



Green stone, hidden life: Bacterial and fungal communities on the weathered serpentinite cladding of Florence Cathedral

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ABSTRACT

Serpentinite is a metamorphic rock widely employed in architecture for its aesthetic value. In Tuscany (Italy), it acquired significant symbolic and cultural relevance during the Gothic and Renaissance periods, becoming a distinctive element of regional architectural identity. Within the region, Florence stands out for its extensive use in the characteristic polychrome cladding of several major monuments, including the Cathedral of Santa Maria del Fiore (SMFC). While the physical and chemical decay processes of serpentinite have been extensively documented, biodeterioration in architectural contexts remains poorly understood. This study aims to characterise both the conservation state and the microbial communities inhabiting the external serpentinite cladding of the SMFC and to compare them with those colonising adjacent white marble surfaces. To this end, an integrated approach combining in situ observations and analyses and microbiological methods (both culture-dependent and independent) was adopted. Microscopic observations revealed surface features such as efflorescence, pitting, and microfracturing, indicative of advanced chemical alteration and mechanical weakening. In situ XRF analysis revealed a relatively high calcium content and appreciable amounts of iron, nickel, and chromium. Bacteria and black meristematic fungi (BMF) cultured from serpentinite showed viable counts of the same order of magnitude across the investigated areas. In addition, strains of BMF were isolated and identified as polyextremotolerant members of the rock-inhabiting genera. Metabarcoding analyses of the 16S rRNA gene, and ITS regions revealed the presence of extremotolerant bacteria, including *Deinococcus*, *Rubrobacter*, and *Truepera*, as well as BMF such as *Coniosporium*, *Knufia*, and *Vermiconidia*. These findings provide new insights into the poorly investigated biodeterioration of serpentinite in cultural heritage, showing that prolonged physicochemical weathering may increase stone bioreceptivity and facilitate the establishment of specialised microbial communities potentially involved in further mineral alteration. Historically weathered serpentinite used as architectural heritage stone can host specialised microbial communities, and we discuss how their functional traits may contribute to subsequent stages of biodeterioration under real urban outdoor conditions. The occurrence of shared rock-inhabiting genera across both lithotypes suggests the presence of a core microbiota adapted to the extreme conditions of exposed stone surfaces, with potential implications for biodeterioration processes. Overall, these findings shed initial light on the understudied status of serpentinite in the cultural heritage context.

1. Introduction

Since prehistoric times, lithic materials have played a fundamental role in human societies, first as tools and later as construction materials and media of artistic expression. Beyond their functional use, stones acquired symbolic and aesthetic values, contributing to the identity of communities. Their durability favoured their use in monuments

intended to endure for centuries; however, the choice of building stones was often governed by availability and accessibility, as long-distance transport was costly and technically challenging. Consequently, local lithotypes became distinctive features of regional architecture, reflecting the underlying geology and shaping unique cultural landscapes. Despite their perceived durability, all stones are ultimately susceptible to weathering and deterioration [e.g., (Siegesmund and Torok, 2014).

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Once extracted from their geological context and used as construction materials, stones become exposed to new environmental stressors, including temperature fluctuations, rainfall, air pollution, biological activity, and human interventions. Consequently, stone decay represents a major challenge in the conservation of cultural heritage. Within this framework, the choice of lithotypes reflects a balance between availability, functional properties, and aesthetic value. While stones such as granite and sandstone, despite their different mechanical properties, have been widely used for construction purposes, serpentinite has been mainly valued for its distinctive colour, aesthetic appeal, and workability, which favoured its use as an ornamental stone despite its susceptibility to alteration [e.g., [Pereira et al., 2010](#); [Santo et al., 2022](#); [Punturo et al., 2023](#)]. In Tuscany (Italy), serpentinite became a distinctive element of architectural identity during the Gothic and Renaissance periods ([Fratini et al., 2013](#)). In the city of Florence, it was extensively employed in the polychrome decoration of major monuments, including the Basilica of San Miniato al Monte, the Basilica of Santa Croce, the Basilica of Santa Maria Novella, the Baptistery of San Giovanni, and the Cathedral of Santa Maria del Fiore (SMFC). In the latter, serpentinite was combined with white Carrara marble and red marly and ammonitic limestones ([Malesani et al., 2003](#); [Pecchioni et al., 2019](#)), creating the characteristic three-colour façade that became a defining feature of the city's monumental heritage. However, its mineralogical and structural characteristics, together with prolonged exposure to the urban environment, make serpentinite particularly susceptible to physical, chemical, and biological weathering, ultimately leading to progressive stone decay. At present, the serpentinite elements of the cathedral display several deterioration features, including granular disintegration, surface exfoliation, lacunae, and features potentially associated with microbial colonisation. These phenomena threaten both the material integrity and the visual legibility of the monuments, posing serious challenges to conservation efforts. A comprehensive understanding of the degradation mechanisms, particularly in relation to the intrinsic mineralogical and textural features of serpentinite, together with a careful assessment of its current conservation state at the SMFC, is therefore essential for the development of effective preservation strategies. In this study, particular attention is given to the microbial groups inhabiting serpentinite. Although this lithology generally provides limited nutrients for microbial communities, biological colonisation can nonetheless play a relevant role in its alteration. Compared to physical and chemical weathering, biodeterioration of serpentinite remains underexplored, especially in an architectural context, with only a limited number of studies addressing its mechanisms and implications on natural rock samples [e.g., [Favero-Longo et al., 2013b](#); [Gulotta et al., 2018](#); [Sun and Lian, 2023](#)]. This limited body of research still constrains a full understanding of the role of biological agents in serpentinite decay and, consequently, the development of targeted conservation approaches. By investigating the microbial community inhabiting the serpentinite at the SMFC, this study aims to improve our understanding of the biological factors involved in the degradation of this lithotype and to provide new insights into the conservation of one of Florence's most iconic monuments.

2. Serpentinite overview

2.1. Formation, mineralogy and uses of serpentinite

Although often misrepresented in the commercial market as “Green Marble”, serpentinite has no mineralogical, geochemical, or physical correspondence to true marble (metamorphosed limestone or dolostone). This mislabeling can be misleading in both architectural and geological contexts.

Serpentinite is a dark green to black, fine-grained metamorphic rock primarily composed of serpentine-group minerals. It originates from the low-to intermediate-temperature (100–700°C) hydrothermal alteration of olivine-rich ultramafic protoliths, particularly peridotites. This

serpentinization process involves the replacement of anhydrous magnesium-rich minerals such as olivine, orthopyroxene, and clinopyroxene by hydrated silicates (mainly serpentine-group minerals) and subordinate phases like magnetite, brucite, talc, calcite, and magnesite ([Evans, 2004](#)). The transformation results in significant changes in rock properties: volume expansion, reduced density compared to the peridotitic protolith, high porosity, and changes in mechanical strength, which are influenced by the rock's anisotropy. The rock commonly shows vein networks of variable composition and a high tendency toward shear and mechanical failure ([Ismael and Hassan, 2008](#)). The serpentine-group minerals share a general chemical formula of approximately $\text{Mg}_3\text{Si}_2\text{O}_5(\text{OH})_4$, and occur as three polymorphs: lizardite, chrysotile, and antigorite, each with distinct crystal structures and stability fields ([Evans, 2004](#); [Capitani and Mellini, 2004](#)). Lizardite is typically stable at low temperature (<300°C) and pressure (<1 GPa), often forming flat, platy crystals in mesh textures replacing olivine ([Pereira et al., 2008](#)). Chrysotile, which becomes more stable than lizardite at high water/rock ratios ([Harrell, 2007](#)), is characterised by cylindrical fibres that may reach several centimeters in length. These fibres frequently fill secondary microfractures within the rock. Despite its historical industrial use for thermal, acoustic, and electrical insulation, chrysotile is a known health hazard due to its fine airborne particles ([Fubini and Fenoglio, 2007](#)). Antigorite is the high-temperature, high-pressure stable phase of serpentine. It possesses a structurally distinct, modulated crystal lattice with strong Si–O bonds, contributing to its greater hardness, higher seismic velocities, and reduced cleavage relative to lizardite and chrysotile ([Evans, 2004](#); [Capitani and Mellini, 2004](#)). It commonly occurs in recrystallised and metamorphosed serpentinites. The serpentinization process may obliterate the primary mineralogy, modify the rock's structure, and influence its mechanical behaviour.

Despite their fragile mechanical properties and moderate weathering resistance, which are sensitive to environmental conditions and degree of carbonation, serpentinites have been extensively used as building and ornamental stones since antiquity. This historical usage is largely attributed to their aesthetic qualities and workability rather than durability ([Harrell, 2007](#); [Aston et al., 2000](#)). In Egypt, serpentinite from the Eastern Desert has been widely used in architecture and sculpture since ancient times ([Aston et al., 2000](#)). Commercial varieties of serpentinite include Rajasthan Green and Emerald Green (India), which are often carbonated, and Vermont Verde Antique (USA) and Verde Pirineo (Spain), which retain more of their primary mineralogy ([Ismael and Hassan, 2008](#); [Pereira et al., 2008](#)).

In Italy, serpentinite outcrops are widespread and geologically significant, particularly along the Alpine and Apennine chains. In Tuscany, serpentinites, associated with the Northern Apennine Ophiolitic Complex, have historically supplied material for local monuments, including those in Florence, Impruneta, Prato, Pistoia, Siena, and Volterra ([Santo et al., 2020, 2025a](#)). These rocks formed during the Jurassic period through the serpentinization of ultrabasic ocean-floor rocks in a marine hydrothermal environment ([Principi et al., 2004](#)). Historically exploited serpentinites were later substituted by other green rocks such as Verde Alpi, which differ in both composition and mechanical properties ([Santo et al., 2022](#)), due to quarry closures or resource exhaustion.

2.2. Composition and degradation processes of serpentinite

Serpentinites are metamorphic rocks formed through the hydrothermal interaction between seawater and mantle-derived peridotites. Geochemically, they are characterised by high MgO contents (typically 33–42 wt%), reflecting the magnesium-rich nature of their ultramafic protoliths, and relatively low silica contents, with SiO_2 generally ranging between 34 and 45 wt%. Total iron contents are relatively high ($\text{FeO} + \text{Fe}_2\text{O}_3 \approx 8\text{--}14$ wt%), as iron is mobilized from primary silicates and precipitates mainly as magnetite during serpentinization. A distinctive feature of serpentinites is their high loss on ignition (LOI),

which can reach up to ~15 wt% due to the abundance of structurally bound water in serpentine minerals. Serpentinites are also enriched in compatible trace elements such as nickel (Ni) and chromium (Cr), whereas aluminum (Al) and calcium (Ca) are generally depleted due to their removal during alteration of olivine- and pyroxene-rich precursors.

Serpentine used in architectural and monumental contexts elements may undergo a range of weathering and decay processes [e.g., Meierding, 2005; Pereira et al., 2010; Siegesmund and Torok, 2014; Gulotta et al., 2017; Navarro et al., 2021; Santo et al., 2022]. Discolouration is frequently observed, producing darkening or heterogeneous surface patterns linked to chemical alteration, particulate deposition, and secondary mineral formation. Fracturing, linked to structural anisotropy, pre-existing microcracks, and environmental stresses, such as thermal fluctuations and moisture ingress, is frequently observed. Material loss typically occurs through granular disintegration and surface exfoliation, which represent among the most pervasive deterioration mechanisms in outdoor conditions. Exfoliation, often following natural planes of weakness associated with foliation, veining, or the lamellar structure of serpentine-group minerals (represents a critical conservation issue as it leads to progressive, often irreversible, loss of original material. Differential erosion produces irregular and rough surfaces, while lacunae are common in heavily weathered areas, affecting decorative integrity. Veins are common features in serpentinites and act as preferential pathways for fluid circulation, thereby enhancing weathering processes. Additionally, serpentinites are frequently affected by carbonation, driven by atmospheric CO₂ dissolved in meteoric water. This process promotes the transformation of Mg-rich silicates into secondary carbonates such as magnesite and, locally, calcite or dolomite, particularly along fractures, microfractures, and foliation planes, depending on the availability of Ca and Mg in the precursor rock (Pereira, 2012; Kelemen and Matter, 2008). Carbonation may significantly modify the physical-mechanical behaviour of the rock through mineral precipitation, recrystallisation, and progressive chemical alteration. In particular, carbonate-filled veins may undergo selective dissolution or recrystallisation, promoting local detachment and fissure widening [e.g., Schwarzenbach et al., 2021].

Serpentine surfaces may also be affected by biodeterioration processes. They are generally not considered highly bioreceptive lithotypes under fresh, unaltered conditions due to their geochemistry and mineralogy, limited porosity, and low water retention capacity. The presence of heavy metals such as Ni and Cr, together with limited availability of essential nutrients (Ca, K, P, N), further constrains microbial colonisation. Nevertheless, bioreceptivity increases significantly following weathering processes, which promote microfracturing, surface roughening, and enhanced moisture retention. Carbonation and secondary mineral precipitation may locally modify geochemical conditions, increasing the availability of reactive surfaces and promoting potential microbial attachment [e.g., Lima-Zaloumis et al., 2022]. In this context, water availability is a key controlling factor, often overriding bulk mineralogical constraints by enabling metabolic activity and stabilisation of biofilms.

Most research on serpentine-associated microbiota has focused on soils, where serpentine environments host distinct and metal-tolerant microbial communities compared to non-serpentine systems (Botha et al., 2024; Canonica et al., 2025). In contrast, fewer studies have addressed microbial colonisation of serpentine rocks, and even fewer have investigated the built environment. Soils therefore represent an important ecological baseline, although their direct transferability to cultural heritage materials remains limited.

Existing research on microbial colonisation and biodeterioration of serpentine exposed to atmospheric conditions has mainly focused on laboratory experiments on natural rock samples. For example, Sun and Lian (2023) demonstrated enhanced magnesium (Mg) release from serpentine under microbial activity in controlled conditions. Favero-Longo et al. (2005a) showed that lichens contribute to biogeochemical weathering of serpentine, promoting

mineral alteration and biomineral formation at the rock–organism interface. Additional studies have highlighted the role of fungi and lichens in the alteration of chrysotile, through acid and chelating metabolite production, leading to Mg and silicon (Si) mobilisation (Daghino et al., 2009; Favero-Longo et al., 2013b). These processes may alter fibre reactivity and toxicity, with implications for environmental risk and potential applications in bioremediation strategies. More recently, interest has emerged in the potential biotechnological exploitation of serpentine-associated microorganisms. Saraba et al. (2025) isolated bacteria from serpentine and limestone substrates and tested them in a functional screening on lignocellulosic and plastic substrates, although their identification was limited.

To the best of our knowledge, only one study has investigated the biodeterioration of serpentine in the context of cultural heritage. Gulotta et al. (2018) evaluated the subaerial biofilm (SAB) formation by confocal laser scanning microscopy, on different lithotypes of the façade of the Cathedral of Monza (Italy) exposed to similar environmental polluted conditions. While calcitic and dolomitic marbles showed a different colonisation extent of SAB communities dominated by phototrophs, the Oira stone, a very compact meta-peridotite deriving from the serpentinization of ultramafic rocks, showed no significant signs of colonisation. According to previous studies relating mineralogical features to biosusceptibility, the authors attribute to the presence of a high amount of carbonate compounds the more intense biocolonization of marbles compared to that of the silicatic Oira stone.

Overall, the colonisation and biodeterioration of serpentine in cultural heritage contexts remain poorly understood. This represents a significant research gap, particularly given the increasing recognition of biological processes as active agents in the long-term alteration of building stones.

3. Site description and sampling

The city of Florence is internationally celebrated for its exceptional artistic heritage and historical significance, particularly as the birthplace of the Renaissance. Its historic centre embodies a remarkable fusion of medieval urban fabric and Renaissance ideals, offering an outstanding representation of both European and Italian cultural evolution. This singular cultural and artistic legacy led to its designation as a UNESCO World Heritage Site in 1982. At the heart of the city lies the Cathedral of Santa Maria del Fiore, the most iconic and representative monument of Florence. Conceived by Arnolfo di Cambio in 1296 in the Gothic tradition and crowned in 1436 by Brunelleschi's revolutionary dome, the cathedral is an architectural masterpiece that bridges Gothic and early Renaissance design. Its exterior is characterised by elaborate polychrome stone panels (Fig. 1), with the present neo-Gothic façade, completed in the 19th century by Emilio De Fabris, contributing to its striking visual identity. Despite its grandeur, the SMFC has long faced significant conservation challenges. The stone cladding shows extensive weathering phenomena, including crust formation, chromatic alteration, surface erosion, mechanical fracturing, and granular disintegration, all visible to the naked eye [e.g., 18]. The preservation of this monument is therefore not only a local priority but also a matter of international cultural concern.

In our previous work, we focused on the SMFC microbial communities inhabiting the darkened external marble (Santo et al., 2021; Checucci et al., 2022) and the use of essential oils to control their growth (Santo et al., 2023, 2025b). In the present study, we focus on the microbial communities inhabiting the SMFC external serpentine and the adjacent marble slabs located in the study area of previous work, i.e., the external gallery encircling the apses (about 36 m above ground; Fig. 1).

Due to the advanced state of surface degradation and restrictions on sampling from the façade of the Cathedral of Santa Maria del Fiore, it was not possible to collect material suitable for the preparation of thin sections or for bulk chemical analyses. Sampling was limited to

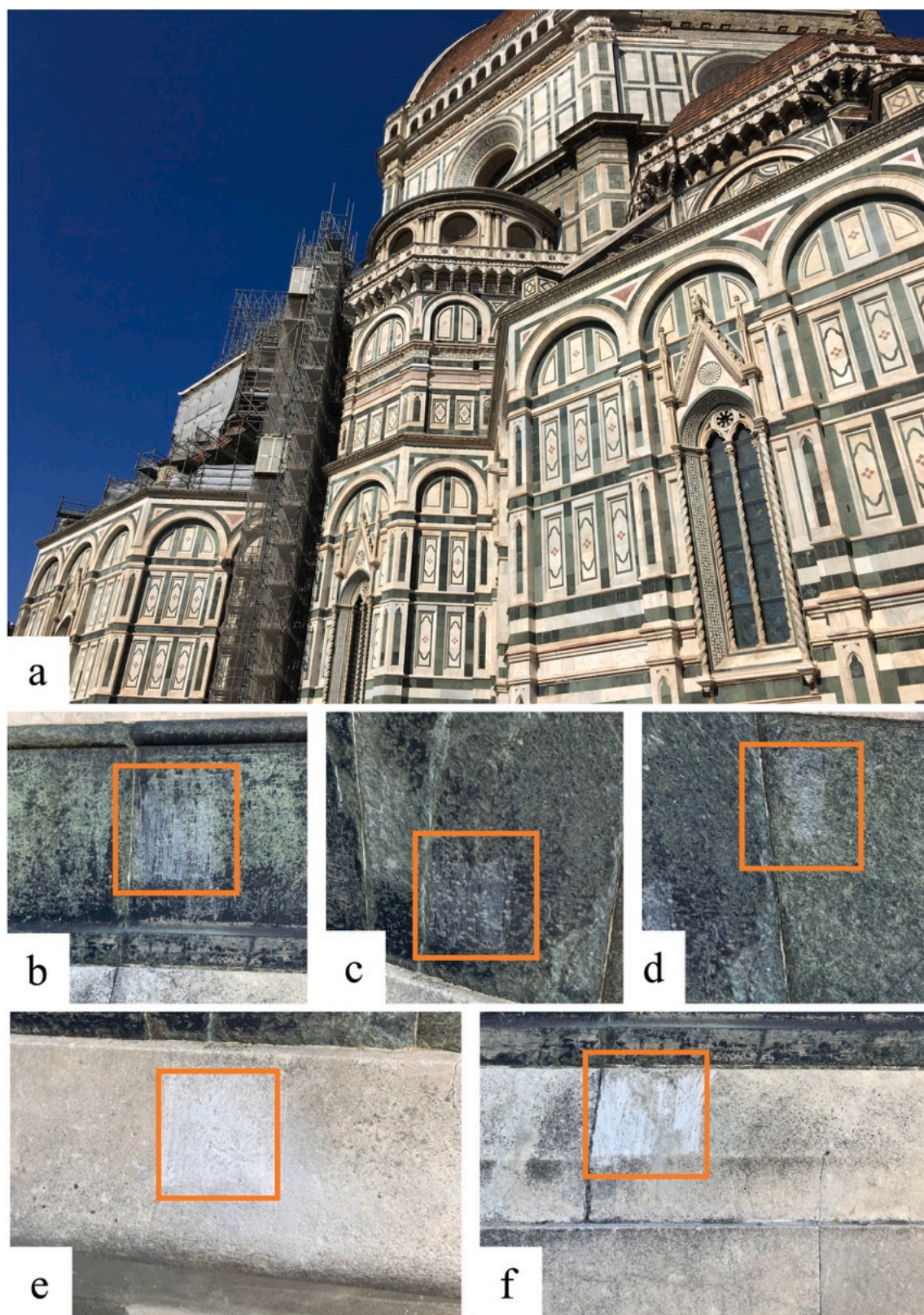


Fig. 1. Panoramic view of the Florence Cathedral showing the terrace along which the sampling areas are located (a). Serpentine sampling areas S1 (b), S3 (c), and S4 (d); white marble sampling areas M1 (e) and M2 (f). Orange squares indicate the sampling areas.

superficial scrapings, which were used exclusively for biological investigations. Consequently, the characterisation of the stone was carried out through non-destructive and minimally invasive approaches, including macroscopic in situ observation of decay features, Dino-Lite

digital microscopy for microtextural analysis, and portable X-ray fluorescence (pXRF) for semi-quantitative elemental characterisation of the altered surface.

The sampled areas of serpentine were named S1, S3, S4, S5, and S6;

the sampled areas of adjacent marble slabs were named M1 and M2 (Fig. 1). Superficial material up to 200 mg was gently scraped from the stone surface using a sterile spatula, collected into sterile tubes, and then brought to the laboratory and processed for the following analyses. For cultivation, the samples used were S3, S4, S5, and S6; for DNA extraction and metataxonomic analysis, samples S1, S3, S4 from serpentinite and M1, M2 from marble were used. The description of the collected samples and corresponding analyses are reported in Table 1.

4. Analytical methods

4.1. In situ observation of serpentinite

An initial analysis of the in situ morphology of serpentinite was carried out using the portable Dino-Lite Edge Digital Microscope AM7915MZT with a magnification range from 10x to 220x. The acquisition and processing of images was performed using DinoCapture 2.0 software.

4.2. Portable X-ray fluorescence

In situ X-ray fluorescence measurements on serpentinite were performed using a Bruker Elio portable XRF spectrometer (Rh anode, <50 kV, <0.2 mA, 1 mm collimation) equipped with a silicon drift detector (SDD, 50 mm² active area, <140 eV resolution at Mn K α , 1–40 keV range). Spot analyses were conducted at 50 kV and 80 μ A for at least 180 s, maintaining a constant working distance of approximately 7–10 mm. The results are expressed as elemental intensities (net peak area, background, and net counts).

4.3. Microbial cultivation, isolation and identification of black meristematic fungi

20 mg of sample collected from the serpentinite sites named S3, S4, S5 and S6 (Fig. 1) were suspended in 2 ml of phosphate-buffered saline (PBS; 8 g/L NaCl, 0.2 g/L KCl, 1.44 g/L Na₂HPO₄, 0.24 g/L KH₂PO₄, pH 7.4) added with 0.001% (v/v) Tween 80 and vortexed for 1h. 0.1 ml of S3 and S4 suspensions were plated on R2A agar (VWR Chemicals, Milan, Italy) in triplicate for cultivation of bacteria; 0.1 ml of S3, S5 and S6 suspensions were plated on Dichloran Bengal Rose Chloramphenicol agar (DRBC; VWR Chemicals, Milan, Italy) in triplicate for cultivation of black meristematic fungi; 0.1 ml of sterile PBS were plated on each kind of medium in duplicate as control. Plates were incubated at 20°C for 4 days in the case of bacteria, and up to four weeks in the case of BMF. At the end of incubation, colonies were counted for the determination of the viable titer. BMF were observed under the stereomicroscope Olympus SZX9 (Olympus, Tokyo, Japan) for morphological description. To classify them, characteristics such as shape, edges, section and colour were observed. Successively, colonies were isolated from S5 and S6 samples onto fresh DRBC medium. DNA was extracted from the isolated colonies employing the Wizard® Genomic DNA Purification kit (Promega, Madison, WI, USA) according to the yeast protocol. ITS region was

amplified with primers ITS1 and ITS4 (0.2 μ M) using VWR 2X Taq polymerase Master Mix as described in Berti et al. (2023). DNA concentration was measured with Qubit 4 Fluorometer (ThermoFisher Scientific, Waltham, MA, USA). Sanger sequencing was performed by Bio-Fab Research s.r.l. (Rome, Italy). Sequences were analysed with Basic Local Alignment Search Tool (BLAST) using the National Center for Biotechnology Information (NCBI) database (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) and deposited under the accession numbers PZ054914, PZ054915, PZ569910-PZ569913.

4.4. Environmental DNA extraction, library preparation and targeted metagenomic sequencing

Environmental DNA was extracted from approximately 100 mg of serpentinite (samples S1, S3, S4) and marble (samples M1, M2) powder using the DNeasy Powersoil Kit (Qiagen, Hilden, Germany), following the manufacturer's instructions. Extractions were performed in triplicate for each sample. DNA quality was assessed by 1.5% (w/v) agarose gel electrophoresis, and DNA concentration was quantified using a Qubit™ 4 Fluorometer with the dsDNA High Sensitivity Assay Kit (Thermo Fisher Scientific). Amplicon libraries were prepared according to Illumina protocols (details described in Renzi et al. (2025)) targeting the bacterial 16S rRNA gene (V3-V4 region) and the fungal Internal Transcribed Spacer 2 (ITS2) region. Sequencing was performed on the Illumina MiSeq platform at the Department of Biology, University of Florence (Italy), using the MiSeq Reagent Kit v3 (600 cycles) with 300 bp paired-end reads.

4.5. Amplicon sequence processing and taxonomic assignment

Raw sequencing data from bacterial 16S rRNA gene and fungal ITS2 region datasets were pre-processed, quality-filtered, trimmed, denoised, merged, modelled, and analysed using QIIME2 (Bolyen et al., 2019) and DADA2 (Callahan et al., 2016). Chimeras were checked and removed using the 'consensus method' with DADA2 (Callahan et al., 2016). Amplicon sequence variants (ASV) were clustered into Operational Taxonomic Units (OTUs) using VSEARCH within QIIME2, with a 97% similarity threshold (Rognes et al., 2016). Finally, taxonomy was assigned using the UNITE + INSD database (v10.0) for fungal OTUs, and the SILVA database (v138.2) for bacteria (Quast et al., 2012). All statistical analyses were performed in the R environment. Alpha diversity was evaluated using richness, Shannon and Simpson indices with the vegan package (Oksanen et al., 2025). Normality was evaluated using the Shapiro–Wilk test, followed by one-way ANOVA and Tukey's post hoc test ($p < 0.05$). Homogeneity in community variance was tested using PERMDISP (Anderson, 2005). Principal Coordinates Analysis (PCoA), based on Bray–Curtis distances, was performed to highlight the differences in the microbial communities between the different sites. Statistical significance of community differences was assessed using permutational multivariate analysis of variance (PERMANOVA) (Anderson, 2001). Data visualisation was performed using the ggplot2 package (Wickham, 2016).

5. Results

5.1. Composition and degradation state of the SMFC serpentinite

Due to the lack of available samples for petrographic and mineralogical analyses, the interpretation of the mineralogical features discussed in this section is based on the integration of macroscopic observations, microscopic imaging, and in situ elemental data. Therefore, mineralogical attributions should be considered indicative rather than conclusive, while the collected information remains valuable for understanding the alteration processes affecting the serpentinite surface.

At close-up view (Fig. 2), the rock displays a fine-to microcrystalline

Table 1

List of the collected and studied samples.

Sample	Stone	Analysis	
		Cultivation	16S/ITS Metabarcoding
S1	serpentinite	NP	P
S3	serpentinite	P	P
S4	serpentinite	P	P
S5	serpentinite	P	NP
S6	serpentinite	P	NP
M1	marble	NP	P
M2	marble	NP	P

P = analysis performed; NP = analysis not performed.

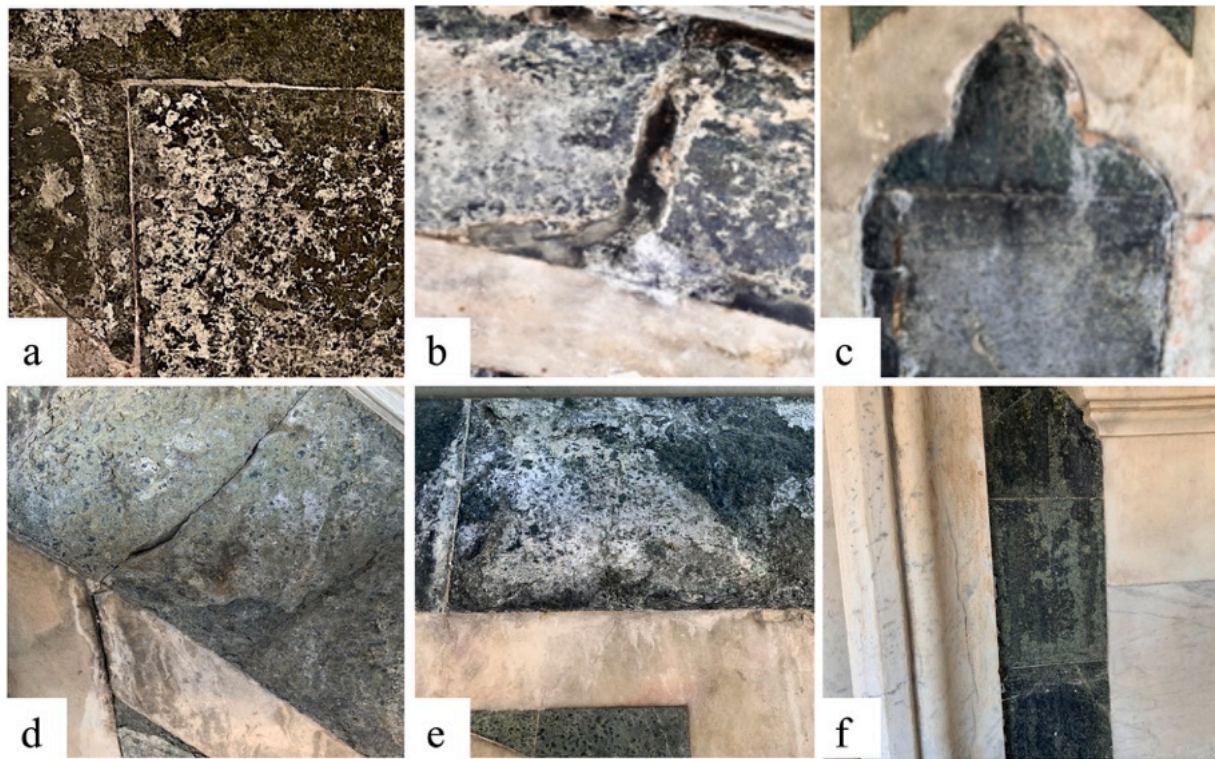


Fig. 2. Examples of degradation in SMF serpentinite cladding: (a) surface flaking; (b) granular disintegration; (c) edge erosion and joint widening; (d) fracturing along natural foliation; (e) surface whitening and efflorescence; (f) colour fading and differential weathering.

matrix with scattered dark inclusions, likely corresponding to Fe-oxides (e.g., magnetite) and possibly relict chromite, together with pseudomorph textures preserving the morphology of primary olivine and pyroxene. These pseudomorph textures reflect the extensive

replacement of the primary silicate assemblage by serpentine-group minerals during serpentinization.

The surface shows pronounced roughness and porosity, with numerous pits and evidence of granular disaggregation, which are

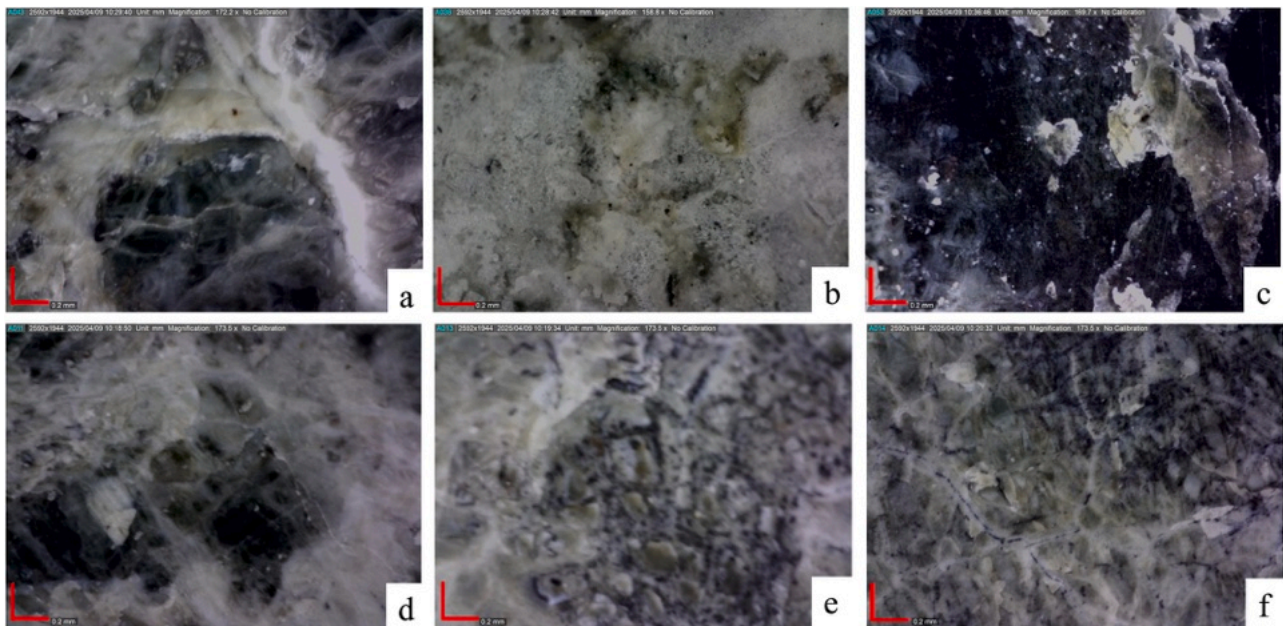


Fig. 3. Dino-Lite micrographs of the serpentinite surface showing different alteration features. (a, d) Dark compact areas bordered by light fibrous zones and bright deposits along fractures, consistent with secondary mineral precipitation and possible biofilm development; (b, e) Lighter surfaces with yellowish patches and granular textures, indicative of heterogeneous chemical alteration and possible microbial colonisation; black spots could be due to black meristematic fungi; (c) Dark serpentinite matrix with lighter lamellar areas, interpreted as secondary carbonate deposits. Bright zones along fractures and edges indicate localised chemical alteration and potential early-stage mineral precipitation, possibly associated with biofilm activity; (f) Irregular surface with light deposits within microcracks, pointing to surface alteration processes and possible biodeterioration under prolonged environmental exposure. The red bar corresponds to 0.2 mm.

typical features of advanced chemical weathering. A distinct colour zonation is observable: lighter zones, ranging from white to pale grey, correspond to areas of intense alteration and leaching, possibly enriched in carbonates or clay minerals, whereas darker green to black patches likely preserve portions of the primary serpentine mineral assemblage. The occurrence of whitish efflorescence suggests the evaporation of pore waters followed by precipitation of salts, probably magnesium or calcium carbonates, or silica-rich residues. Multiple alteration processes appear to be involved. Carbonation reactions have likely promoted the formation of magnesium carbonates and silica, while weathering-induced transformation into clay minerals may have further weakened the rock fabric. Salt crystallisation, potentially enhanced by atmospheric pollution, may also contribute to surface disruption. In some areas, oxidation of accessory minerals (e.g., magnetite transforming to hematite or goethite) may produce reddish or brownish tones, although these are not yet widespread. Structural discontinuities, such as veins and fractures, often bordered or filled by alteration products, likely act as preferential pathways for moisture and pollutants, thereby accelerating deterioration. Overall, these observations point to a serpentine surface undergoing advanced chemical alteration and mechanical weakening, in which efflorescence, surface pitting, and micro-fracturing represent the dominant decay features, consistent with prolonged environmental exposure.

Images acquired with a Dino-Lite digital microscope directly on the serpentine surface (Fig. 3) reveal a heterogeneous texture characterised by dark compact areas alternating with lighter fibrous or microgranular zones. Fractures and discontinuities are often filled with secondary materials, and colour variations range from dark green to whitish-yellow tones. These features, together with the occurrence of irregular patinas and superficial deposits, indicate advanced physical and chemical alteration processes, as well as possible signs of surface biodeterioration, likely related to microbial biofilm development or secondary salt precipitation.

The elemental composition obtained by portable XRF (Table 2) provides valuable information on the detectable elemental constituents of the serpentine surface. However, the results must be interpreted considering the analytical limitations of in situ measurements performed in air, particularly the limited detection of light elements such as Mg. Therefore, Mg is absent from the dataset despite being a major constituent of serpentine. Within this limitation, the relatively high Ca content suggests the presence of secondary Ca-bearing phases, likely related to carbonation processes rather than primary mineralogy. Iron, nickel, and chromium are present in appreciable amounts; however, their possible role in microbial colonisation and biodeterioration processes should be interpreted with caution, as pXRF does not provide information on their speciation or bioavailability.

5.2. Cultivation of bacteria and meristematic fungi

To verify the presence of microorganisms hosted by the SMFC serpentinites and quantify their abundance, bacteria and black meristematic fungi were cultivated from serpentine powders. The bacterial

titer was determined for samples S3 and S4, while the BMF titer was determined for samples S3, S5, and S6; the results are reported in Table 3. Sample S3 showed a viable bacterial titer very similar to that of S4 (about 5×10^5 CFU/g) and one order of magnitude lower than the bacterial titer previously found for the external SMFC marble with similar exposure (4.4×10^6 CFU/g in the northwest area of the terrace in Santo et al. (2023)), even if after different incubation conditions. For BMF, the viable titer ranged from 10^5 CFU/g (the same order of magnitude as that of bacteria) to 10^6 CFU/g (sample S5), the latter being more similar to that previously determined for BMF on the SMFC marble with similar exposure (1.2×10^6 CFU/g in the northwest area of the terrace), in this case after a longer incubation (Berti et al., 2023).

BMF are considered Rock Inhabiting Fungi (RIF), a term highlighting their integral role in the ecosystems on rock surfaces (Celi et al., 2025). BMF are among the most stress-resistant organisms on Earth and show a remarkable ability to withstand the harsh environmental conditions present on rocks, such as solar radiation, desiccation, and extreme temperature fluctuations (Sterflinger and Krumbein, 1995; Tessei, 2022). The presence of a melanized cell wall represents a pivotal feature providing protection against ionising radiation, extreme temperatures and metal toxicity (Casadevall et al., 2017). They also represent one of the most damaging groups of microorganisms, causing the deterioration of outdoor-exposed stone monuments, mainly due to the darkening of their surface (Marvasi et al., 2012), cracking, and bio-pitting (Sterflinger and Krumbein, 1997), also due to acid production (Berti et al., 2023). For these reasons, we were interested in isolating BMF from a harsh environment such as serpentine rock.

BMF colonies were observed and classified into apparently different morphotypes. Three strains coming from the sample S5 (m1.3, m1.5 and m2.2) and three from the S6 sample (m3.1, m3.2 and m4.1) were identified through ITS rDNA amplification and sequencing. Taxonomic assignment indicates that the isolates all belong to the *Coniosporium* and *Exophiala* genera regardless of the sampling area, except for one belonging to *Knufia* (Table 4).

5.3. Analysis of the microbial communities

To analyse the bacterial and fungal communities, DNA was extracted and sequenced in triplicate from the three serpentine areas named S1, S3, and S4, as well as from the adjacent white marble areas M1 and M2. Samples were grouped as serpentine (S1-1, S1-2, S1-3; S3-1, S3-2, S3-3;

Table 3

Viable titer of bacteria and black meristematic fungi (BMF) from serpentine samples.

Sample	Microorganisms	Viable titer (CFU/g) $\pm$ SD
S3	Bacteria	$4.8 \times 10^5 \pm 7.8 \times 10^4$
	BMF	$1.1 \times 10^5 \pm 7.0 \times 10^4$
S4	Bacteria	$5.1 \times 10^5 \pm 3.2 \times 10^4$
S5	BMF	$1.0 \times 10^6 \pm 7.2 \times 10^4$
S6	BMF	$3.7 \times 10^5 \pm 1.0 \times 10^4$

Table 2

Intensities obtained by pXRF on different areas of serpentine SMFC façade. The intensities are reported for the area, background, and peak of each element.

Element	Point 1			Point 2			Point 3			Point 4			Point 5		
	Nac	Bc	NPc	Nac	Bc	NPc	Nac	Bc	NPc	Nac	Bc	NPc	Nac	Bc	NPc
Si - K α	4886	872	605	3503	773	455	3431	773	456	3942	724	544	1889	364	223
S - K α	2712	841	299	2320	877	268	3802	918	428	2552	916	270	715	259	104
Ca - K α	19856	1494	1957	36765	1489	3709	30142	1468	2973	18277	1338	1898	8815	578	886
Ti - K α	3008	3292	222	1811	2005	165	1869	2469	154	2396	2647	188	1009	1118	77
Cr - K α	1714	3753	172	20378	3254	1526	3364	2503	305	511	3511	35	723	1029	75
Fe - K α	342743	3976	27287	125408	3594	10038	134200	3571	10403	330148	3934	25973	117876	1663	9262
Ni - K α	16550	3929	1238	6571	3467	494	8616	3443	676	15328	3313	1202	5669	1400	436
Nac= Net Area counts; Bc= Background counts; NPc= Net Peak counts.															

Table 4

Identification of black meristematic fungi isolated from LG and DG areas of SMFC serpentinite, based on ITS region analyses.

Strain name	Provenance	Organisms with the most similar rDNA Sequences (ITS1) (GeneBank accession number)	Identity (%)	Nucleotides Sequenced
m1.3	S5	<i>Knufia petricola</i> / <i>Knufia marmoricola</i> (NR_111871.1/NR_145016.1)	99.29	420
m1.5	S5	<i>Coniosporium uncinatum</i> (NR_145343.1)	94.56	329
m2.2	S5	<i>Exophiala xenobiotica</i> (NR_111203.1)	99.30	427
m3.1	S6	<i>Exophiala xenobiotica</i> (NR_111203.1)	98.22	449
m3.2	S6	<i>Coniosporium uncinatum</i> (NR_145343.1)	98.10	421
m4.1	S6	<i>Coniosporium uncinatum</i> (NR_145343.1)	98.16	379

S4-1, S4-2, S4-3) and marble (M1-1, M1-2, M1-3; M2-1, M2-2, M2-3) for the analysis of the microbial community using R.

Concerning bacterial communities, richness was highest in S4 and differed significantly from M1, S1, and S3 (all grouped as b; Fig. 4). M2 (ab) showed intermediate richness and did not differ significantly from the other groups. Shannon diversity displayed a similar result, with S4 (a) significantly higher than all other sites (all b). Simpson index values were largely homogeneous across sites, with M1, M2, S1, and S4

belonging to the same statistical group (a), while only S3 (b) showed significantly lower evenness, indicating dominance. Overall, alpha-diversity results did not reveal a clear substrate-driven differentiation between marble and serpentinite communities, suggesting comparable community structures.

In contrast, fungal taxa richness showed a clear site-driven differentiation (Fig. 5). S4 area exhibited significantly higher richness (a) than all other sites (all b), which did not differ significantly from each other, indicating that both marble (M1, M2) and the remaining serpentinite sites (S1, S3) supported similarly lower numbers of taxa. Shannon diversity revealed a more complex pattern: M2 displayed the highest value (a), significantly exceeding M1 and S4 (both b), whereas S3 (c) showed the lowest diversity; S1 and S4 (bc) occupied intermediate positions and did not differ significantly from the other groups. A comparable trend was observed for the Simpson index, with M2 (a) showing significantly greater evenness than M1 (b), while S3 (c) again exhibited the lowest values, and S1 and S4 (bc) remained intermediate. Overall, although richness was strongly influenced by the elevated values at S4, diversity and evenness indices suggest that marble, particularly at site M2, supports more evenly distributed fungal communities, whereas serpentinite sites, especially S3, tend to host less diverse communities characterised by higher dominance, highlighting pronounced site-specific variability.

For both fungal and bacterial communities, PERMANOVA indicated a significant global difference among sites and among the two substrates (Fig. 6). However, the pairwise post-hoc comparisons did not identify significant differences between any specific site pairs. This pattern suggests that while there is a global difference among groups, as also supported by the high F and R² values, the statistical power is limited,

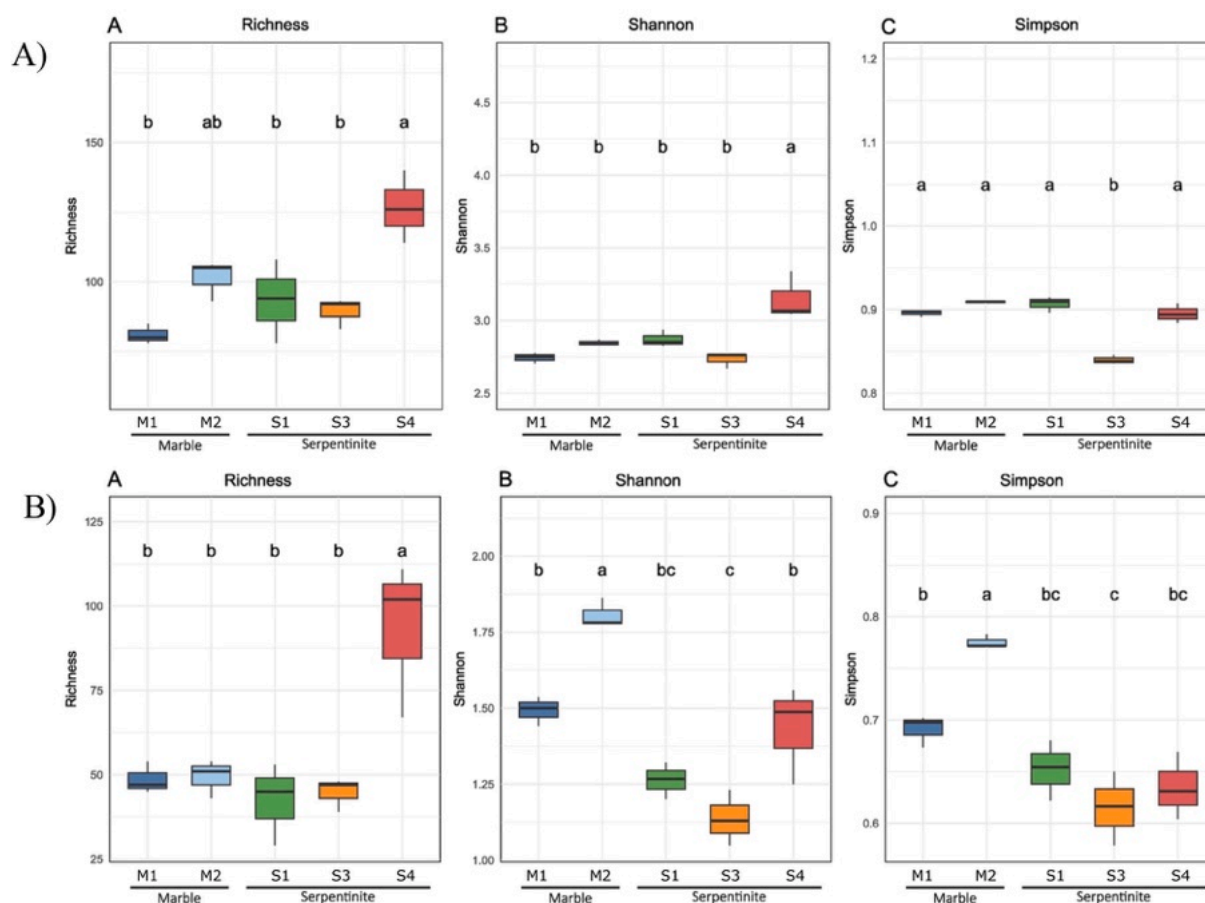


Fig. 4. A) Bacterial alpha diversity indices calculated for the different sites. A: Richness; B: Shannon index; C: Simpson index. To test the statistical significance ANOVA test was performed with a Tukey post-hoc test ($p < 0.05$). The different letters indicate the statistical class. B) Fungal alpha diversity indices calculated for the different sites. A: Richness; B: Shannon index; C: Simpson index. To test the statistical significance ANOVA test was performed with a Tukey post-hoc test ($p < 0.05$). The different lowercase letters indicate the statistical class.

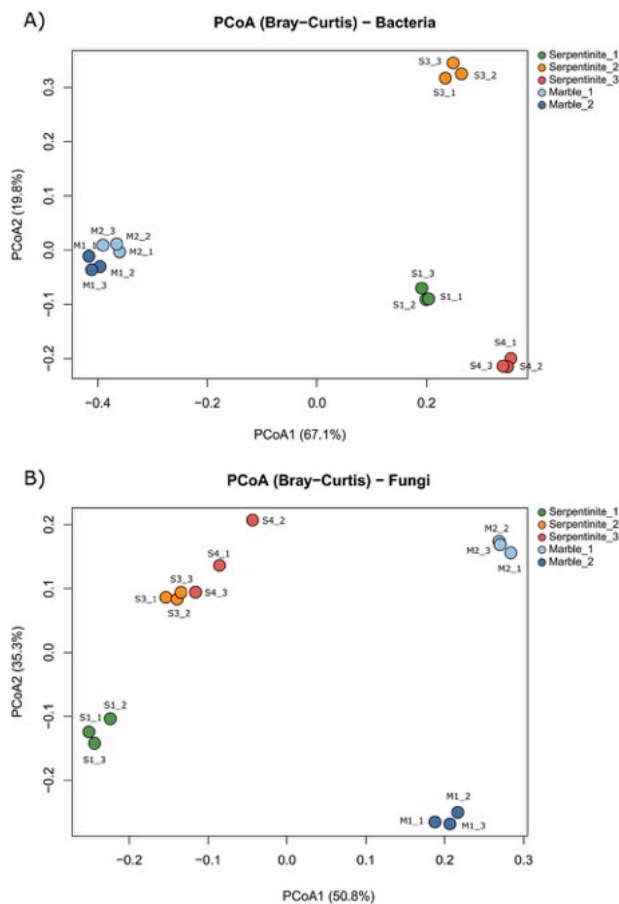


Fig. 5. Principal Coordinates Analysis (PCoA) based on Bray–Curtis distances of bacteria (A) and fungi (B), showing the distribution of samples according to microbial community composition. Each point in the graphic represents one sample, while different colours indicate different sampling sites.

likely due to the small number of replicates per site. This is further supported by the PCoA based on Bray–Curtis dissimilarity, showing that samples from marble and serpentinite sites cluster into two distinct macro-groups for both communities. Overall, community composition differs among sites, but these differences are diffuse and not driven by any single site-to-site contrast.

The taxonomic composition and relative abundance of bacterial communities differed between marble and serpentinite samples (Fig. 6A). The common dominant genera across both lithotypes were *Truepera* and *Modestobacter*. Marble was dominated by *Sphingomonas* and, to a lesser extent, by *Quadrisphaera*, *Acidiphilium* and *Hymenobacter* (Fig. 6A). In addition, their relative abundances appear to be homogeneous across the marble replicates. Concerning serpentinite, it was dominated by *Rubrobacter* across all samples. Other dominant genera are *Actinomycetospora* and *Sphingomonas*. *Deinococcus* was found among the dominant genera of serpentinite sites S1 and S4, but not S3.

The taxonomic composition of fungal communities showed similarities between marble and serpentinite samples (Fig. 6B). The common dominant genera across both lithotypes were *Vermiconidia* (formerly *Vermiconia*) and *Knufia*. Concerning marble, it was dominated by the lichen *Flavoplaca*, with different relative abundances between the two sites, M1 and M2, while it was absent among the dominant genera of the serpentinite environment. Serpentinite was also dominated by the black meristematic fungus *Coniosporium*, which was present to a lesser extent in the marble environment.

6. Discussion and concluding remarks

According to a range of historical and recent sources (Coli et al., 2023) and references therein], the serpentinite used in Tuscan monuments, locally referred to as *Verde di Prato*, *Nero di Prato*, *Marmo Nero*, and *Paragone*, was quarried in the Monteferrato (Prato) and Impruneta (Florence) areas. In particular, documents preserved in the Archivio dell'Opera di Santa Maria del Fiore and in the Archivio di Stato of Florence and Prato indicate that most of the serpentinite used in the monuments of the Santa Maria del Fiore complex (Baptistery, Cathedral, and Giotto's Bell Tower) was sourced from the Monteferrato quarry district. Cava Bagnolo, one of the historical quarries of this district, exploited since the Middle Ages, was investigated by Santo et al. (2018, 2025c), who compared quarry serpentinite with samples collected from the pavement of the Florence Baptistery.

The studied quarry material shows the typical mineralogical and geochemical features of ophiolitic serpentinite and displays strong similarities with the stone employed in the monument. Furthermore, the physical properties of the quarry stone, including capillary water absorption, open porosity, apparent density, and imbibition coefficient (Santo et al., 2025c), are consistent with those measured for the Baptistery serpentinite and fall within the range reported in the literature for serpentinites from different geographical areas [e.g., Santo et al., 2025a; Pereira et al., 2007; Diamantis et al., 2009]. Given these similarities, the Cava Bagnolo material provides a useful mineralogical and geochemical reference framework for interpreting the serpentinite used in the cathedral. The mineralogical characterisation of the Cava Bagnolo reference material by X-ray diffraction and electron-microprobe analyses identified chrysotile and lizardite as the main serpentine-group minerals, together with magnetite (Santo et al., 2025c). Microprobe analyses further showed that the analysed serpentine-group mineral domains contain appreciable amounts of Fe, Cr, and Ni, providing a relevant mineralogical and geochemical framework for interpreting the stone used in the SMFC complex. Because direct sampling of the Cathedral cladding was not possible, its composition was assessed in situ by portable X-ray fluorescence (pXRF). These measurements characterise the exposed surface rather than the bulk composition of the fresh stone and should therefore not be quantitatively equated with the high-precision analyses of the quarry reference material.

The SMFC serpentinite nevertheless displays clear evidence of substantial surface alteration, including carbonation, pitting, microfracturing, and the development of secondary surface features (Fig. 2), indicating a marked departure from the properties of the fresh quarry material. This altered condition is particularly relevant when considering the microbial colonisation of the stone. Although fresh serpentinite may represent a chemically selective substrate for microbial growth because of its mineralogical composition, limited availability of some essential nutrients, and enrichment in potentially toxic elements, weathering can modify the physicochemical characteristics of the exposed surface and increase its bioreceptivity. The occurrence of a diverse community of extremotolerant bacteria and black meristematic fungi on the SMFC serpentinite may therefore reflect the adaptation of these microorganisms to an altered mineral–surface environment, while their metabolic activities may in turn contribute to ongoing mineral–biofilm interactions.

Gulotta et al. (2018) showed that extensive biological growth preferentially affected already altered lithic substrates, whereas silicatic surfaces in a better state of conservation remained substantially uncolonized. Accordingly, the present results support the hypothesis that pre-existing weathering processes play a primary role in promoting microbial colonisation. In the SMFC serpentinite, the relatively high Ca concentration detected by pXRF may reflect the occurrence of secondary Ca-bearing phases at the exposed surface, potentially associated with carbonation processes, fluid-mediated alteration, and deposits rather than to primary mineralogy of the rock. Nevertheless, the occurrence of Ca-bearing secondary minerals may be relevant to bioreceptivity, as

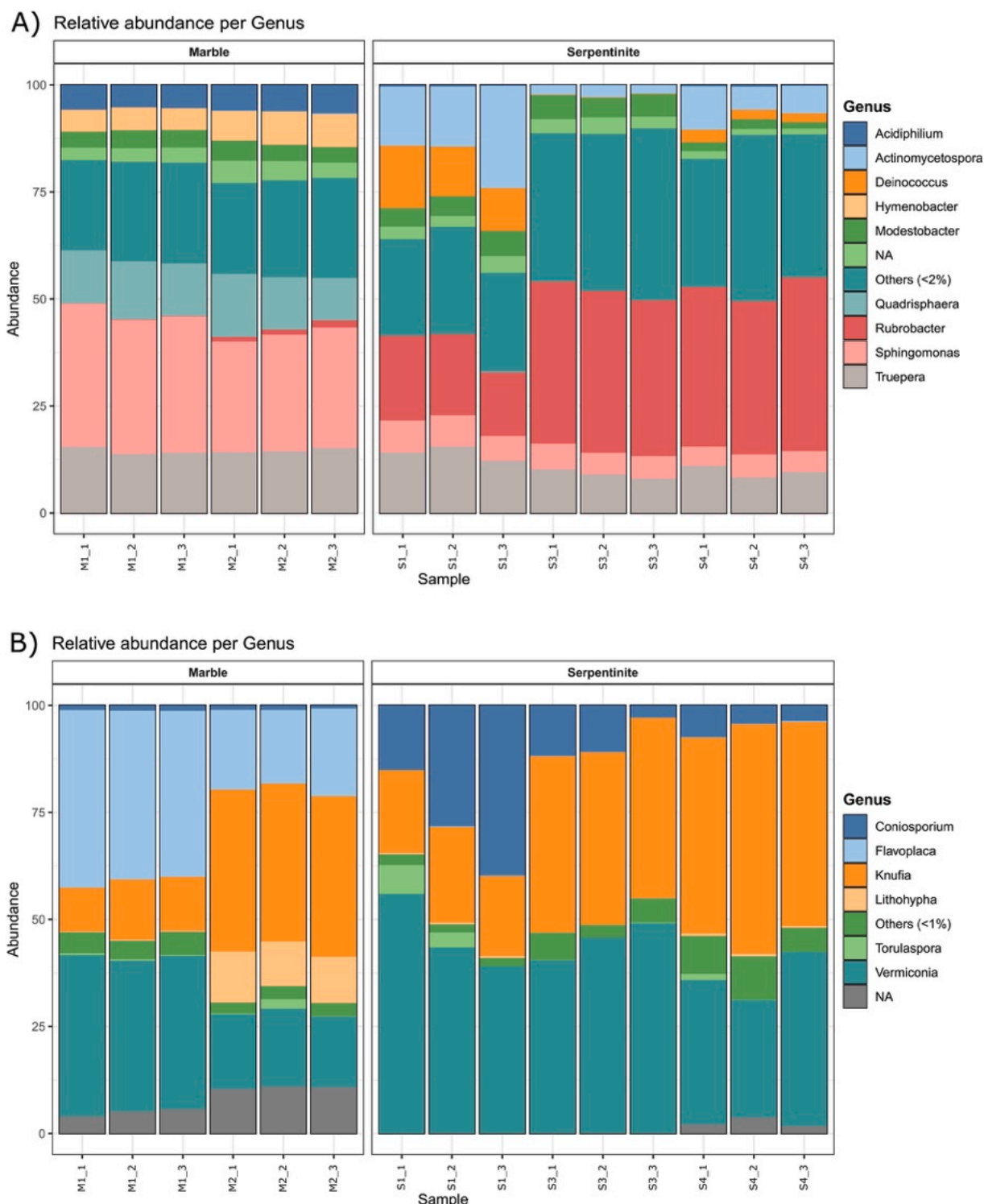


Fig. 6. Bar plots representing the most abundant genera of bacteria (A) and fungi (B). NA = not assigned. Samples labels: M = marble; S = serpentinite.

altered and secondary mineral assemblages can differ substantially from the fresh serpentinite in terms of solubility, surface reactivity, and nutrient availability. Conversely, microbial activity can also contribute to localised mineral transformation, through biologically mediated carbonate precipitation (MICP) [e.g., [Marvasi et al., 2020](#)] as well as carbonate dissolution processes, potentially resulting in the formation or redistribution of Mg- and Ca-bearing carbonate phases.

Some bacterial taxa detected on SMFC serpentinite, such as *Deinococcus* and *Sphingomonas*, have also been reported to be able to

precipitate calcium carbonate ([Mondal et al., 2020](#); [Checcucci et al., 2022](#)), whereas black meristematic fungi are known to promote carbonate dissolution through organic-acid production ([Berti et al., 2023](#)). These observations support the possibility of bidirectional interactions between microbial colonisation and carbonate phases, although such processes were not directly demonstrated in the present study.

Iron represents another important component of the mineral-microorganism interface. In serpentinites, Fe can occur both within serpentine-group minerals and in accessory Fe-bearing phases such as

magnetite, as indicated by the mineralogical and microprobe characterisation of the Cava Bagnolo reference material. Fe-bearing minerals may influence microbial colonisation both through their physicochemical properties and through the availability of Fe at the mineral surface. Microorganisms can interact with Fe-bearing phases through several mechanisms, including production of organic acids, extracellular polymeric substances (EPS), chelating compounds, and redox-active metabolites. In particular, siderophores are high-affinity Fe-chelating compounds that can contribute to Fe acquisition and mobilisation from mineral surfaces under suitable environmental conditions (Knapp and Giddings, 2026). All the meristematic fungi and most bacterial genera dwelling on SMFC serpentinite (*Actinomycetospora*, *Hymenobacter*, *Rubrobacter*, *Sphingomonas*) have the capability, tested or deduced from genomic analysis, to produce siderophores and then the potential for Fe acquisition in the mineral–biofilm interface, increasing the bioavailability of this essential nutrient, and, at the same time, for weakening the mineral lattice in Fe-serpentinite.

Nickel and chromium require a different interpretation because both are characteristic trace elements of ultramafic and serpentinitic rocks but may display substantially different behaviour during weathering. Their presence may also represent an important ecological factor because, while nickel is needed at low concentrations as an essential nutrient, elevated concentrations of Ni and Cr can exert toxic effects on microorganisms and selective pressure on microbial communities. Serpentine-derived materials are commonly enriched in both elements, but their mobility and environmental availability depend strongly on their mineralogical hosts and on weathering conditions (Quantin et al., 2008; Vallalar et al., 2019). Recent work on serpentinite rocks and serpentinite-derived sediments from Tuscany showed that Ni is generally more mobile than Cr, with Ni being substantially associated with Mn oxides and amorphous Fe oxides, whereas Cr is more strongly retained in primary rock-derived phases and well-crystallised Fe oxides (Franceschini, 2026). These differences indicate that total Ni and Cr concentrations should not be interpreted as equivalent measures of their bioavailability. The comparatively greater mobility may make Ni more responsive to changes in pH, redox conditions, mineral dissolution, and complexation processes (Vallalar et al., 2019). Nickel can become redistributed among secondary and more reactive phases during weathering. At elevated concentrations, Ni may exert toxic effects on microorganisms and may consequently contribute to the selection of metal-tolerant populations (Canonica et al., 2025; Daghino et al., 2009; Vallalar et al., 2019). Chromium requires an even more cautious interpretation because its environmental behaviour is strongly dependent on oxidation state. Chromium in fresh serpentinites is predominantly present as Cr(III), which is generally poorly soluble under circumneutral conditions and may be associated with Cr-bearing silicates, spinel-group minerals, and other primary phases. During weathering, interactions with Fe- and Mn-bearing secondary phases can modify Cr retention and redistribution, while Mn-bearing oxides may contribute to the oxidation of Cr(III) to the more mobile Cr(VI) species. Nevertheless, the potential for redox-dependent Cr mobilisation provides a relevant geochemical context for microbial colonisation because microorganisms can modify local chemical conditions, including pH and redox state, and may interact with metal species through metabolic and extracellular processes.

Microorganisms are intimately involved in metal biogeochemistry with a variety of processes, determining mobility, and therefore bioavailability of metals, as well as immobilization (Gadd, 2004). Among mechanisms of mobilization, siderophores, beyond iron, can also bind various heavy metals, including nickel, especially in metal-rich environments (Knapp and Giddings, 2026). Metals toxic to microorganisms can be mobilized, solubilized, and precipitated or immobilized by sorption or uptake and intracellular sequestration, in addition to increasing the metal tolerance of microorganisms (Gadd, 2004). Black meristematic fungi, dominant on SMFC serpentinite, are known for their great resistance to environmental stressors (Celi et al., 2025); their

heavy metal resistance, however, is little known. The capacity to bind heavy metal to their melanized cell wall was recently demonstrated; for example, the melanin extracts of a hyper-metallotolerant strain of *Exophiala* can adsorb Cr(VI) (Medina-Armijo et al., 2024).

Many identified bacterial genera on serpentinite were identified on SMFC marble affected by red stains associated with the presence of lead (*Acidiphilium*, *Deinococcus*, *Modestobacter*, *Quadrisphaera*, *Shingomonas*, *Truepera*) and their tolerance to heavy metal was previously discussed [687]. Concerning *Rubrobacter*, the genus dominant on SMFC serpentinite (Fig. 6A), it was identified on biodeteriorated monuments and as the aetiological agent of rosy discoloration on mural paintings (Basile et al., 2025). While its resistance to gamma and UV radiation is well known, no information is available on its relationship with heavy metals; metagenomic analysis, however, showed the potential for siderophores biosynthetic pathways (Basile et al., 2025). Given its dominance on the SMFC serpentinite and the genomic potential for siderophore biosynthesis, *Rubrobacter* represents a promising target for future studies investigating interactions between serpentinite mineral phases, metal acquisition, and bacterial activity.

Our results highlight a close relationship between lithological composition, surface alteration, and microbial colonisation, with weathering processes acting as major drivers of microbial colonisation and potentially influencing subsequent biodeterioration processes. In this respect, the SMFC serpentinite differs significantly from the silicatic Oira stone investigated by Gulotta et al. (2018). Although both are silicate-rich lithologies, the Oira stone showed a relatively well-preserved surface and negligible biological colonisation, whereas the SMFC serpentinite investigated here exhibits extensive alteration features. These differences suggest that the degree of pre-existing chemical–physical weathering may be more important than lithology alone in determining the extent of microbial colonisation. The microbial activity may, in turn, contribute to further modifications of the local mineral–surface environment, potentially enhancing or modifying ongoing biodeterioration processes. Both SMFC marble and serpentinite host microbial communities dominated by extremophilic taxa, including rock-inhabiting fungi such as *Coniosporium*, *Knufia* and *Vermiconidia* (Celi et al., 2025), and bacteria such as *Rubrobacter*, *Truepera*, and *Deinococcus*. These bacterial genera are characterised by efficient DNA repair systems, resistance to oxidative stress and radiation, and protective pigmentation (Ferreira et al., 1999; Albuquerque et al., 2005; de Groot and Blanchard, 2023). Their occurrence, consistent with previous reports from stone substrates [e.g., Chen et al., 2021], suggests that stress tolerance represent an important ecological trait contributing to microbial assembly on exposed architectural stone. In serpentinite, the enrichment in elements such as Ni and Cr may impose additional selective pressure on microbial populations and favour the persistence of metal-tolerant taxa, although the present data do not allow a direct relationship between metal concentrations and community composition to be established. Culture-dependent analyses showed that SMFC serpentinite hosts bacterial and BMF communities in abundances comparable to those previously reported for SMFC marble. With respect to rock-inhabiting fungi isolated from serpentinite, *Coniosporium* and *Knufia* are among the most abundant genera found by metabarcoding analysis, confirming that BMF are viable components of the serpentinite-associated mycobiota, rather than being detected only as environmental DNA.

All BMF identified in this study are frequently reported as colonizers of heritage stone substrates and are characterised by pronounced ecological plasticity and the ability to thrive under oligotrophic and stressful conditions, typical of exposed lithic environments (Gostinčar et al., 2012), as well as by marked resistance to antifungal biocides (Kirchhoff et al., 2017). These stress-resistance traits may contribute to their persistence despite conservation treatments (Celi et al., 2025) and make them suitable candidates for further investigation of resilience mechanisms in stone-colonising fungi. Most of the abundant bacterial and fungal genera detected on marble and serpentinite in this study have

previously been reported in investigation of SMFC marble (Checcucci et al., 2022; Berti et al., 2023). The presence of common rock-inhabiting genera across both lithotypes suggests the existence of a shared “core microbiota” adapted to the harsh environmental conditions of the SMFC exposed stone surface. However, the observed differences in community composition and relative abundance between the two lithotypes (Fig. 6) indicate that lithological and weathering-related factors still play an important role in shaping microbial assembly. Given the spatial proximity of the marble and serpentinite sampling areas, major differences in climatic conditions are unlikely to account for the observed patterns. The observed differences may therefore be more plausibly related to lithological, structural, and mineralogical characteristics, although microenvironmental effects cannot be excluded. Based on the present and previous data, the lichen-forming fungus *Flavoplaca* (syn. *Caloplaca*) can be considered a common inhabitant of the SMFC external marble, as it has been repeatedly detected in independent surveys of the same substrate (Santo et al., 2021; Checcucci et al., 2022). Although lichens are known to colonise both carbonate substrates (Matteucci et al., 2019) and serpentinite (Favero-Longo et al., 2005b), the absence of *Flavoplaca* on the serpentinite investigated in this study suggests that lithological and geochemical factors may limit its establishment on this substrate. Similarly, the bacteria *Acidiphilium* and *Qudrisphaera* appear to be more closely associated with the marble microbiota (Checcucci et al., 2022; Celi et al., 2026), whereas *Rubrobacter* appears to be more strongly associated with the serpentinite environment (Fig. 6A). Further data are required to confirm these substrate preferences and to identify the environmental factors underlying them.

Overall, the present results support the interpretation that microbial colonisation on the SMFC serpentinite is strongly influenced by the altered state of the substrate. Although serpentinite mineralogy may intrinsically limit biological colonisation under pristine conditions, prolonged environmental weathering and secondary mineral transformations may progressively increase stone bioreceptivity, enabling the establishment of specialised microbial communities adapted to oligotrophic, metal-rich, and stress-exposed lithic environments. These communities may subsequently contribute to further mineral–biofilm interactions, highlighting the dynamic relationship between stone weathering and microbial colonisation in the long-term deterioration of serpentinite used as architectural heritage material.

CRedit authorship contribution statement

Alba P. Santo: Writing – review & editing, Writing – original draft, Project administration, Funding acquisition, Conceptualization. **Domenico Celi:** Writing – original draft, Investigation. **Luigimaria Borruso:** Writing – review & editing, Formal analysis. **Maria Landolfi:** Writing – review & editing, Formal analysis. **Sonia Renzi:** Writing – review & editing, Investigation. **Brunella Perito:** Writing – review & editing, Writing – original draft, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

References

- Albuquerque, L., Simões, C., Nobre, M.F., Pino, M.N., Battista, J.R., Silva, M.T., Rainey, F.A., de Costa, M.S., 2005. *Truepera radiovictrix* gen. nov., sp. nov., a new radiation resistant species and the proposal of trueperaceae fam. nov. FEMS Microbiol. Lett. 247 (2), 161–169. <https://doi.org/10.1016/j.femsle.2005.05.002>.
- Anderson, M., 2001. A new method for non-parametric multivariate analysis of variance. Austral. Ecol. 26 (February), 32–46. <https://doi.org/10.1111/j.1442-9993.2001.01070>.
- Anderson, M.J., 2005. Distance-based tests for homogeneity of multivariate dispersions. Biometrics 62 (1), 245–253. <https://doi.org/10.1111/j.1541-0420.2005.00440.x>.
- Aston, B.G., Harrell, J.A., Shaw, I., 2000. Stone. In: Nicholson, P.T., Shaw, I. (Eds.), *Ancient Egyptian Materials and Technology*. Cambridge University Press, Cambridge, pp. 5–77.
- Basile, A., Riggio, F.P., Tescari, M., Chebbi, A., Sodo, A., Bartoli, F., Imperi, F., Caneva, G., Visca, P., 2025. Metagenome-resolved functional traits of *Rubrobacter* species implicated in rosy discoloration of ancient frescoes in two Georgian cathedrals. Sci. Total Environ. 958, 178135. <https://doi.org/10.1016/j.scitotenv.2024.178135>, 2025.
- Berti, L., Marvasi, M., Perito, B., 2023. Characterization of the community of black meristematic fungi inhabiting the external white marble of the florence cathedral. J. Fungi 9 (6). <https://doi.org/10.3390/jof9060665>.
- Bolyen, E., Rideout, J.R., Dillon, M.R., et al., 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat. Biotechnol. 37 (8), 852–857. <https://doi.org/10.1038/s41587-019-0209-9>.
- Botha, D., Barnard, S., Claassens, S., Rajakaruna, N., Venter, A., Ismail, A., Allam, M., Siebert, S.J., 2024. Soil type and precipitation level have a greater influence on fungal than bacterial diversity in serpentine and non-serpentine biological soil crusts. Ecol. Res. 39 (6), 862–878. <https://doi.org/10.1111/1440-1703.12500>.
- Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P., 2016. DADA2: High-resolution sample inference from Illumina amplicon data. Nat. Methods 13 (7), 581–583. <https://doi.org/10.1038/nmeth.3869>.
- Canonica, L., Cecchi, G., Comba, S., Di Piazza, S., Gianoglio, F., Marescotti, P., Voyron, S., Zotti, M., 2025. Fungi and potentially toxic elements (PTEs): exploring mycobiota in serpentinite soils. Soil Syst. 9 (4). <https://doi.org/10.3390/soilsystems9040129>.
- Capitani, G., Mellini, M., 2004. The modulated crystal structure of antigorite: the $m = 17$ polysome. Am. Mineral. 89, 147–158.
- Casadevall, A., Cordero, R.J.B., Bryan, R., Nosanchuk, J., Dadachova, E., 2017. Melanin, radiation, and energy transduction in fungi. Microbiol. Spectr. 5 (2). <https://doi.org/10.1128/microbiolspec.FUNK-0037-2016>.
- Celi, D., Marvasi, M., Perito, B., 2025. Conventional and innovative approaches to black fungi control for stone heritage preservation. IUBMB Life 77 (3), e70010. <https://doi.org/10.1002/iub.70010>.
- Celi, D., Perito, B., Landolfi, M., Sonia Renzi, S., Ricci, M., Santo, A.P., 2026. Red stains and associated microbial community of the external white marble of florence cathedral. J. Cult. Herit. 79, 56–165. <https://doi.org/10.1016/j.culher.2026.03.002>.
- Checcucci, A., Borruso, L., Petrocchi, D., Perito, B., 2022. Diversity and metabolic profile of the microbial communities inhabiting the darkened white marble of florence cathedral. Int. Biodeterior. Biodegrad. 171, 105420. <https://doi.org/10.1016/j.ibiod.2022.105420>.
- Chen, X., Bai, F., Huang, J., Lu, Y., Wu, Y., Yu, J., Bai, S., 2021. The organisms on rock cultural heritages: growth and weathering. Geoh Heritage 13, 56. <https://doi.org/10.1007/s12371-021-00588-2>.
- Coli, M., Compagnoni, R., Cossio, R., Del Ventisette, C., Vannucchi, P., 2023. The Monteferrato Serpentinized Peridotite (Figline Di Prato, Prato) In The Northern Apennines, Italy: a Witness Of The Ligurian Ocean Margin In The Monumental Religious Buildings Of Tuscany. Ofioliti 48 (2), 105–120. <https://doi.org/10.4454/Ofioliti.V48i2.564105>.
- Daghino, S., Turci, F., Tomatis, M., Girlanda, M., Fubini, B., Perotto, S., 2009. Weathering of chrysotile asbestos by the serpentine rock-inhabiting fungus verticillium leptobactrum. FEMS (Fed. Eur. Microbiol. Soc.) Microbiol. Ecol. 69 (1), 132–141. <https://doi.org/10.1111/j.1574-6941.2009.00695.x>.
- de Groot, A., Blanchard, L., 2023. DNA repair and oxidative stress defense systems in radiation-resistant *Deinococcus murrayi*. Can. J. Microbiol. 69 (11), 416–431. <https://doi.org/10.1139/cjm-2023-0074>.
- Diamantis, K., Gartzos, E., Migros, G., 2009. Study on uniaxial compressive strength, point load strength index, dynamic and physical properties of serpentinites from Central Greece: test results and empirical relations. Eng. Geol. 108, 199–207.
- Evans, B.W., 2004. The serpentine multisystem revisited: Chrysotile is metastable. Int. Geol. Rev. 46, 479–506.

- Favero-Longo, S.E., Castelli, D., Salvadori, O., Belluso, E., Piervittori, R., 2005a. Pedogenetic action of the Lichens *Lecidea atrobrunnea*, *Rhizocarpon geographicum* gr. and *Sporastatia testudinea* on serpentinized ultramafic rocks in an alpine environment. *Int. Biodeterior. Biodegrad.* 56 (1), 17–27. <https://doi.org/10.1016/j.ibiod.2004.11.006>.
- Favero-Longo, S.E., Castelli, D., Salvadori, O., Belluso, E., Piervittori, R., 2005b. Pedogenetic action of the Lichens *Lecidea atrobrunnea*, *Rhizocarpon geographicum* gr. and *Sporastatia testudinea* on serpentinized ultramafic rocks in an alpine environment. *Int. Biodeterior. Biodegrad.* 56 (4), 250–251. <https://doi.org/10.1016/j.ibiod.2004.11.006>.
- Favero-Longo, S.E., Turci, F., Fubini, B., Castelli, D., Piervittori, R., 2013b. Lichen deterioration of asbestos and asbestiform minerals of serpentinite rocks in Western alps. *Int. Biodeterior. Biodegrad.* 84, 342–350. <https://doi.org/10.1016/j.ibiod.2012.07.018>.
- Ferreira, A.C., Nobre, M.F., Moore, E., Rainey, F.A., Battista, J.R., da Costa, M.S., 1999. Characterization and radiation resistance of new isolates of *Rubrobacter radiotolerans* and *Rubrobacter xylanophilus*. *Extremophiles* 3 (4), 235–238. <https://doi.org/10.1007/s007920050121>.
- Franceschini, F., 2026. Speciation and mobility of Cr and Ni in serpentine rocks and derived sediments (Tuscany, Italy). *Standards* 6, 5. <https://doi.org/10.3390/standards6010005>.
- Fratini, F., Resic, S., 2013. The stone materials of the historical architecture of Tuscany, Italy. In: Cassar, J., Winter, M.G., Marker, B.R., Walton, N.R.G., Entwistle, D.C., Bromhead, E.N., Smith, J.W.N. (Eds.), *Stone in Historic Buildings: Characterization and Performance*, 391. Geological Society, London, Special Publications, pp. 71–92.
- Fubini, B., Fenoglio, I., 2007. Toxic potential of mineral dusts. *Elements* 3, 407–414.
- Gadd, G.M., 2004. Microbial influence on metal mobility and application for bioremediation. *Geoderma* 122, 109–119. <https://doi.org/10.1016/j.geoderma.2004.01.002>.
- Gostinčar, C., Muggia, L., Grube, M., 2012. Polyextremotolerant Black fungi: oligotrophism, adaptive potential, and a link to lichen symbioses. *Front. Microbiol.* 3, 390. <https://doi.org/10.3389/fmicb.2012.00390>.
- Gulotta, D., Bontempi, E., Bugini, R., Goidanich, S., Toniolo, L., 2017. The deterioration of metamorphic serpentinites used in historical architecture under atmospheric conditions. *Q. J. Eng. Geol. Hydrogeol.* 50, 402–441. <https://doi.org/10.1144/qjehg2016-133>.
- Gulotta, D., Villa, F., Cappitelli, G., Toniolo, L., 2018. Biofilm colonization of metamorphic lithotypes of a renaissance cathedral exposed to urban atmosphere. *Sci. Total Environ.* 639, 1480–1490, 2018.
- Harrell, J.A., 2007. Stones in Ancient Egypt. Department of Environmental Science, Univ. of Toledo, USA. World Wide Web Address: <http://www.eeescience.utoledo.edu/egypt/>.
- Ismael, I.S., Hassan, M.S., 2008. Characterization of some Egyptian serpentinites used as ornamental stones. *Chin. J. Geochem.* 27, 140–149. <https://doi.org/10.1007/s11631-008-0140-0>.
- Kelemen, P.B., Matter, J., 2008. In situ carbonation of peridotite for CO₂ storage. *Proc. Natl. Acad. Sci.* 105 (45), 17295–17300.
- Kirchhoff, L., Olsowski, L., Zilmans, K., Dittmer, S., Haase, G., Sedlacek, L., Steinmann, E., Buer, J., Rath, P.-M., Steinmann, J., 2017. Biofilm formation of the black yeast-like fungus *Exophiala dermatitidis* and its susceptibility to anti-infective agents. *Sci. Rep.* 7, 42886. <https://doi.org/10.1038/srep42886>.
- Knapp, M., Giddings, L.A., 2026. Bacterial siderophore production in metal-rich environments: underexplored sources of siderophores and insights into bioremediation. *J. Nat. Prod.* 89, 846–863. <https://doi.org/10.1021/acs.jnatprod.5c01586>, 2026.
- Lima-Zaloumis, J., Neubeck, A., Ivarsson, M., Bose, M., Greenberger, R., Templeton, A.S., Czaja, A.D., Kelemen, P.B., Edvinsson, T., 2022. Microbial biosignature preservation in carbonated serpentine from the Samail Ophiolite, Oman. *Commun. Earth Environ.* 3, 231. <https://doi.org/10.1038/s43247-022-00551-1>.
- Malesani, P., Pecchioni, E., Cantisani, E., Fratini, F., 2003. Geolithology and provenance of materials of some historical buildings and monuments in the centre of Florence (Italy). *Episodes* 26 (3), 250–255. <https://doi.org/10.18814/epiugs/2003/v26i3/017>, 2003.
- Marvasi, M., Donnarumma, F., Frandi, A., Mastromei, G., Sterflinger, K., Tiano, P., Perito, B., 2012. Black microcolonial fungi as detritogens of two famous marble statues in Florence, Italy. *Int. Biodeterior. Biodegrad.* 68, 36–44. <https://doi.org/10.1016/j.ibiod.2011.10.011>.
- Marvasi, M., Mastromei, G., Perito, B., 2020. Bacterial calcium carbonate mineralization in situ strategies for conservation of stone artworks: from cell components to microbial community. *Front. Microbiol.* 11, 1386. <https://doi.org/10.3389/fmicb.2020.01386>.
- Matteucci, E., Scarcella, A.V., Croveri, P., Marengo, A., Borghi, A., Benelli, C., Hamdan, O., Favero-Longo, S.E., 2019. Lichens and other lithobionts on the carbonate rock surfaces of the heritage site of the tomb of Lazarus (Palestinian territories): diversity, biodeterioration, and control issues in a semi-arid environment. *Ann. Microbiol.* 69 (10), 1033–1046. <https://doi.org/10.1007/s13213-019-01465-8>.
- Medina-Armijo, C., Isola, D., Illa, J., Puerta, A., Viñas, M., Prenafeta-Boldú, F.X., 2024. The metal tolerance and biosorption of As(V) and Cr(VI) by black fungi. *J. Fungi* 10 (1), 47. <https://doi.org/10.3390/jof10010047>.
- Meierding, T.C., 2005. Weathering of Serpentine Stone Buildings in the Philadelphia, Pennsylvania, Region: a Geographic Approach Related to Acidic Deposition, 390. Geological Society of America Special Publication: Stone Decay in the Architectural Environment, pp. 17–25.
- Mondal, S., Das, P., Datta, P., Ghosh, A.D., 2020. *Deinococcus radiodurans*: a novel bacterium for crack remediation of concrete with special applicability to low-temperature conditions. *Cement Concr. Compos.* 108, 103523. <https://doi.org/10.1016/j.cemconcomp.2020.103523>.
- Navarro, R., Pereira, D., Fernández de Arévalo, E., Sebastián-Pardo, E.M., Rodríguez-Navarro, C., 2021. Weathering of serpentinite stone due to in situ generation of calcium and magnesium sulfates. *Constr. Build. Mater.* 280, 122402.
- Oksanen, J., Simpson, G.L., Blanchet, F.G., et al., 2025. Vegan: community ecology package. <https://vegandevs.github.io/vegan/>.
- Pecchioni, E., Magrini, D., Cantisani, E., Fratini, F., Garzonio, C.A., Nosengo, C., Santo, A.P., Vettori, S., 2019. A Non-Invasive Approach for the Identification of “Red Marbles” from Santa Maria Del Fiore Cathedral (Firenze, Italy). *Int. J. Architect. Herit.* <https://doi.org/10.1080/15583058.2019.1629045>.
- Pereira, D., 2012. A report on serpentinites in the context of heritage stone resources. *Episodes* 35 (4), 478–480. <https://doi.org/10.18814/epiugs/2012/v35i4/003>.
- Pereira, D., Peinado, M., Yenes, M., Monterrubio, S., Nespereira, J., Blanco, J.A., 2010. Serpentinites from Cabo Ortegal (Galicia, Spain): a search for correct use as ornamental stones. *Geol. Soc. Lond. Spec. Publ.* 333, 81–84.
- Pereira, D., Yenes, M., Sanche, J.A.B., Peinado Moreno, M.M., 2007. Characterization of serpentinites to define their appropriate use as dimension stone. *Geol. Soc. Spec. Publ.* 271, 55–62.
- Pereira, M.D., Peinado, M., Blanco, J.A., Yenes, M., 2008. Geochemical characterization of serpentinites at Cabo Ortegal, Northwestern Spain, the. *Can. Mineral.* 46, 317–327.
- Principi, G., Bortolotti, V., Chiari, M., Cortesogno, L., Gaggero, L., Marcucci, M., Saccani, E., Treves, B., 2004. The pre-orogenic volcano-sedimentary covers of the western Tethys oceanic basin. a review. *Ofioliti* 29 (2), 177–211.
- Punturo, R., Visalli, R., Cirrincione, R., 2023. A review of the mineralogy, petrography, and geochemistry of serpentinite from Calabria regions (Southern Italy): problem or georesource? *Minerals* 13, 1132. <https://doi.org/10.3390/min13091132>.
- Quantin, C., Ettler, V., Garnier, J., Šebek, O., 2008. Sources and extractibility of chromium and nickel in soil profiles developed on Czech serpentinites. *C. R. Geosci.* 340, 872–882. <https://doi.org/10.1016/j.crte.2008.07.013>.
- Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner, F.O., 2012. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res.* 41 (D1), D590–D596. <https://doi.org/10.1093/nar/gks1219>.
- Renzi, S., Russo, A., D'Alessandro, A., Ciattini, S., Soliman, S.C., Nistri, A., Pretti, C., Cavalieri, D., Ugolini, A., 2025. Microbial communities' composition of supralittoral and intertidal sediments in two east African beaches (Djibouti republic). *Microbiol. Res.* 16 (8), 173. <https://doi.org/10.3390/microbiolres16080173>.
- Rognes, T., Flouri, T., Nichols, B., Quince, C., Mahé, F., 2016. VSEARCH: a versatile open source tool for metagenomics. *PeerJ* 4, e2584. <https://doi.org/10.7717/peerj.2584>.
- Santo, A.P., Agostini, B., Checucci, A., Pecchioni, E., Perito, B., 2020. An interdisciplinary study of biodeterioration of the external marbles of Santa Maria del Fiore Cathedral, Florence (IT). *IOP Conf. Ser. Mater. Sci. Eng.* 949.
- Santo, A.P., Agostini, B., Cuzman, O.A., Michelozzi, M., Salvatici, T., Perito, B., 2023. Essential oils to contrast biodeterioration of the external marble of Florence cathedral. *Sci. Total Environ.* 877, 162913. <https://doi.org/10.1016/j.scitotenv.2023.162913>.
- Santo, A.P., Agostini, B., Garzonio, C.A., Pecchioni, E., Salvatici, T., 2022. Decay process of serpentinite: the case of the San Giovanni Baptistery (Florence, Italy) pavement. *Appl. Sci.* 12 (2), 861. <https://doi.org/10.3390/app12020861>, 2022.
- Santo, A.P., Cuzman, O.A., Petrocchi, D., Pinna, D., Salvatici, T., Perito, B., 2021. Black on white: microbial growth darkens the external marble of Florence cathedral. *Appl. Sci.* 11 (13). <https://doi.org/10.3390/app11136163>.
- Santo, A.P., Cuzman, O.A., Salvatici, T., Agostini, B., Perito, B., 2025b. In situ long-term monitoring of microbial patinas on the external marble of Florence cathedral after treatments with essential oils. *J. Cult. Herit.* 74, 246–256. <https://doi.org/10.1016/j.culher.2025.06.012>.
- Santo, A.P., Garzonio, C.A., Pecchioni, E., Salvatici, T., 2025a. Petrophysical characterisation and suitability of serpentinites from the Monteferrato area (Tuscany, Italy) for architectural restoration. *Minerals* 15 (11), 1105. <https://doi.org/10.3390/min15111105>.
- Santo, A.P., Garzonio, C.A., Pecchioni, E., Salvatici, T., 2025c. Petrophysical characterisation and suitability of serpentinites from the Monteferrato area (Tuscany, Italy) for architectural restoration. *Minerals* 15 (11). <https://doi.org/10.3390/min15111105>.
- Santo, A.P., Pecchioni, E., Garzonio, C.A., 2018. The San Giovanni Baptistery in Florence (Italy): characterisation of the serpentinite floor. *IOP Conf. Ser. Mater. Sci. Eng.* 364 (1), 012069. <https://doi.org/10.1088/1757-899X/364/1/012069>.
- Šaraba, V., Trtić-Petrović, T., Kostić, B., Dabić, P., Janković, V., Nikodinović-Runic, J., Ciric, M., 2025. Diversity and biotechnological potential of limestone and serpentinite microorganisms. *Geomicrobiol. J.* 42 (6), 523–529. <https://doi.org/10.1080/01490451.2025.2485466>.
- Schwarzenbach, E.M., Vogel, M., Fröh-Green, G.L., Boschi, C., 2021. Serpentinization, carbonation and metasomatism of ultramafic sequences in the Northern Apennine ophiolite (NW Italy). *J. Geophys. Res. Solid Earth* 126, e2020JB020619. <https://doi.org/10.1029/2020JB020619>.
- Siegesmund, S., Torok, A., 2014. Building stones. In: Siegesmund, E., Snethlage, R. (Eds.), *Stone in Architecture. Properties, Durability*. Springer, Berlin/Heidelberg, Germany; New York, NY, USA; Dordrecht, The Netherlands; London, UK.
- Sterflinger, K., Krumbein, W.E., 1995. Multiple Stress Factors Affecting Growth of Rock-Inhabiting Black Fungi. <https://api.semanticscholar.org/CorpusID:85191481>.
- Sterflinger, K., Krumbein, W.E., 1997. Dematiaceous fungi as a major agent for biopitting on Mediterranean marbles and limestones. *Geomicrobiol. J.* 14 (3), 219–230. <https://doi.org/10.1080/01490459709378045>.

- Sun, M., Lian, B., 2023. Comparative analysis of weathering rate of serpentine by microbial pure culture and field burial tests: a case study. *Int. Biodeterior. Biodegrad.* 184, 105670. <https://doi.org/10.1016/j.ibiod.2023.105670>.
- Tesei, D., 2022. Black fungi research: Out-of-This-world implications. *Encyclopedia* 2 (1), 212–229. <https://doi.org/10.3390/encyclopedia2010013>.
- Vallalar, B., Meyer-Dombard, D.R., Cardace, D., Arcilla, C.A., 2019. Multimetal resistant, alkali-tolerant bacteria isolated from serpentinizing fluid-associated sediments and acid mine drainage in the Zambales ophiolite, the Philippines. *Geomicrobiol. J.* 36 (9), 792–809. <https://doi.org/10.1080/01490451.2019.1628132>.
- Wickham, H., 2016. Data analysis. In: *Ggplot2: Elegant Graphics for Data Analysis*. Springer International Publishing. https://doi.org/10.1007/978-3-319-24277-4_9.