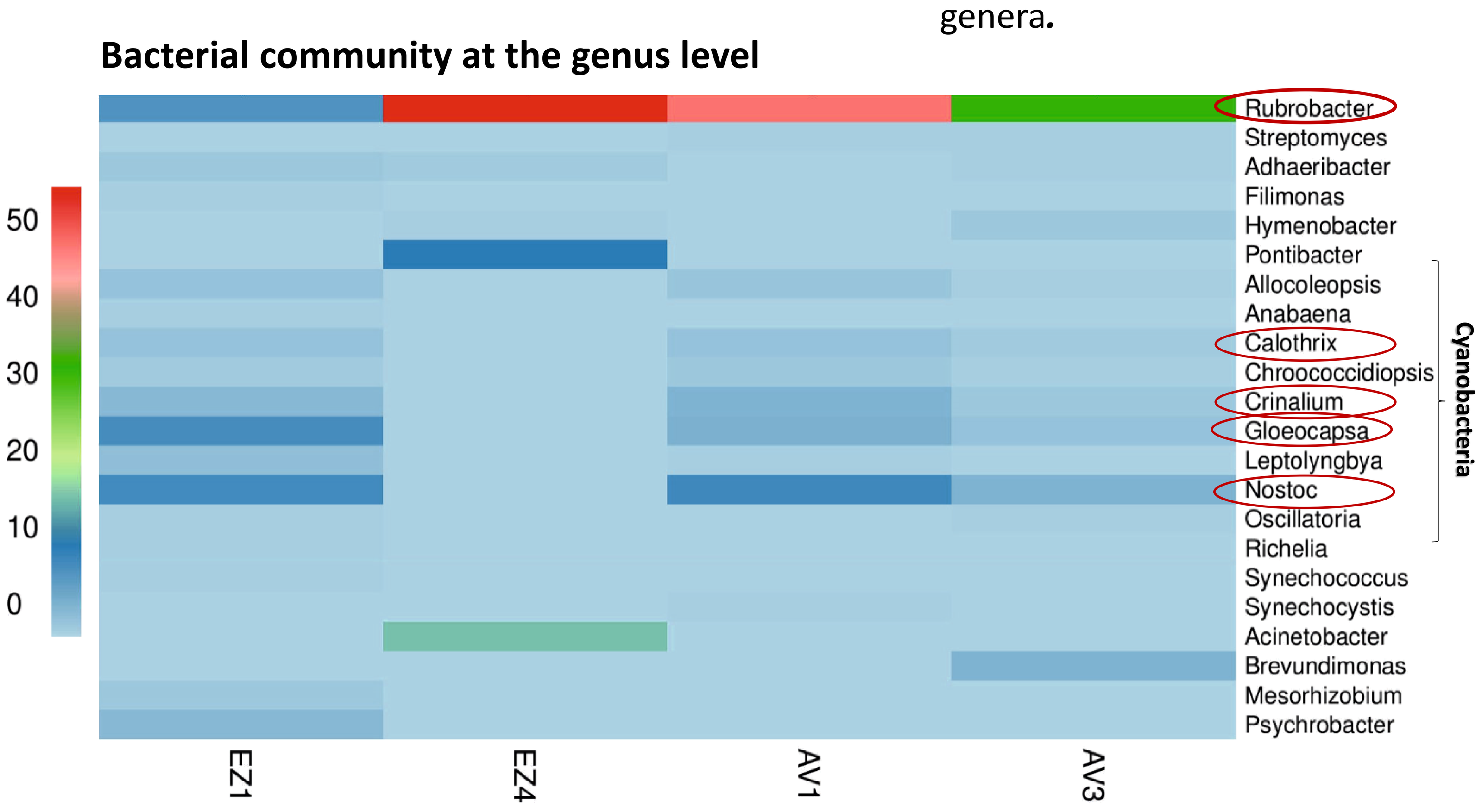
Normalized Raman spectra collected on the orange layer beneath the black crust (a) and on a spot colonized by cyanobacteria (b) in the range from 900 cm^{-1} to 1700 cm^{-1}. (λ = 532 nm).

The peaks around 1000 (δ C=CH), 1150 (ν C-C) and 1505 (ν C=C) are characteristic of carotenoids pigments; spectrum collected on the orange spot might be assigned to *Bacterioruberin* (→ *Rubrobacter*), spectrum collected on the green spot might be assigned to β-*carotene* (→ *Cyanobacteria*).

Microbial communities: metagenomics

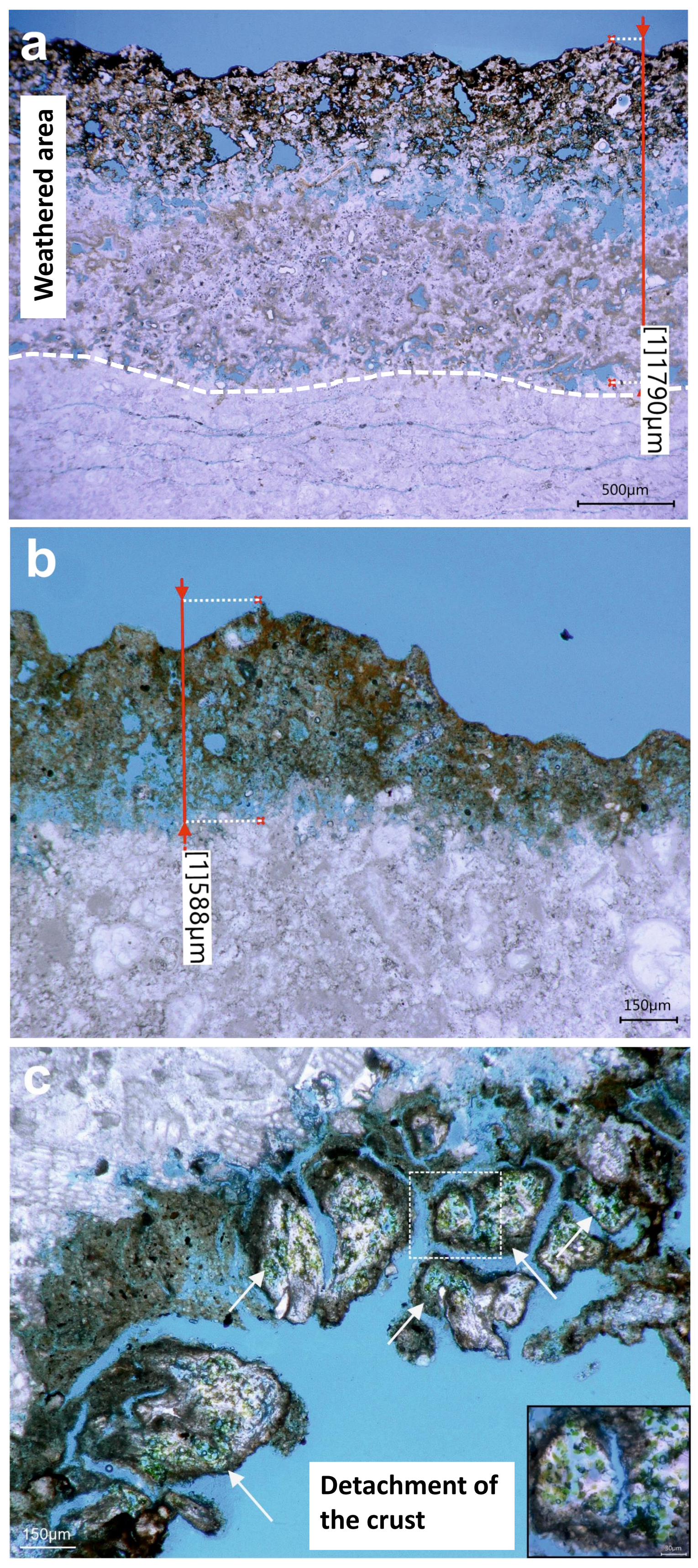


The metagenomic analysis were performed with the **Oxford Nanopore technology** (MinION platform) following a Whole Genome Amplification (WGA) protocol, which allows to obtain simultaneously the real proportions of different groups of microorganisms (fungi, bacteria and archaea) in a sample. The results showed that the microbiomes of the samples are dominated by **bacteria**, with very high proportions of the genus ***Rubrobacter*** and high biodiversity of ***Cyanobacteria*** genera**.**



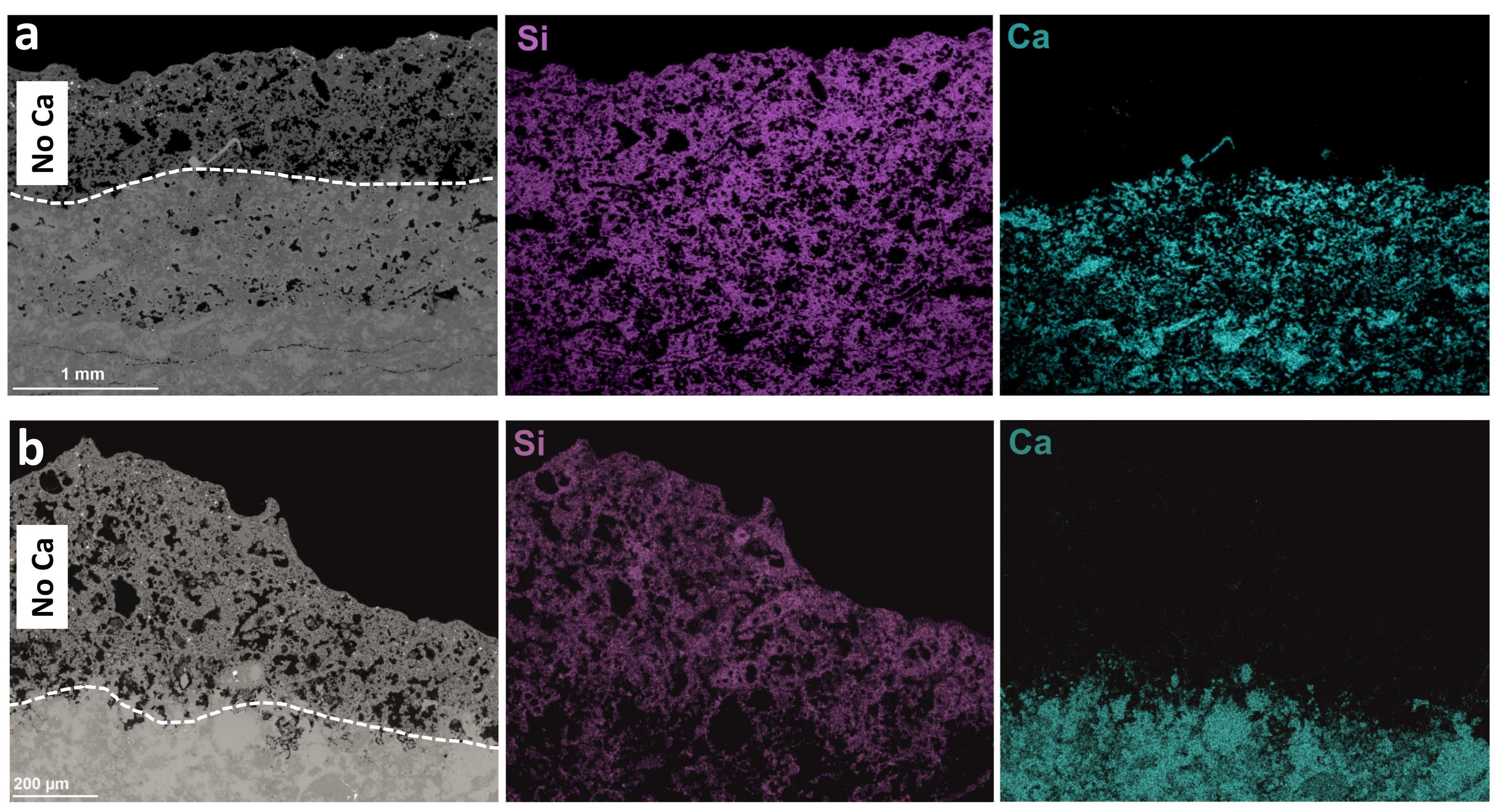
Heatmap displaying the relative abundance (%) of genera in each sample; the genera represent an abundance greater than 0.5% in at least one sample.

Weathered areas: microscopy



Microscope pictures of the thin sections: (a) a ***weathered area*** *beneath the black crust, (b) the orange crust of sample EZ4, (c) a* ***weathered area*** *colonized by* ***cyanobacteria****.*

Weathered areas: SEM-EDX mappings



SEM images in backscattered electron mode of a weathered area beneath the black crust (a) and of the orange crust of sample EZ4 (b), with EDX elemental mappings (Si and Ca).

CONCLUSIONS

- most of the microorganisms detected are taxa adapted to live in **harsh environmental condition**, with a high resistance to **radiation** and **desiccation**.
- we observe **patterns of deterioration** in the bedrock and in the black crust, feasibly connected to **microbial action**:
  - the weathering of the stone beneath the black crust was associated with the **potential of the species detected to leach the calcareous matrix of the rock** to find optimal conditions of growth (Krumbein 1981);
  - some species might take advantage of the **Mn and Fe reach crust** and uptake this cations to survive oxidative stress (Webb and DiRuggiero 2012), leading to a very slow deterioration of the crust, enhanced by mechanical detachment;
- in sample EZ4 the **orange layer** is probably a **weathered area** of the bedrock and it might represent a **later stage of weathering** in which the black crust is completely disappeared.

Acknowledgements

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References

- Y. Goldsmith *et al.*, Geochimica et Cosmochimica Acta 126, (2014)
- U.F. Lingappa *et al.*, PNAS, vol.118 No.25, (2021)
- I. Nir *et al.*, Environ. Microbiol., (2021)
- W.Krumbein and K. Jens, Oecologia, vol.50, (1981)
- K.M Webb and J. DiRuggiero, Archaea, (2012)

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