

## **Weakening of gneiss surfaces colonized by endolithic lichens in the temperate climate area of NW Italy**

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## Abstract

A role of lithobionts in geomorphological processes is increasingly argued, but the spatio-temporal scale of their impact is largely unexplored in many ecosystems. This study first characterizes in the temperate zone (NW-Italy) the relationships between lithobiontic communities including endolithic lichens and the hardness of their siliceous rock substrate (Villarfocchiardo Gneiss). The communities are characterized, on humid and xeric quarry surfaces exposed for decades and natural outcrops exposed for centuries, in terms of lichen and microbial constituents, using a combined morphological and molecular approach, and with regard to their development on and within the gneiss.

A lichen species belonging to Acarosporaceae (*Polysporina-Sarcogyne-Acarospora* group, needing taxonomic revision) chasmoendolithically colonizes both the humid and xeric quarry surfaces, on which epilithic cyanobacterial biofilms and epilithic pioneer lichens respectively occur. Light and electron microscopic observations show the development of the endolithic thalli within rock microcracks and the hyphal penetration along crystal boundaries down to depths of 1-3 mm, more pronounced within the humid surfaces. Such colonization patterns are likely related to biogeophysical deterioration, while no chemical alteration characterizes minerals contacted by the endolithic lichen. By contrast, on natural outcrops, where the endolithic colonization is negligible, a reddish rind below epilithic lichens indicates chemical weathering processes.

Schmidt Hammer measurements highlight that the endolithic lichens deeply affect the hardness of the gneiss (down to -60% with respect to fresh controls and surfaces only colonized by cyanobacteria), exerting a significantly higher weakening effect with respect to the associated epilithic lithobionts. The phenomenon is more

remarkable on humid than on xeric quarry surfaces and natural outcrops, where epilithic lichens are likely involved in long-term hardening processes supporting surface stabilization.

Endolithic lichens are thus active biogeomorphological agents at the upper millimetric layer of siliceous rocks in temperate areas, exerting their weakening action during the early decade-scaled stages of surface exposure.

**Keywords:**

biogeomorphology, endolithic lichens, gneiss, Schmidt Hammer, temperate climates

**Running title:**

Biogeomorphological impact of endolithic lichens on siliceous rocks

**For Twitter:**

Endolithic lichens are biogeomorphological agents on siliceous rock surfaces at the early decade-scaled stages of exposure in temperate areas.

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## Introduction

The role of lithobionts as agents of geomorphic change, especially in extreme environments, is increasingly argued, although information on their multiple effects on geomorphological processes and on the temporal and spatial scale of their impact is still poor (Viles et al., 2008). Molecular investigations recently improved the knowledge on the richness and variability of epi- and endolithic microbial communities (*i.e.* living on and in rocks, respectively), but the equilibria between their weathering and protective effects, having geomorphological implications, is yet largely unknown, mostly in the case of endoliths (Viles, 2012).

### *Endolithic life style*

Endolithism is a successful life strategy in extremely hot and cold environments, sheltering microorganisms from excessive temperature, irradiation and drought conditions (Walker and Pace, 2007; Antony et al., 2012). Communities of lichenized and non-lichenized fungi, green algae and cyanobacteria inhabiting existing cracks and fissures (chasmoendolithism) and structural cavities (cryptoendolithism) or actively penetrating the substrate (euendolithism) have been indeed reported as predominant forms of colonization for both igneous and sedimentary substrates in hot and cold deserts (Golubic et al., 1981; Gorbushina, 2007; Wierzchos et al., 2012), periglacial environments (McCarroll and Viles, 1995; Etienne, 2002) and tropical inselbergs (Porembski, 2007). Related biogeophysical and biogeochemical processes affecting the mineral substrate have also been unveiled (Büdel et al., 2004; de los Ríos et al., 2005a; Davila et al., 2008; Guglielmin, 2012). On the other hand, although the endolithic habitat is ubiquitous (Walker and Pace, 2007), minor attention has been devoted to the presence and ecological role of

endoliths in the warm-temperate zone (Matthes-Sears et al., 1997; Weber and Büdel, 2011): some researches have studied the endolithic colonization of stone building materials, considering its relevance for biodeterioration issues, but the related weathering processes and their effects on the substrate durability have been only partially clarified (e.g. de los Ríos et al., 2012; Gaylarde et al., 2012; Caneva et al., 2014). With regard to endolithic lichens, in particular, studies mostly focused on growth patterns, physiological rates and deterioration activity within carbonate rocks (Tretiach and Pecchiari, 1995; Pinna et al., 1998; Favero-Longo et al., 2009, 2011; Weber et al., 2011; Casanova-Municchia et al., 2014), while their colonization and deterioration of siliceous lithotypes and related biogeomorphological processes have been widely neglected. This is rather surprising as lichens are generally recognized as remarkable agents of biodeterioration on siliceous rocks through a wide range of climatic and urbanization conditions (Adamo and Violante 2000; Gazzano et al. 2009a; Scheer et al. 2009) and several silicicolous species with obligate/facultative endolithic thallus development are included in lichen floras of temperate areas (e.g. Nimis and Martellos, 2008; Smith et al., 2009).

#### *Lichen deterioration of carbonate and siliceous lithotypes*

Endolithic growth of several lichen species (euendoliths) within carbonate rocks is related to active penetration processes (Golubic et al., 1981; Hoppert et al., 2004). Although these have still not been definitely clarified, different metabolic activities potentially responsible of the pervasive dissolution of carbonate minerals have been reported, including the release of chelating compounds, as oxalic acid (Garvie et al., 2008) and siderophore-like molecules (Favero-Longo et al., 2011), and a dissolution-promoting role of the enzyme carbonic anhydrase (Tretiach et al., 2008).

Lichen metabolites with acidic and chelating functions, as oxalic acid and polyphenolic secondary metabolites, are also active in the leaching of several silicate minerals: their release has been widely related to the chemical modification of minerals at the interface between epilithic lichens and several silicate lithotypes, also accounting for the appearance of secondary minerals (Adamo and Violante, 2000; Gadd et al., 2012). However, the biogeochemical activity of lichens on silicate minerals has been more documented in terms of chemical modification of the surface crystalline layers and partial conversion to clay minerals (Barker and Banfield, 1996; Wierzchos and Ascaso, 1998), rather than as pervasive dissolution process. Accordingly, the penetration of epilithic lichens within the colonized silicate substrates has been mostly characterized as a hyphal growth along spaced mineral boundaries, porosities and fractures, but it is not definitely clear (and likely depends on the examined lichen-lithotype case and on the temporal stage of the colonization) how much the hyphae exploit intrinsic discontinuities of the substrate as passageway and/or in what measure they physically and chemically contribute to originate and improve such discontinuities (Ascaso and Wierzchos, 1995; Gadd et al., 2012). In the case of the endolithic growth within silicate rocks observed in the extreme Antarctic conditions, lichens of the genus *Lecidea* were shown to be active in chemically modifying minerals in the proximity of penetrating hyphae (de los Ríos et al., 2005a). However, colonization of cracks, fissures and porosities (chasmo- and cryptoendolithic behaviour) generally appears as the dominant behaviour of endolithic lichens in both cold and hot desert environments, where they are recognized as prominent colonizers (Wierzchos et al., 2012).

### *The effect of lithobionts on the rock hardness*

Field-based measurements of rock hardness have been used since the 1960s in geomorphological research as proxy indices of the degree of rock surface weathering and, by extension, of the date of surface exposure (Goudie, 2006).

Heritage scientists subsequently extended to rock building materials their non-destructive application to detect deterioration phenomena, including the formation of soft secondary minerals, microcracks and flaws (Viles et al., 2011; Fort et al., 2013). Accordingly, rock hardness studies also investigated the impact and rates of biological weathering, with a main focus on endolithic microorganisms and lichens in periglacial and desert environments (McCarroll and Viles, 1995; Viles and Goudie, 2004; Matthews and Owen, 2008; Guglielmin et al., 2012) and minor emphasis on the stone cultural heritage (Garcia-Vallès et al., 2003). Different effects, ranging from a decrease of the rock hardness to a case hardening were reported, likely related to the different climates, lithotypes and microbial communities considered in each study, but such processes were still not considered in temperate areas.

### *Objectives*

This study investigated patterns and effects of the lichen and microbial colonization on and in the Villarfocchiardo Gneiss, a siliceous metamorphic lithotype from NW Italy, widely used as structural and ornamental stone in the local architecture (Borghetti et al., 2014). We aimed to verify the hypothesis that lichens growing as chasmoendoliths within siliceous rocks, together with the associated microbial biofilms, exert geomorphologically-significant effects when considered in the less-explored temperate environments. Moreover, we aimed to consider the temporal scale of the biodeterioration impact.

Communities of epi- and endolithic lichens and co-occurring microbial biofilms on the gneiss were examined on abandoned quarry surfaces, exposed since 25 and 75 years, and on natural outcrops, coupling microscopy and molecular analyses. The physical and chemical interaction of the microorganisms with the lithic substrate was characterized, with a particular focus on the spread and the depth of the hyphal penetration component (*sensu* Favero-Longo et al., 2005) of lichens and on the chemical composition of minerals contacted by hyphae. The microbial geomorphological impact was evaluated with reference to the variation in the rock surface hardness, determined with a Schmidt Hammer rebound tester.

## **Material and methods**

### *Rock material and study sites*

The Villarfocchiardo Gneiss is a leucocratic orthogneiss of white to light greyish colour with quartz, microcline, albite and phengite as fundamental components, and tourmaline as characterizing mineral (Borghi et al., 2014). The most common variety of the gneiss is characterized by a greater amount of tourmaline and a foliation less noticeable. A second variety, outcropping in smaller quantities, is characterized by an increased amount of white mica, which gives the rock a more foliated appearance.

Active and historical quarries of the Villarfocchiardo Gneiss are located in the middle Susa Valley (Dora-Maira Massif, a continental crust unit of the Western Alps; Bussoleno and Villarfocchiardo municipalities), nearly 50 km west of Turin (av. annual rainfall: 740 mm in Bussoleno; av. temperature: 11-12°C). The wide usage of Villarfocchiardo Gneiss in historical architecture in Turin is exemplified by the stone



bridges Princess Isabella and Umberto I (end of 19<sup>th</sup> century) on the Po river and by parts of the stone covering of the façade of the Royal Palace (17<sup>th</sup> century).

Microbial and lichen colonization on/within the gneiss was surveyed on (a) (sub-)vertical surfaces abandoned since 25 years in the active quarry of San Basilio (UTM ED50, N 4998807, E 354323; 600 m a.s.l.) and (b) since about 75 years in the dismissed historical quarry of Banda (UTM ED50, N 4997226, E 359015; 700 m a.s.l.), and (c) on natural outcrops adjacent to the active quarry (630 m a.s.l.). In the two quarries, the survey was performed separately on xeric (x) and humid (h) rock surfaces, the latter differing by a longer period of water flow after rain. Only xeric surfaces characterized the natural outcrops. In the active quarries of San Basilio and Picapera (UTM ED50, N 4998668, E 354077), (f) fresh rock surfaces were additionally selected as representative of the tourmaline-rich/fengite-poor (f1) and of the tourmaline-poor/fengite rich (f2) varieties of the Villarfocchiardo Gneiss, respectively, to be used as controls.

#### *Sampling and characterization of lithobionts*

Lithobiotic communities were surveyed and sampled in April 2012 throughout the investigated sites (a-c). Micro-fragments (approx. 1 g in weight) of the black biofilm characterizing all the sites were collected by gently scraping the rock surface, with the aid of a lancet, at least one meter above ground level in order to avoid soil contaminations. The samples were examined under a light microscope (Nikon Eclipse 50i), identifying at the genus level dominant cyanobacteria and green algae following Komárek and Anagnostidis (1986) and Bellinger and Sigeo (2010).

Moreover, for at least three samples (0.8 g) from each site, the supernatant obtained by the overnight incubation of the pulverized black patina in 1 ml of physiological

solution (0.8% NaCl) was plated on suitable media to isolate and obtain pure sub-cultures of oligotrophic non-lichenized fungi (BBM, then MEA; Favero-Longo et al., 2011), cyanobacteria (BG11; Gaylarde et al., 2012) and green algae (MKM; Gaylarde et al., 2012) for subsequent molecular analyses. Fungal DNA was extracted using the DNeasy plant minikit (Qiagen S.A.; Courtabouef, France) and the ITS region of rDNA (primers ITS1F-ITS4; Gardes and Bruns, 1993) was PCR amplified. Cyanobacterial and algal DNA was obtained using a 100 mM Tris-HCl, 10 mM EDTA extraction buffer (10 min boiling, 5 min in ice, 10 min centrifugation at 14000 rpm and final collection of the DNA-containing supernatant) and the 18S (primers 1.2F-ALG2R; Cutler et al., 2013) and 16S (primers 27F-408R; Neilan et al., 1997) regions of rDNA were PCR amplified, respectively. PCR products were sequenced by Beckman-Coulter (London). The BLASTn tool (Altschul et al., 1997) was used to search sequences with high similarity available in NCBI (National Centre for Biotechnology Information, Bethesda, MD, USA) GenBank, being informative for the fungal identification and to confirm the morphological identification of cyanobacteria and green algae.

Centimetric rock fragments, which revealed the occurrence of endolithic microorganisms after being hit with a geologist's hammer, were also sampled. Micro-fragments of the endolithic component were picked with sterile lancets, avoiding contamination from the epilithic layer. DNA was directly extracted using the DNeasy plant minikit from these micro-fragments and from potentially-related apothecia on the rock surface. The ITS region was characterized and examined according to the above protocol and through an additional phylogenetic analysis (see Supplementary material SM1).

Epilithic lichens characterizing each site were collected and identified in laboratory following Smith et al. (2009) and Wirth (1995) and, for the nomenclature, Nimis and Martellos (2008).

#### *Analysis of lichen penetration within the rock and related mineral weathering*

Gneiss fragments were sampled in the quarries to analyze the colonization patterns within the substrate of different lithobiontic consortia recognized on humid (ah/i-ii and bh) and xeric (ax and bx) surfaces, respectively. Rock samples colonized by the epilithic lichens *Candelariella vitellina* (crustose) and *Xanthoparmelia* gr. *stenophylla* (foliose), dominating the lichen vegetation of the natural outcrops (cx/i-ii), and samples from the fresh control surfaces (f1 and f2) were also examined for comparison. Polished cross sections (approx. 1 × 2-5 × 0.5 cm h × l × w, at least 5 sections per site per study case) were cut with a diamond saw, PAS stained and observed under reflected light microscopy (Favero-Longo et al., 2005). Along the colonized transect, measuring points were established at each millimetre from the cross section vertex and the hyphal penetration was measured perpendicularly beneath each measuring point (Favero-Longo et al., 2011). High definition images of the sections were analyzed using the WinCAM Pro 2007d software (Regent's Instruments Canada Inc.) to quantify the hyphal spread within the substrate, according to Gazzano and colleagues (2009b). Results were statistically analyzed by means of ANOVA with the post-hoc Tukey's test. SYSTAT 10.2 (SYSTAT, Evanston, IL, USA) was used to perform the test (P<0.05 was considered significant).

Petrographic thin cross sections were also prepared and microscopically observed under transmitted light using a polarizing microscope Olympus BX4 to analyze the lichen penetration patterns with reference to the rock microstructure. After coating

with carbon, the sections were examined by scanning electron microscopy (SEM) in both the secondary electron and back-scattered electron mode, using a Cambridge S-360 electron microscope equipped with a Oxford INCA Energy 200 EDS apparatus. EDS analyses were performed on plagioclase and alkali-feldspar crystals, either uncolonized (controls; n=10) and contacted by endolithic lichens (n=25) in quarry sites and beneath epilithic lichen thalli (n=5) on the natural outcrop. The results were statistically analyzed by means of ANOVA with the post-hoc Tukey's test.

Polished and petrographic thin cross sections are conserved in the Lichen-Petrographic Collection of the Herbarium of the University of Torino (Gazzano et al., 2007).

#### *Schmidt Hammer measurements*

Measurements of rock hardness were performed on the abandoned surfaces of the active and historical quarries, colonized by different lithobiontic consortia, on the natural outcrops, below epilithic thalli of the dominant lichen species, and on the fresh rock surfaces, as control.

A Schmidt-Hammer type L (Proceq, Schwerzenbach, CH) was used with an impact pressure of 0.735 Nm, which is appropriate for rocks with a thin weathering crust (Viles et al., 2011). For the following case studies, fourteen readings were made on at least three 400 cm<sup>2</sup> parcels ( $n_{\text{tot}} \text{ parcels}=42$ ): fresh tourmaline-rich/fengite-poor gneiss surface (f1), fresh tourmaline-poor/fengite-rich gneiss surface (f2), active quarry/humid surface/epilithic biofilm only (ah/i), active quarry/humid surface/epi-endolithic lithobiontic consortium (ah/ii), active quarry/xeric surface/epi-endolithic lithobiontic consortium (ax), historical quarry/humid surface/epi-endolithic lithobiontic

consortium (bh), historical quarry/xeric surface/epi-endolithic lithobiontic consortium (bx), natural outcrop/xeric surface/*Candelariella vitellina* (cx/i), natural outcrop/xeric surface/*Xanthoparmelia* gr. *stenophylla* (cx/ii). The mean of all the rebound values (R-values) was used for each parcel. According to the producer's operating instructions, we also calculated the means by discarding the extreme values, but no differences resulted. R-values were calculated by one operator only, in dry conditions, in points displaying homogeneous roughness and absence of cracks. Both colonized and control test surfaces were cleaned with a soft small brush, removing crustose lichen thalli and epilithic biofilms and/or inorganic detritus. Foliose lichen thalli (where present) were gently removed with the aid of a lancet before the cleaning procedure. The use of the Proceq supplied carborundum grindstone to smoothen the test surfaces was not adopted to avoid possible disturbance on potential case-hardened layers below the epilithic colonization (see Viles et al., 2011). Results were statistically analyzed by means of ANOVA with the post-hoc Tukey's test.

## **Results**

### *Lithobiontic colonization on Villarfochiardo Gneiss*

Gneiss surfaces display prominent lithobiontic colonization throughout the investigated sites, with the exception of the fresh control surfaces of the active quarries (Table 1). The extension and localization of different microbial communities appear related with microclimatic features, mostly water availability, due to surface morphologies (Fig. 1).

The abandoned surfaces of the active and historical quarries show metric to decametric-sized black biofilms, localized on subvertical planes on which water flows

from overhanging vegetated soils following rain events (Fig. 1a, b, c; Table 1ah, bh). *Gloeocapsa* sp. was microscopically recognized as the dominant component of the black biofilms in both sites (Fig. 1d). Other coccoid and filamentous cyanobacteria and dematiaceous fungi subordinately characterize the biofilms, green algae being also abundantly detected in the historical quarry. Geologist's hammer strokes unveiled in both sites the widespread occurrence beneath the epilithic black biofilm of endolithic lichen thalli having decimetric to metric size (Fig. 1e; see the next subchapter for thallus organization and penetration patterns). They discontinuously colonize the 25-years old humid surfaces of the active quarry (~ 20% of the surveyed surfaces), while quite continuously colonize the humid surfaces of the historical quarry (>90% of the surveyed surfaces). Molecular analyses on the endolithic thalli indicated that they belong to a non-monophyletic group including species of the genera *Polysporina*, *Sarcogyne* and *Acarospora* in the family Acarosporaceae (highest phylogenetic relationship with *Polysporina simplex*; phylogenetic analysis in Supplementary material SM1). Accordingly, *Sarcogyne*-like apothecia (*i.e.* with thin true exciple and paraphyses simple to sparsely branched) were locally observed in areas of the rock surface displaying cracks, substantially appearing as the only epilithic trace of lichen growth above the black biofilm (Fig. 1c, f). However, it is worth noting that the apothecium-morphology does not completely fulfil the descriptions of the *Sarcogyne* species reported on siliceous rocks in Europe (see *e.g.* Nimis and Martellos, 2008; Smith et al., 2009), as the apothecia display brown hypothecium (reported for *Sarcogyne clavus*), but a non-crenulate true exciple (reported for *Sarcogyne privigna*).

More xeric vertical surfaces of the two quarries were not covered by the black biofilm and displayed a more conspicuous epilithic lichen colonization (Table 1ax, bx). In the

active quarry, *Candelariella vitellina* was the dominant species (Fig. 1g), but apothecia of *Polysporina simplex* were also widely observed in lines following microcracks on the gneiss surface, *Sarcogyne*-like apothecia being instead rare. Higher lichen diversity characterized the 75-years old xeric surfaces of the historical quarry, with *C. vitellina* as one of the dominant species and a minor occurrence of *P. simplex* and of small, young thalli of *Xanthoparmelia* gr. *stenophylla*. In both the quarries, endolithic growth of lichen thalli was also detected beneath these epilithic components during the sampling work (Fig. 1h). A high diversity of dematiaceous meristematic fungi was detected in the historical quarry (Table 1bx).

The examined natural outcrops displayed microclimatic conditions similar to those of the xeric surfaces in the quarries. Lichen cover was higher than 95%. A mature xerophytic saxicolous community dominated by *X. gr. stenophylla* was observed. *C. vitellina* and *P. simplex* also occurred in this site as companions, while the occurrence of endolithic thalli and microbial biofilms was not prominent in the field and only rarely was observed in laboratory investigations (Table 1cx).

#### *Lichen growth within the Villarfocchiardo Gneiss*

The interior of the fresh control samples did not display any biological colonization (f1-f2; Fig. 2a). On the humid surfaces abandoned since 25 years, sparse hyphae penetrating down to  $0.5 \pm 0.2$  mm were discontinuously observed beneath the epilithic black biofilms where the endolithic lichen colonization did not occur (ah/i; Fig. 2b and 3a).

In both the active and historical quarries, the endolithic lichen component developed beneath the epilithic black biofilms as a  $300 \pm 100$   $\mu$ m thick thallus at approx.  $1.1 \pm 0.3$  mm from the rock surface, including a rather continuous upper algal layer (80-100



$\mu\text{m}$ ) and a lower medulla (100-150  $\mu\text{m}$ ) (ah/i and bh; Fig. 2c, e). A hyphal penetration component growing more than 2 mm in depth below the endolithic thalline layer was also observed (Fig. 3a).

The endolithic thallus developed in microcracks parallel to the rock surface, along the sides of which broken crystals were recognizable (Fig. 4a, b, c). Hyphal structures growing below the endolithic thallus were instead localized along the crystal boundaries, intracrystalline penetration being not observed. Entrapment of mineral fragments within the endolithic thallus was abundantly detected (Fig. 4e, f). A similar colonization pattern was observed beneath the abandoned xeric surfaces. The hyphal penetration component developing in continuity with the epilithic thalli of *C. vitellina* appeared subordinate in biomass to an endolithic lichen component, which, however, had a less continuous development and a significantly lower thickness ( $150 \pm 50 \mu\text{m}$ ) than that observed beneath the black biofilms (ax and bx; Fig. 2d, f). Moreover the hyphal penetration component developing below the endolithic thalline patches displayed a 2 mm penetration depth only in the historical quarry, while significantly lower values (1.3 mm) were measured beneath the 25-years old surfaces of the active quarry (Fig. 3a).

Hyphal penetration depths of approx. 2 mm were also observed beneath *C. vitellina* and *X. stenophylla* thalli on natural xeric outcrops, where the occurrence of an endolithic lichen component, although sporadically observed, was substantially negligible (cx/i-ii; Fig. 4d).

According to the different size of the endolithic thalli, the hyphal spread (*sensu* % of rock affected by the penetration of lichen structures) was significantly higher beneath the black biofilms than below the *Candelariella* epilithic thalli in both the quarry sites (Fig. 3b).



Chemical analyses were performed on alkali-feldspar and plagioclase crystals contacted by the endolithic thalli in both humid and xeric sites along the fissure borders or directly on entrapped fragments (Fig. 5). No significant differences with respect to control analyses performed on the same minerals in areas far from the rock surface were detected. In the case of the natural outcrops, the upper part of the rock was characterized by a reddish rind extended for several millimetres below the surface, beneath the epilithic lichen thalli: high Fe contents in EDS analyses of feldspars at that layer (Fe up to 11 wt%) suggest the diffuse occurrence of iron oxides (Fig. 4d).

### 3.3 Rock hardness

R-values of the fresh surfaces of Villarfocchiardo Gneiss (controls f1-f2) ranged around 50 and did not show significant differences between the tourmaline-rich variety and the other one, richer in mica, although this latter showed a slightly lower average value (Fig. 6). Similar values were also measured in the active quarry on the surfaces covered by black biofilms without associated endolithic lichens (ah/i) and on the xeric surfaces colonized by *C. vitellina* with associated thin endolithic thalli (ax). Differently, in both the quarry sites, the hardness of the gneiss was significantly lower than the fresh controls where black biofilms and thick endolithic lichen thalli co-occurred, R-values around 25 and 35 characterizing the historical (bh) and active quarries (ah/ii), respectively. Average values around 40 characterized the surfaces of the historical quarry colonized by *C. vitellina* with the associated endolithic component (bx) and also the natural outcrops colonized by *C. vitellina* (cx/i) and *X. stenophylla* (cx/ii) without associated endolithic thalli.

#### 4. Discussion

Endolithic lichens are recognized as a prominent feature of carbonate outcrops in the temperate regions, Schneider and colleagues (in Schneider and le Campion-Alsumard, 1999; Pohl and Schneider, 2002) having estimated in Central Europe they colonize 60% of available surfaces. Deterioration of carbonate rock by endolithic lichens has been mostly investigated with reference to their potential impact on the stone cultural heritage (Favero-Longo et al., 2009), but their contribution to the development of mesoscale weathering landforms was also recently demonstrated (McIlroy de la Rosa et al., 2012). In this paper, investigations on the microbial colonization of gneiss surfaces in quarry and natural sites first allowed us to highlight that colonization by endolithic lichens also deeply affects the hardness of siliceous lithotypes in temperate regions, exerting a significantly higher deterioration impact with respect to the associated epilithic lithobionts. In particular, a significant weakening effect of the endolithic thalli on humid rock surfaces (*i.e.* exposed to a long period of water flow after rain) is shown at the timescale of decades and related to physical rather than chemical processes.

##### *Complexity of the lithobiontic communities at the gneiss bedrock-atmosphere interface*

Our investigation characterizes endolithic lichens as remarkable deteriorogenic component in lithobiontic communities colonizing siliceous rocks in temperate areas, where the abundance of epilithic lichens and/or microbial crusts, absent or less prominent in extreme regions, may cause them to be overlooked. The examined endolithic colonization characterizes both humid and xeric rock surfaces, shared with other microorganisms according to the common richness of lithobiontic communities

(Gorbushina, 2007; Wierzbos et al., 2012), which often include co-dominant lichens and cyanobacteria (e.g. Wessels and Büdel, 1989; Porembski, 2007; de los Ríos et al., 2012). The molecular analyses on the endolithic thalli, associated to the microscopical observation of spatially related epilithic *Sarcogyne*-like apothecia, allow to assign them to a group of Acarosporaceae including common silicolous, pioneer, chasmoendolithic species of the non-monophyletic genera *Polysporina* and *Sarcogyne* (Crewe et al., 2006). It is worth noting that these described species do not completely fulfil the features of the observed specimens, but in this case the specific identification goes beyond the aims of the work, as the whole genera *Sarcogyne* and *Polysporina* are in need of a critical revision using molecular data to evaluate morphologies and to form natural genera (Knudsen et al., 2009). The high complexity of the relationships between the diversity of lichenized- (and non-lichenized) fungi inside a rock and that observed on the corresponding surfaces, not always resulting univocally related (Bjelland and Ekman, 2005), represents an alternative explanation to the observed incongruences.

In xeric conditions, the observed endolithic lichen is associated to pioneer, nitrophilous epilithic lichen species, according to the reported occurrence of *Sarcogyne* in nitrophilous communities on stone cultural heritage (e.g. on granite churches in NW Spain: Carballal et al., 2001). In humid conditions, it is associated to black biofilms including the most common cyanobacteria reported for lithophylic floras (Macedo et al., 2009), which are known to show an astonishing similarity in species from temperate to tropic regions (Büdel, 2001). Dematiaceous fungi, already reported on natural outcrops and stonework as free inhabitant (Onofri et al., 2014 with refs. therein) and in association with lichen thalli (Harutyunyan et al., 2008), also occur, while cyanolichens, which elsewhere dominate rock crusts on old surfaces

(e.g. on inselbergs; Büdel, 2001), are absent. Such complexity of the characterized lithobiontic communities indicates that the study case can be considered representative of interactions between lithobiontic communities and silicate lithotypes being significant for weathering dynamics in temperate natural environments, but also for the conservation of cultural heritage.

*Deterioration processes at the gneiss bedrock-atmosphere interface putatively related to the lithobiontic colonization*

Absence of chemical deterioration in the feldspars directly contacted by the endolithic lichen thalli indicates the scarce relevance of chemical deterioration processes in the examined lithotype-lichen species study case and/or in the considered colonization time-scale (not more than 25-75 years in the two quarries). In other studies, chemical deterioration of feldspars contacted by lichen species was detected (Prieto et al., 1994). However, species of the *Polysporina-Sarcogyne-Acarospora* group are known to not secrete secondary metabolites with chelating functions (e.g. Smith et al., 2009), potentially responsible of acidolysis or complexolysis of the contacted minerals. Moreover, oxalates, which characterize some *Sarcogyne* species, were not observed on the apothecia surface and when the endolithic thalli were scraped and spectroscopically examined (data not shown). The contribution of the observed endolithic thalli to the rock deterioration should be thus related to biogeophysical processes. With this regard, the direct measure of the force (see Wright and Armstrong, 2006) exerted by the hyphal penetration component of lichens within different colonized lithotypes still represents a future target in biodeterioration research. However, the occurrence of mineral fragments entrapped in the basal parts of thalli and among penetrating hyphae, and the hyphal

penetration itself, have been long considered markers of a lichen mechanical action supporting rock disaggregation (e.g. Fry, 1927; Ascaso and Wierzbos, 1995).

Accordingly, the occurrence of mineral fragments entrapped within the examined endolithic thalli confidently supports that their growth has an active role in the gneiss disaggregation. Nevertheless, a circular causality in the relationships between the existence of rock discontinuities/planes of weakness and the penetration of hyphal structures has generally to be taken into account (see the next sub-chapter).

The presence of epilithic lichens and other lithobionts was supposed to induce in granites the formation of microhabitats suitable to the endolithic colonization by algae and non-lichenized fungi (de los Ríos et al., 2002). Significant higher thickness of the endolithic thalli observed within the humid surfaces, with respect to those in the xeric ones, suggests that black-biofilms, together with the high water supply, may favour the(ir) expansion of the colonized discontinuities. Thalline expansion and contraction, exerting mechanical stress, is indeed a consequence of wetting and drying processes (Adamo and Violante, 2000). Because of the black colour, cyanobacterial biofilms are known to affect the temperatures of the colonized surfaces (Garty, 1990), a process which may be involved in modulating the strength of physical weathering dynamics (e.g. freezing-thawing cycles) involved in the early step of weakening of the surface rock layers. Similarly, rock blocks colonized by dark epilithic lichen thalli were shown to experience higher thermal gradients than bare blocks, likely increasing their susceptibility to breakdown (Carter and Viles, 2004). On the other hand, the intercrystalline hyphal penetration of epilithic lichens does not seem compatible with the development of the 100-300  $\mu\text{m}$  thick discontinuities occupied by the endolithic lichens, excluding that they act a main role in increasing the rock bioreceptivity to endoliths.

*Influence of petrographic features on the timescale of the endolithic lichen  
colonization*

Chasmoendolithic silicicolous lichen species were already reported to characterize early colonization steps on the surfaces of abandoned mines (Favero-Longo et al., 2006). The remarkable observation that the examined “young” surfaces (25-75 years) host wide endolithic thalli is in agreement with previous observations on endolithic communities on recently exposed carbonates: in relatively few years they developed to depths and volumes characterizing communities in rocks exposed for approx. one century, suggesting a quick initial colonization step until they reach an equilibrium carrying capacity that is then maintained (Hoppert et al., 2004; Walker and Pace, 2007). Processes starting the availability of the surface-parallel discontinuities which are quickly colonized by lichens can be only speculated, but it is likely that surfaces exposed/denuded by quarry activities, but also by natural processes as the glacial retreat or landslip events, can suffer mechanical stress developing bioreceptive planes of weakness. The development of the observed wide pluridecimetric thalli, rather than the inconspicuous ones commonly reported for lichen species with endolithic behaviour in siliceous rocks (e.g. Smith et al., 2009; McCarthy and Kantvilas, 2013) or the discontinuous patches observed in polar environments (de los Ríos et al. 2005a; Guglielmin et al., 2011), appears strictly related to the structural habit of the investigated lithotype. In all the investigated sites, the decimetric- to metric-wide planar discontinuities occupied by the thalli (1- mm below the rock surface) follow the orientation of the main rock foliation, which is parallel to the outcrop surface. This indicates the extent of planes of weakness favouring the development of discontinuities as a primary factor controlling the availability and dimension of microniches suitable for the (chasmo-)endolithic

colonization. Similarly, de los Ríos and colleagues (2005b) observed that the endolithic distribution of symbiont cells in Antarctic granites was related not only to external conditions, but also to physico-chemical properties of the substrate. Lower continuity in the planes of weakness in other lithotypes (e.g. just in the massively-structured granites) likely justifies the poor dimension frequently reported for endolithic silicicolous thalli. Moreover, in the gneiss, the fact that the discontinuity planes are parallel to the surface, allows the development of thalli having their algal component covered by a fixed thickness of rock (approx. 1 mm), allowing a light penetration compatible with photosynthetic requirements (300 W/m<sup>2</sup> were measured 1 mm below a granite surface; Hall et al., 2008).

#### *Lithobiontic influence on the gneiss hardness*

Rebound measures performed on the young humid surfaces of the gneiss quarries highlight that the occurrence of endolithic lichens (ah/ii and bh) is associated with a significant decrease of the rock hardness with respect to fresh surfaces (f1 and f2), a weakening process not observed where only epilithic cyanobacteria occur (ah/i). The decrease of the R-values with respect to the controls develops at the time-scale of decades (approx. -25% after 25 years; -60% after 75 years). Similarly, on the moraine of a valley glacier in Norway, the endolithic *Lecidea auriculata*, having a rather dissimilar thallus structure and penetration pattern with respect to the analyzed species (from top to bottom: 200 µm thick flakes of rocks; 250-500 µm thick thallus; 1-5 mm deep hyphal penetration; McCarroll and Viles, 1995), determined an approx. 30% decrease in the R-values of gneiss surfaces exposed from 20 years, while minor lowering was observed in those exposed from more decades (Matthews and Owen, 2008). A significant decrease in the hardness of diorites and

granodiorites was also calculated in the Antarctic peninsula on surfaces colonized by epilithic fruticose and foliose thalli (Guglielmin et al., 2012), although the different measuring approach (e.g. without removing the epilithic thalli) prevents a complete comparison of the results. On the other hand, Schmidt Hammer measurements even suggested a bioprotective role of epilithic lichens on the Cappadocian monuments made of tuff, the lichen coating likely modifying the presence of water inside the rocks and reducing the water-related physical decay (Garcia-Vallés et al., 2003). A protective effect of cryptoendolithic communities of cyanobacteria and fungi, estimated on the basis of rock hardness measurements, was instead related to a biologically driven case-hardening process (Viles and Goudie, 2004), frequently described in both cold and hot deserts (Guglielmin et al., 2011 with refs. therein). The fact that all these studies were performed on different lithotypes, colonized by different epilithic and endolithic lichen species, under different macro- and microclimatic conditions and on surfaces exposed for different times mostly prevents general comparisons. However, our results on quarry surfaces confirm previous results by Matthews and Owen (2008) on the exceptionally fast weakening of gneiss surfaces driven by endolithic lichens, extending their value from a periglacial environment in N-Europe to the temperate areas.

On the other hand, young and old xeric surfaces of quarries and natural outcrops, colonized by pioneer (ax, bx) and mature (cx) lichen communities, respectively, did not show significant lower values of rock hardness with respect to controls, even where thin endolithic thalli occurred. In the case of the young quarry surfaces, lower thickness of endolithic thalli on xeric surfaces than on humid ones suggests that different microclimatic conditions can determine at the time-scale of decades a different effectiveness of the biogeophysical cycles which regulate the expansion of



rock discontinuities as suitable endolithic microniche. In a circular relationship, more and less developed endolithic biomasses in humid and xeric sites, respectively, are likely to support more and less effective biogeophysical processes, thus accounting for the different decrease in the rock hardness. In the case of the old surfaces of natural outcrops, the endolithic component is extremely localized and subordinate to epilithic lichen species. This suggests a progressive decay of the mineral surface together with the endolithic thalli ( $\gamma$  species, yielding rapid weathering, *sensu* Höppert and Koenig, 2006), associated with an increasing effect of epilithic thalli ( $\kappa$  species, enhancing crust formation, *sensu* Höppert and Koenig, 2006) in the case-hardening of the surface, which likely prevents in the long-term the expansion and durability of the endolithic colonization. Accordingly, the case of the examined gneiss likely follows the pattern suggested by Viles (2012) for the lithobiontic effect at the decade-century time-scale, implying an initial denudation stage followed by stabilization. In this context, endolithic lichens result the pioneer lithobiontic component supporting weakening and denudation processes involved in the early micromorphological development of young rock surfaces, particularly on humid ones, while at the mature stage of the colonization they become subordinate to the epilithic component. This latter is likely involved in long-term hardening processes on dry surfaces, as suggested by the oxidation rind observed below mature thalli on the examined natural outcrops (see Guglielmin et al., 2011; Guglielmin, 2012; Mergelov et al., 2012), or is more related to external supply factors rather than to the substrate, as in the case of cyanolichens, elsewhere widely reported in mature rock crusts on periodically humid surfaces (see Büdel, 2001).

## Conclusions

Geomorphic roles of lithobiotic communities are nowadays progressively unveiled, with a particular focus on extreme environments (Viles, 2012). Here we highlight the surface weathering of gneissic siliceous rocks in temperate areas which is related to the endolithic colonization, and the consequent physical deterioration, by lichens. In extreme environments, endoliths, including lichens, often represent the only colonizers of siliceous rocks and thus received remarkable attention with regard to their colonization patterns and weathering activity (Wierzchos et al., 2012). Our results indicate that endolithic lichens may also represent a remarkable and biogeomorphologically active component of lithobiotic communities in temperate areas, although their occurrence can be hidden by rich epilithic biofilms including cyanobacteria, green algae, lichenized and non-lichenized fungi. In this context, the reported community complexity and the fact that the different components potentially exert different effects on the rock substrate highlight the importance of a deep characterization of lithobionts in biogeomorphological researches.

Microstructural features of the investigated gneiss support the chasmoendolithic growth (*i.e.* growth within fissures) of the lichen thalli, which in turn are likely to determine physical stress at the rock surface layer and its weakening, indicated by rebound measures. According to previous reports on periglacial environments (Matthews and Owen, 2008), the examined endolithic lichen colonization yields a significant decrease in the hardness of gneissic rocks exposed from few decades. This rapid biogeomorphological process, however, only affects the young gneiss surfaces (*i.e.* quarry surfaces exposed from 25 and 75 years) periodically exposed to a long period of water flow after rain, while it does not characterize the xeric quarry surfaces, where the endolithic growth is less pervasive, and the old xeric surfaces of

natural outcrops, where case-hardening processes have likely reduced the availability of planes of weakness suitable for the chasmoendolithic colonization. In conclusion, endolithic lichens are active biogeomorphological agents at the upper millimetric layer of siliceous rocks in temperate areas, their action being prominent on humid surfaces at the early decade-scaled stages of exposure (denudation stage, *sensu* Viles, 2012). Beyond the comprehension of natural dynamics of “microbial geomorphology”, these findings also support the evaluation of the lichen and microbial impact on the stone cultural heritage, which can be reasonably considered young rock surfaces exposed to lithobiontic colonization.

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## References

- Adamo P, Violante P. 2000. Weathering of rocks and neogenesis of minerals associated with lichen activity. *Applied Clay Science* **16**: 229-256.
- Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research* **25**: 3389-3402.
- Antony CP, Cockell CS, Shouche YS. 2012. Life in (and on) the rocks. *Journal of Biosciences* **37**: 3-11.
- Ascaso C, Wierzos J. 1995. Study of the biodeterioration zone between the lichen thallus and the substrate. *Cryptogamic Botany* **5**: 270-281.
- Barker WW, Banfield JF. 1996. Biologically versus inorganically mediated weathering reactions: relationships between minerals and extracellular microbial polymers in lithobiontic communities. *Chemical Geology* **132**: 55-69.
- Bellinger EG, Sigeo DC. 2010. *Freshwater algae*. Wiley-Blackwell: Chichester, UK.
- Bjelland T, Ekman S. 2005. Fungal diversity in rock beneath a crustose lichen as revealed by molecular markers. *Microbial Ecology* **49**: 598-603.
- Borghi A, d'Atri A, Martire L, Castelli D, Costa E, Dino G, Favero-Longo SE, Ferrando S, Gallo LM, Giardino M, Groppo C, Piervittori R, Rolfo F, Rossetti P, Vaggelli G. 2014. Fragments of the Western Alpine Chain as historic ornamental stone in Turin (Italy): enhancement of urban geological heritage through geotourism. *Geoheritage* **6**: 41-55.
- Büdel B. 2001. Diversity and ecology of biological crusts. *Progress in Botany* **63**: 386-404.

Büdel B, Weber B, Kühl M, Pfan­z H, Sültemeyer D, Wessels D. 2004. Reshaping of sandstone surfaces by cryptoendolithic cyanobacteria: bioalkalization causes chemical weathering in arid landscapes. *Geobiology* **2**: 261-268.

Caneva G, Lombardozzi V, Ceschin S, Casanova Municchia A, Salvadori O. 2014. Unusual differential erosion related to the presence of endolithic microorganisms (Martvili, Georgia). *Journal of Cultural Heritage* **15**: 538-545.

Carballal R, Paz-Bermúdez G, Sánchez-Biezma MJ, Prieto B. 2001. Lichen colonization of coastal churches in Galicia: biodeterioration implications. *International Biodeterioration & Biodegradation* **47**: 157-163.

Carter NEA, Viles HA. 2004. Lichen hotspots: raised rock temperatures beneath *Verrucaria nigrescens* on limestone. *Geomorphology* **62**: 1-16.

Casanova Municchia A, Percario Z, Caneva G. 2014. Detection of endolithic spatial distribution in marble stone. *Journal of Microscopy* **256**: 37-45.

Crewe AT, Purvis OW, Wedin M. 2006. Molecular phylogeny of *Acaroporaceae* (*Ascomycota*) with focus on the proposed genus *Polysporinopsis*. *Mycological Research* **110**: 521-526.

Cutler NA, Viles HA, Ahmad S, McCabe S, Smith BJ. 2013. Algal 'greening' and the conservation of stone heritage structure. *Science of the Total Environment* **442**: 152-164.

Davila AF, Gómez Silva B, de Los Ríos A, Ascaso C, Olivares H, McKay CP, Wierzchos J. 2008. Facilitation of endolithic microbial survival in the hyperarid core of the Atacama Desert by mineral deliquescence. *Journal of Geophysical Research: Biogeosciences* **113**: G1.

de los Ríos A, Wierzchos J, Ascaso C. 2002. Microhabitats and chemical microenvironments under saxicolous lichens growing on granite. *Microbial Ecology* **43**: 181-188.

de los Ríos A, Sancho LG, Grube M, Wierzchos J, Ascaso C. 2005a. Endolithic growth of two *Lecidea* lichens in granite from continental Antarctica detected by molecular and microscopy techniques. *New Phytologist* **161**: 181-190.

de los Ríos A, Wierzchos J, Sancho LG, Green TGA, Ascaso C. 2005b. Ecology of endolithic lichens colonizing granite in continental Antarctica. *Lichenologist* **37**: 383-395.

de los Ríos A, Pérez-Ortega S, Wierzchos J, Ascaso C. 2012. Differential effects of biocide treatments on saxicolous communities: Case study of the Segovia cathedral cloister (Spain). *International Biodeterioration & Biodegradation* **67**: 64-72.

Etienne S. 2002. The role of biological weathering in periglacial areas: a study of weathering rinds in south Iceland. *Geomorphology* **47**: 75-86.

Favero Longo SE, Castelli D, Salvadori O, Belluso E, Piervittori R. 2005.

Pedogenetic action of *Lecidea atrobrunnea*, *Rhizocarpon geographicum* gr. and *Sporastatia testudinea* on serpentinized ultramafic rocks in an alpine environment. *International Biodeterioration & Biodegradation* **56**: 17-27.

Favero-Longo SE, Siniscalco C, Piervittori R. 2006. Plant and lichen colonization in an asbestos mine: spontaneous bio-attenuation limiting fibre airborne dispersion. *Plant Biosystems* **140**: 1-17.

Favero-Longo SE, Borghi A, Tretiach M, Piervittori R. 2009. In vitro receptivity of carbonate rocks to endolithic lichen-forming aposymbionts. *Mycological Research* **113**: 1216-1227.

Favero-Longo SE, Gazzano C, Girlanda M, Castelli D, Tretiach M, Baiocchi C, Piervittori R. 2011. Physical and chemical deterioration of silicate and carbonate rocks by meristematic microcolonial fungi and endolithic lichens (Chaetothyriomycetidae). *Geomicrobiology Journal* **28**: 732-744.

Fort R, Alvarez de Buergo M, Perez-Monserrat EM. 2013. Non-destructive testing for the assessment of granite decay in heritage structures compared to quarry stone. *International Journal of Rock Mechanics and Mining Sciences* **61**: 296-305.

Fry EJ. 1927. The mechanical action of crustaceous lichens on substrata of shale, schist, gneiss, limestone, and obsidian. *Annals of Botany* **3**: 437-460.

Gadd GM, Rhee YJ, Stephenson K, Wei Z. 2012. Geomycology: metals, actinides and biominerals. *Environmental Microbiology Reports* **4**: 270-296.

Garcia-Vallès M, Topal T, Vendrell-Saz M. 2003. Lichenic growth as a factor in the physical deterioration or protection of Cappadocian monuments. *Environmental Geology* **43**: 776-781.

Gardes M, Bruns TD. 1993. ITS primers with enhanced specificity for basidiomycetes – Application to the identification of mycorrhizae and rusts. *Molecular Ecology* **2**: 113-118.

Garty J. 1990. Influence of epilithic microorganisms on the surface temperature of building walls. *Canadian Journal of Botany* **68**: 1349-1353.

Garvie LAJ, Knauth LP, Bungartz F, Klonowski S, Nash III TH. 2008. Life in extreme environments: survival strategy of the endolithic desert lichen *Verrucaria rubrocincta*. *Naturwissenschaften* **95**: 705-712.

Gaylarde CC, Gaylarde PM, Neilan BA. 2012. Endolithic phototrophs in built and natural stone. *Current Microbiology* **65**: 183-188.

Gazzano C, Favero-Longo SE, Matteucci E, Castelli D, Piervittori R. 2007.

Allestimento di una collezione licheno-petrografica presso l'erbario crittogamico di Torino (TO) per lo studio del biodeterioramento di rocce e materiali lapidei. In *Lo stato dell'arte 5: Atti del congresso nazionale IGIC* (Cremona, 11-13 ottobre 2007). Nardini: Firenze; 669-677.

Gazzano C, Favero-Longo SE, Matteucci E, Roccardi A, Piervittori R. 2009a. Index of Lichen Potential Biodeteriogenic Activity (LPBA): A tentative tool to evaluate the lichen impact on stonework. *International Biodeterioration & Biodegradation* **63**: 836-843.

Gazzano C, Favero-Longo SE, Matteucci E, Piervittori R. 2009b. Image analysis for measuring lichen colonization on and within stonework. *Lichenologist* **41**: 299-313.

Golubic S, Friedmann I, Schneider J. 1981. The lithobiontic ecological niche, with special reference to microorganisms. *Journal of Sedimentary Petrology* **51**: 475-478.

Gorbushina AA. 2007. Life on the rocks. *Environmental Microbiology* **9**: 1613-1631.

Goudie AS. 2006. The Schmidt Hammer in geomorphological research. *Progress in Physical Geography* **30**: 703-718.

Guglielmin M, Favero-Longo SE, Cannone N, Piervittori R, Strini A. 2011. Role of lichens in granite weathering in cold and arid environments of continental Antarctica. In *Ice-marginal and periglacial processes and sediments*, Martini IP, French HM, Pérez-Alberti A (eds.) Geological Society, London, Special Publications **354**: 195-203.

Guglielmin M. 2012. Advances in permafrost and periglacial research in Antarctica: A review. *Geomorphology* **155-156**: 1-6.



Guglielmin M, Worland R, Convey P, Cannone N. 2012. Schmidt Hammer studies in the maritime Antarctic: Application to dating Holocene deglaciation and estimating the effects of macrolichens on rock weathering. *Geomorphology* **155-156**: 34-44.

Hall K, Guglielmin M, Strini A. 2008. Weathering of granite in Antarctica: I. Light penetration into rock and implications for rock weathering and endolithic communities. *Earth Surface Processes and Landforms* **33**: 295-307.

Harutyunyan S, Muggia L, Grube M. 2008. Black fungi in lichens from seasonally arid habitats. *Studies in Mycology* **61**: 83-90.

Hoppert M, Flies C, Pohl W, Günzl, Schneider J. 2004. Colonization strategies of lithobiontic microorganisms on carbonate rocks. *Environmental Geology* **46**: 421-428.

Hoppert M, König S. 2006. The succession of biofilms on building stone and its possible impact on biogenic weathering. In *Heritage, weathering and conservation*, Fort R, Alvarez de Buergo M, Gomez-Heras M, Vazquez-Calvo C (eds.) Taylor & Francis, London; 311-315.

Knudsen K, Gökhan Halıcı M, Kocakaya M. 2009. *Sarcogyne magnispora* (Acarosporaceae), a new species in the *nivea* group from Turkey. *Mycotaxon* **107**: 413-417.

Komárek J, Anagnostidis K. 1986. Modern approach to the classification system of cyanophytes 2 – Chroococcales. *Archives of Hydrobiology Supplements* **73**: 157-226.

Macedo MF, Miller AZ, Dionísio A, Saiz-Jimenez C. 2009. Biodiversity of cyanobacteria and green algae on monuments in the Mediterranean Basin: an overview. *Microbiology* **155**: 3476-3490.

- Matthes-Sears U, Gerrath JA, Larson DW. 1997. Abundance, biomass, and productivity of endolithic and epilithic lower plants on the temperate-zone cliffs of the Niagara escarpment, Canada. *International Journal of Plant Sciences* **158**: 451-460.
- Matthews JA, Owen G. 2008. Endolithic lichens, rapid biological weathering and Schmidt Hammer R-values on recently exposed rock surfaces: Sorbreen Glacier Foreland, Jotunheimen, Norway. *Geografiska Annales* **90A**: 287-297.
- McCarroll D, Viles H. 1995. Rock-weathering by the lichen *Lecidea auriculata* in an arctic-alpine environment. *Earth Surface Processes and Landforms* **20**: 199-206.
- McCarthy PM, Kantvilas G. 2013. Two new species of *Sarcogyne* (lichenised Ascomycota: Acarosporaceae) from central and southern Australia. *Journal of the Adelaide Botanic Gardens* **26**: 15-21.
- Mergelov NS, Goryachkin SV, Shorkunov IG, Zazovskaya EP, Cherkinsky AE. 2012. Endolithic pedogenesis and rock varnish on massive crystalline rocks in East Antarctica. *Eurasian Soil Science* **45**: 901-917.
- McIlroy de la Rosa JP, Warke PA, Smith BJ. 2012. Microscale biopitting by the endolithic lichen *Verrucaria baldensis* and its proposed role in mesoscale solution basin development on limestone. *Earth Surface Processes and Landforms* **37**: 374-384.
- Neilan BA, Jacobs D, Blackall LL, Hawkins PR, Cox PT, Goodman AE. 1997. rRNA sequences and evolutionary relationships among toxic and nontoxic cyanobacteria of the genus *Microcystis*. *International Journal of Systematic Bacteriology* **47**: 693-697.
- Nimis PL, Martellos S. 2008. ITALIC – The information system in italian lichens. Version 4.0. University of Trieste, Dept. Of Biology, IN4.0/1 (<http://dbios.univ.trieste.it>; accessed on 26<sup>th</sup> June 2014).

Onofri S, Zucconi L, Isola D, Selbmann L. 2014. Rock-inhabiting fungi and their role in deterioration of stone monuments in the Mediterranean area. *Plant Biosystems* **148**: 384-391.

Pinna D, Salvadori O, Tretiach M. 1998. An anatomical investigation of calcicolous endolithic lichens from the Trieste karst (NE Italy). *Plant Biosystems* **132**: 183-195.

Pohl W, Schneider J. 2002. Impact of endolithic biofilms on carbonate rock surfaces. In *Natural stone, weathering phenomena, conservation strategies and case studies*, Siegesmund S, Weiss T, Vollbrecht A (eds.) Geological Society, London, Special Publications 205: 177-194.

Porembski S. 2007. Tropical inselbergs: habitat types, adaptive strategies and diversity patterns. *Revista Brasileira de Botanica* **30**: 579-586.

Prieto B, Silva B, Carballal R, de Silanes ME. 1994. Colonization by lichens of granite dolmens in Galicia (NW Spain). *International Biodeterioration & Biodegradation* **34**: 47-60.

Scheerer S, Ortega-Morales O, Gaylarde C. 2009. Microbial deterioration of stone monuments – An updated overview. *Advances in applied microbiology* **66**: 97-139.

Schneider J, le Campion-Alsumard T. 1999. Construction and destruction of carbonates by marine and freshwater cyanobacteria. *European Journal of Phycology* **34**: 417-426.

Smith CW, Aptroot A, Coppins BJ, Fletcher A, Gilbert OL, James PW, Wolseley PA. 2009. *The lichens of Great Britain and Ireland*. The British Lichen Society - The Natural History Museum: London.

Tretiach M, Pecchiari M 1995. Gas exchange rates and chlorophyll content of epi - and endolithic lichens from the Trieste Karst (NE Italy). *New Phytologist* **130**: 585-592.

Tretiach M, Favero-Longo SE, Crisafulli P, Gazzano C, Carbone F, Baiocchi C, Giovine M, Modenesi P, Rinino S, Chiapello M, Salvadori O, Piervittori R. 2008. How do endolithic lichens dissolve carbonates? In *Biology of lichens and bryophytes: lichenological abstracts*, Nash III TH, Seaward MRD (eds.). Miscellaneous Publication N. 2008 of the American Bryological and Lichenological Society and the International Association for Lichenology: Tempe, AZ; 72.

Viles H, Goudie A. 2004. Biofilms and case-hardening on sandstones from Al-Quwayra, Jordan. *Earth Surface Processes and Landforms* **29**: 1473-1485.

Viles HA, Naylor LA, Carter NEA, Chaput D. 2008. Biogeomorphological disturbance regimes: progress in linking ecological and geomorphological systems. *Earth Surface Processes and Landforms* **33**: 1419-1435.

Viles H, Goudie A, Grab S, Lalley J. 2011. The use of the Schmidt Hammer and Equotip for rock hardness assessment in geomorphology and heritage science: a comparative analysis. *Earth Surface Processes and Landforms* **36**: 320-333.

Viles HA. 2012. Microbial geomorphology: A neglected link between life and landscape. *Geomorphology* **157-158**: 6-16.

Walker JJ, Pace NR. 2007. Endolithic microbial ecosystems. *Annual Review in Microbiology* **61**: 331-347.

Weber B, Büdel B. 2011. Endoliths. In *Encyclopedia of Geobiology*, Reitner J, Thiel V (eds.). Springer: Berlin; 348-355.

Weber B, Scherr C, Bicker F, Friedl T, Büdel B. 2011. Respiration-induced weathering patterns of two endolithically growing lichens. *Geobiology* **9**: 34-43.

Wessels DCJ, Büdel B. 1989. A rock pool lichen community in Northern Transvaal, South Africa: composition and distribution patterns. *Lichenologist* **21**: 259-277.

Wierzchos J, Ascaso C. 1998. Mineralogical transformation of bioweathered granitic biotite, studied by HRTEM: evidence for a new pathway in lichen activity. *Clays and Clay Minerals* **46**: 446-452.

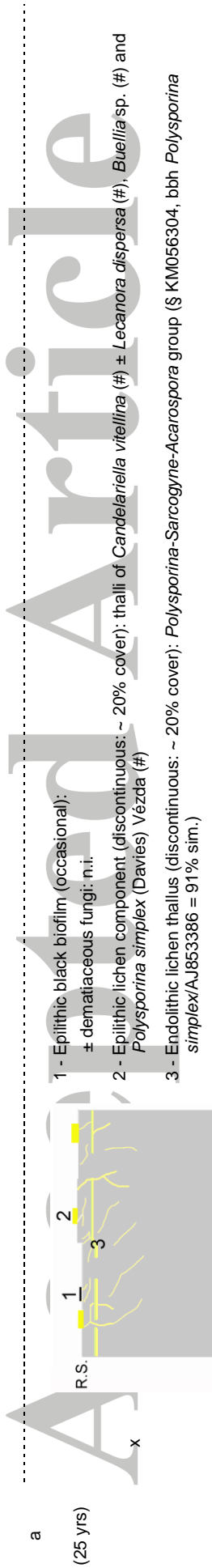
Wierzchos J, de los Ríos A, Ascaso C. 2012. Microorganisms in the desert rocks: the edge of life on Earth. *International Microbiology* **15**: 171-181.

Wirth V. 1995. *Die Flechten*. Baden-Württembergs. Ulmer: Stuttgart.

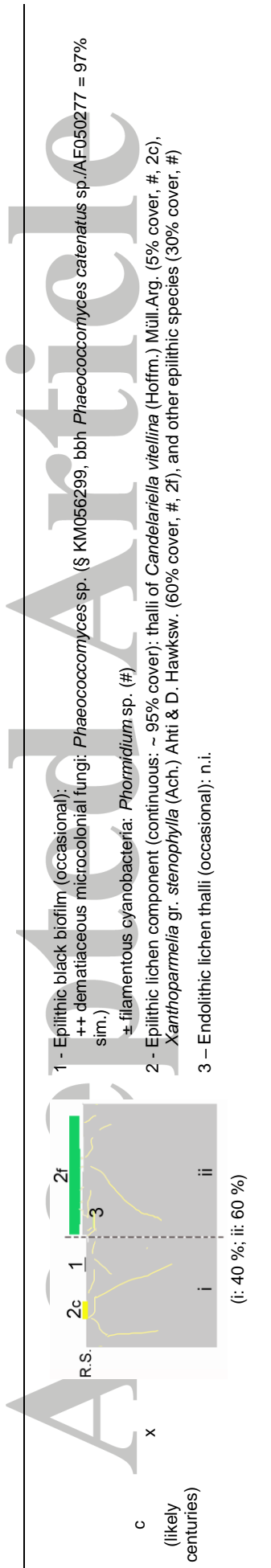
Wright CJ, Armstrong I. 2006. The application of atomic force microscopy force measurements to the characterisation of microbial surfaces. *Surface and Interface Analysis* **38**: 1419-1428.

Table 1. Lithobiontic communities on/within the Villarfocchiaro Gneiss (sites a, b, c and controls f1 and f2): dominant taxa on humid (h) and xeric (x) surfaces. Identifications based on microscopical observations (#) - supported by molecular biology analyses (§ accession number in GenBank of the obtained DNA sequence, best blast hit in GenBank: microorganism/accession number and sequence similarity %) - on isolated strains of microcolonial fungi, cyanobacteria and green algae and on field-sampled lichen thalli. Abundance of microbial and lichen components as cover %; relative abundance of identified species according to the following scale: ubiquitous (+++), abundant (++) , common (+), occasional (±), occasional (-). Hyphal penetration: yellowish lines; rock surface: R.S.

Site (surface age)	x/h	Spatial patterns of lithobiont communities	Microbial and lichen components of the lithobiont communities
f1-f2 (< 1 yr)	-		No epilithic and/or endolithic colonization on/within both the tourmaline-rich/fengite poor and tourmaline-poor/fengite rich gneiss varieties.
h			<p>1 - Epilithic black biofilm (continuous: ~ 100% cover):            +++ coccoid cyanobacteria: <i>Gloeocapsa</i> sp. (#)            + coccoid cyanobacteria: Chroococcales sp. pl. (#, <i>Chroococcidiopsis/Chlorogloea</i> sp.)            + filamentous cyanobacteria: Nostocales sp. (#, § KM056289, bbh <i>Anabaena</i> cfr. <i>cylindrical</i>/AJ293110 = 95% similarity)            ± filamentous cyanobacteria: <i>Phormidium</i> sp. (§ KM056290, bbh <i>Phormiodesmis</i> sp./KC311914 = 97% sim.)            ± dematiaceous fungi: cfr. <i>Rhinochlaetia</i> sp. (§ KM056298, bbh <i>Rhinochlaetia</i> sp./EU139137 = 89% sim.)</p> <p>2 - Epilithic lichen component (occasional): Sarcogyne-like apothecia (#, § KM056301, bbh <i>Polysporina simplex</i>/AJ853386 = 90% sim.)</p> <p>3 - Endolithic lichen thallus (discontinuous: ~ 20% cover): <i>Polysporina-Sarcogyne-Acarospora</i> group (§ KM056300, bbh <i>Polysporina simplex</i>/AJ853386 = 90% sim.)</p>



Site (surface age)	x/h	Spatial patterns of lithobiont communities	Microbial and lichen components of the lithobiont communities
<b>b</b> (75 yrs.)	h		<p>1 - Epilithic black biofilm (continuous; &gt; 90% cover): +++ coccoid cyanobacteria: <i>Gloeocapsa</i> sp. (#) + coccoid cyanobacteria: Chroococcales sp. pl. (#, <i>Chroococcus</i> sp. and cfr. <i>Chroococcidiopsis/Chlorogloea</i> sp.) + filamentous cyanobacteria: Nostocales sp. (#) + dematiaceous fungi: <i>Rhinocladia</i> sp. (§ KM056296, bbh <i>Rhinocladia</i> sp./EU139141 = 98% sim.) ± filamentous green algae: <i>Microspora</i> sp. (#, § KM056293, bbh <i>Microspora stagnorum</i>/EU434028 = 99% sim.)</p> <p>2 - Epilithic lichen component (occasional): <i>Sarcogyne</i>-like apothecia: (#, § KM056302, bbh <i>Polysporina simplex</i>/AJ853386 = 91% sim.)</p> <p>3 - Endolithic lichen thallus (continuous; &gt;90% cover): <i>Polysporina-Sarcogyne-Acarospora</i> group (§ KM056303, bbh <i>Polysporina simplex</i>/AJ853386 = 91% sim.)</p>
	x		<p>1 - Epilithic black biofilm (discontinuous; ~ 15% cover): ++ dematiaceous fungi: <i>Sarcinomyces</i> sp. (§ KM056297, bbh <i>Sarcinomyces</i> sp./JQ061171 = 99% sim.) <i>Capnobotryella</i> sp. (§ KM056294, bbh <i>Capnobotryella</i> sp./AJ972854 = 98% sim.) <i>Cladophialophora</i> sp. (§ KM056295, bbh <i>Cladophialophora chaetospiral</i>/EU035403 = 95% sim.) <i>Rhinocladia</i> sp. (§ = KM056296, bbh <i>Rhinocladia</i> sp./EU139141 = 98% sim.) + coccoid green algae: <i>Trebouxia</i> sp. (#, § KM056291, bbh <i>Trebouxia impressa</i>/Z21551 = 99% sim.) <i>Chlorella</i> sp. (#, § KM056292, bbh <i>Chlorella</i> sp./AB058305 = 100% sim.)</p> <p>2 - Epilithic lichen component (discontinuous; ~ 85% cover): thalli of <i>Candelariella vitellina</i> (Hoffm.) Müll.Arg. (#) ± <i>Polysporina simplex</i> (Davies) Vězda (#), young individuals of <i>Xanthoparmelia</i> gr. <i>stenophylla</i> (Ach.) Ahti &amp; D. Hawksw (#) and other epilithic species (#)</p> <p>3 - Endolithic lichen thallus (discontinuous; ~ 20% cover): <i>Polysporina-Sarcogyne-Acarospora</i> group (§ KM056303, bbh <i>Polysporina simplex</i>/AJ853386 = 91% sim.)</p>



- 1 - Epiithic black biofilm (occasional):  
 ++ dematiaceous microcolonial fungi: *Phaeococcomyces* sp. (§ KM056299, bbh *Phaeococcomyces catenatus* sp./AF050277 = 97% sim.)  
 ± filamentous cyanobacteria: *Phormidium* sp. (#)
- 2 - Epiithic lichen component (continuous: ~ 95% cover): thalli of *Candelariella vitellina* (Hoffm.) Müll.Arg. (5% cover, #, 2c), *Xanthoparmelia* gr. *stenophylla* (Ach.) Antl & D. Hawksw. (60% cover, #, 2f), and other epiithic species (30% cover, #)
- 3 - Endolithic lichen thalli (occasional): n.i.



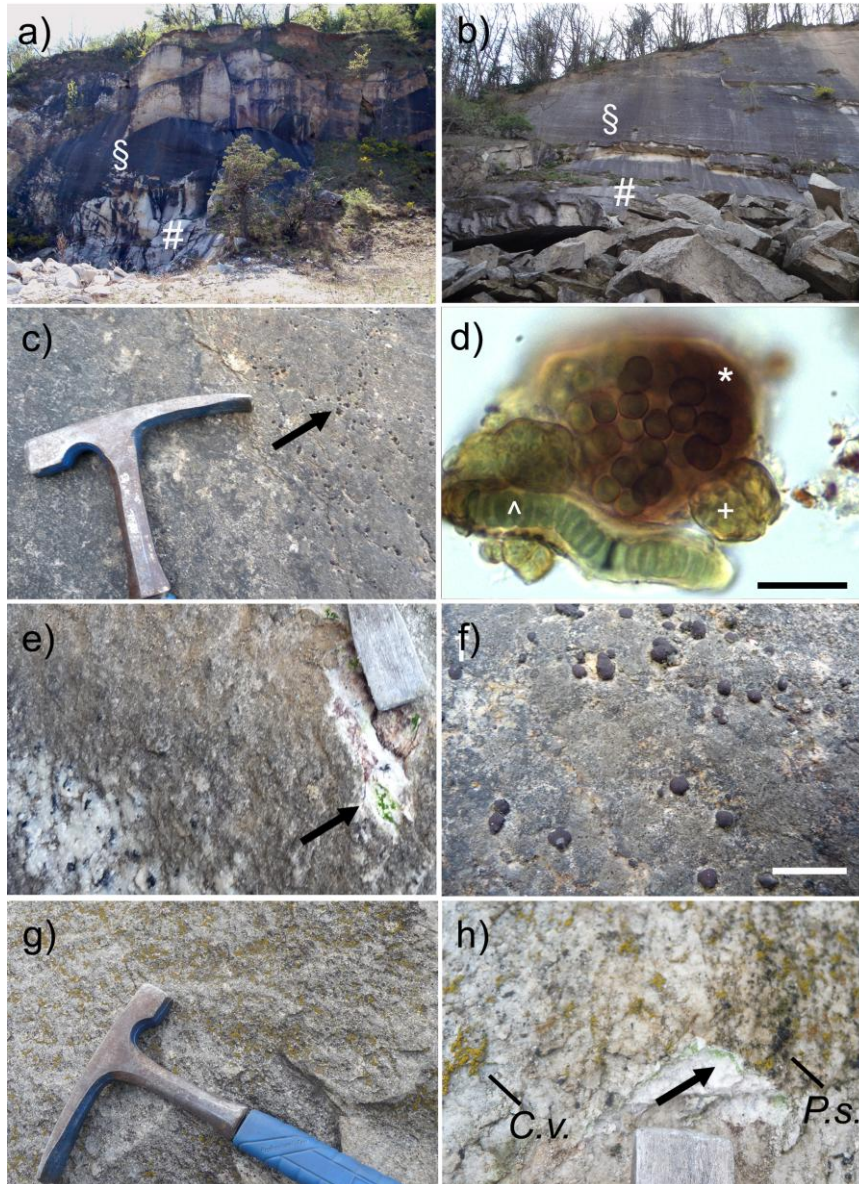


Fig. 1. Microbial colonization on the Villarfocchiardo Gneiss. On quarry surfaces abandoned since (a) 25 and (b) 75 years in the middle Susa Valley (NW Italy), epilithic black biofilms (c-f) develop where water periodically flows from overhanging soils (§), while more xeric surfaces (#) host pioneer communities of epilithic lichens (g-h). (c) Black biofilm with *Sarcogyne*-like apothecia (arrow) growing along microcracks. (d) *Gloeocapsa* sp. (\*) and other coccoid (+) and filamentous (^) cyanobacteria characterizing the black biofilms (scale bar: 20  $\mu$ m). (e) Endolithic lichen thallus (arrow) below the black biofilm, as unveiled by geologist's hammer strokes. (f) *Sarcogyne*-like apothecia (scale bar: 1 cm). (g) Xeric surface colonized by the epilithic lichen *Candelariella vitellina*. (h) Endolithic lichen thallus (arrow) developing beneath the xeric surfaces (*C. vitellina*, *C.v.*; *Polysporina simplex*, *P.s.*).



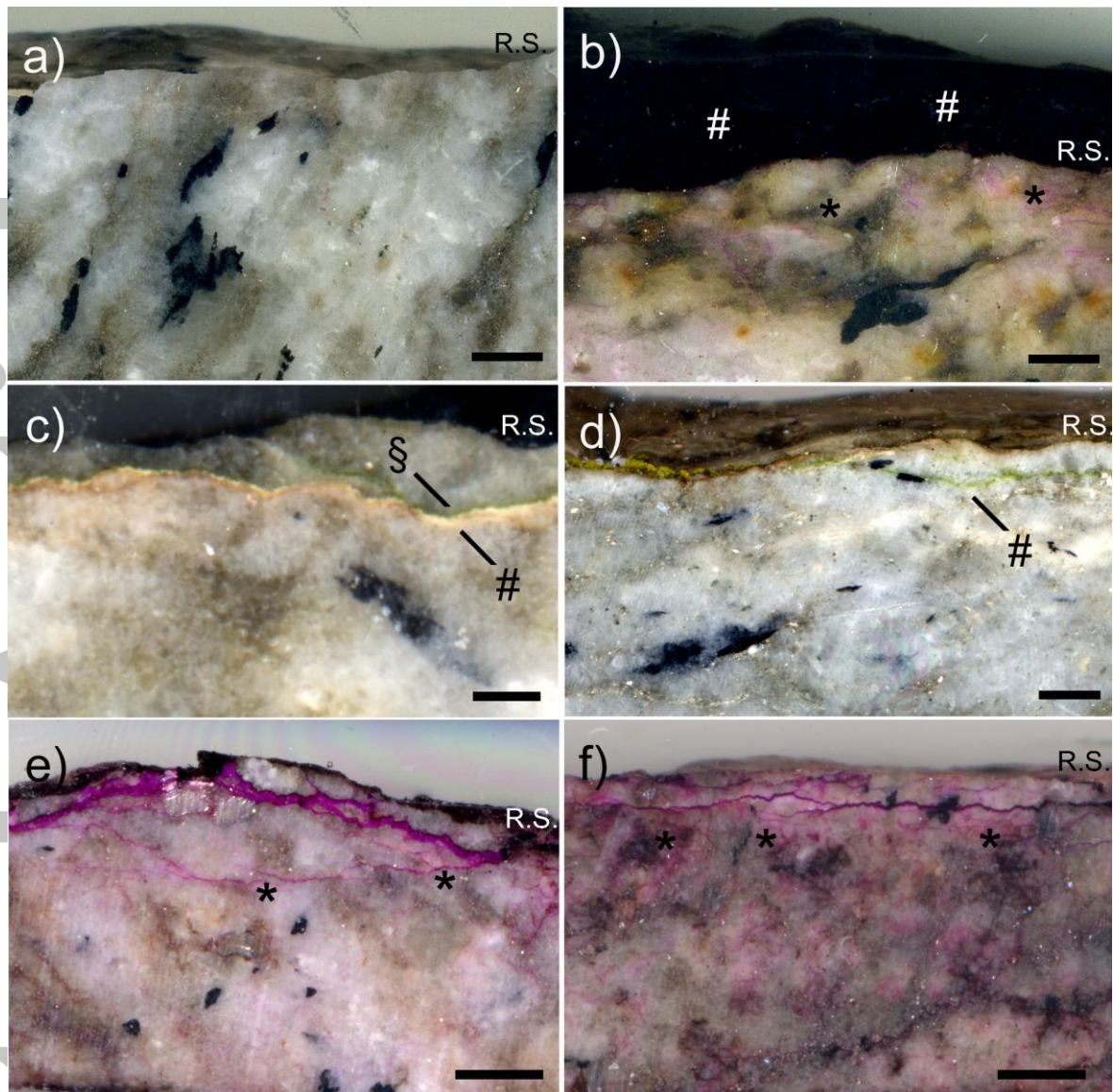


Fig. 2. Lithobiont growth within the Villarfocchiardo Gneiss (polished cross sections observed under reflected light microscopy). (a) Fresh control without lithobionts (f1; stained by PAS). (b) Black biofilm on humid surface (ah/i): occasional hyphal penetration (\*) below the epilithic biofilm (#) stained by PAS. (c, e) Endolithic thalli below the epilithic black biofilm on humid surfaces (ah/ii): (c), algal layer (§) and medulla (#); (e), hyphal penetration component stained by PAS (\*). (d, f) Endolithic thalli beneath the epilithic *Candelariella vitellina* on xeric surfaces (bx): (d), thin endolithic thallus (#); (f), hyphal penetration component stained by PAS (\*). Rock surface: R.S. Scale bars: 3 mm (a, b, e, f), 1 mm (c), 2 mm (d).

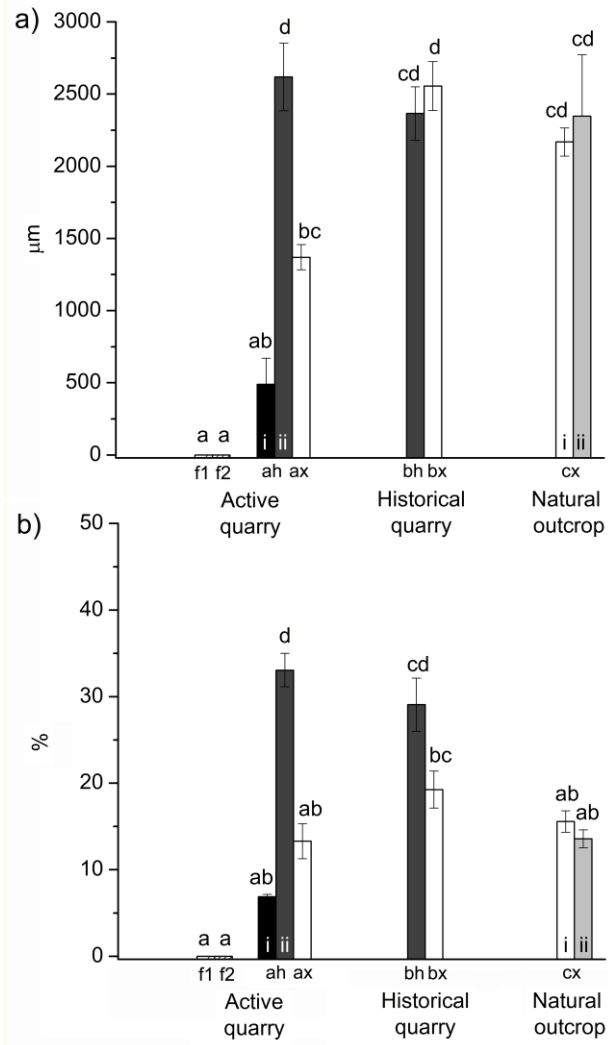


Fig. 3. Average ( $\pm$  standard error) depth (a;  $\mu\text{m}$ ) and spread (b; %) of the hyphal penetration component observed within the Villarfochiardo Gneiss. Active quarry: fresh control surfaces of the tourmaline rich (f1) and white-mica rich (f2) gneiss varieties; humid surfaces, abandoned since 25 years, colonized by an epilithic black biofilm (ah/i) and an epilithic black biofilm associated with endolithic lichen thalli (ah/ii); xeric surfaces, abandoned since 25 years, colonized by *Candelariella vitellina* and endolithic lichen thalli (ax). Historical quarry: humid surfaces, abandoned since 75 years, colonized by epilithic black biofilm associated with endolithic lichen thalli (bh); xeric surfaces, abandoned since 75 years, colonized by *Candelariella vitellina* and endolithic lichen thalli (bx). Natural outcrop: xeric surfaces colonized by *Candelariella vitellina* (cx/i) and *Xanthoparmelia* gr. *stenophylla* (cx/ii). The different lithobiontic communities are detailed in Table 1. According to Tukey's test, columns that do not share at least one letter are statistically different ( $p < 0.05$ ).



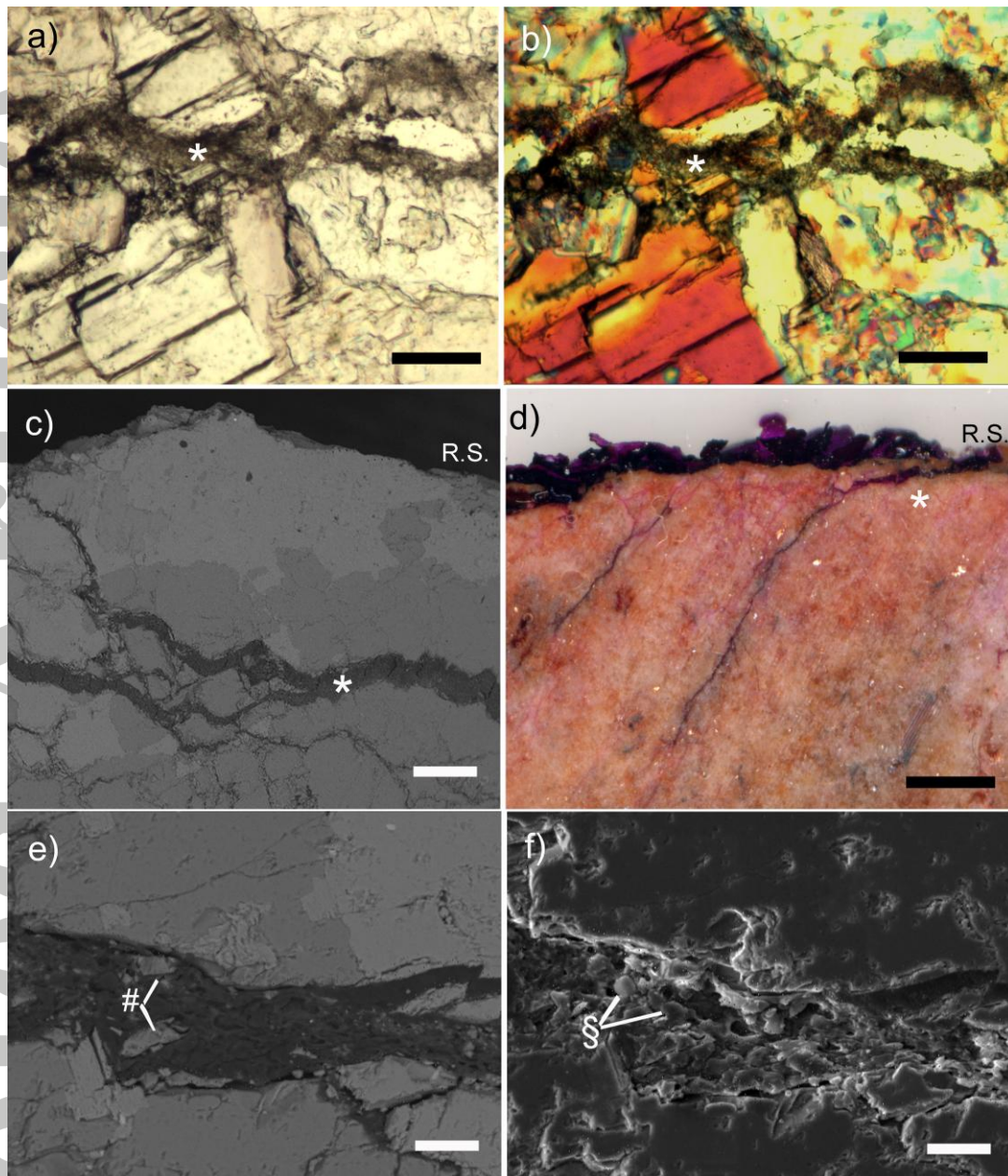


Fig. 4. Endolithic lichen growth in the Villarfochiardo Gneiss. Endolithic (\*) thallus developed in microcracks crossing broken crystals. Thin section observed under transmitted plane polarized (a) and cross polarized (b) light and by scanning electron microscope (c, e, backscattered images; f, secondary electron image). (d) Sporadic occurrence of an endolithic thallus (\*) on the natural outcrop, where the upper part of the bedrock is characterized by a diffuse iron-rich oxidation rind below epilithic thalli of *Xanthoparmelia* gr. *stenophylla* (polished cross sections stained by PAS and observed under reflected light microscopy). Rock surface: R.S. Scale bars: 500  $\mu$ m (a, b, c); 3.5 mm (d); 200  $\mu$ m (e, f).

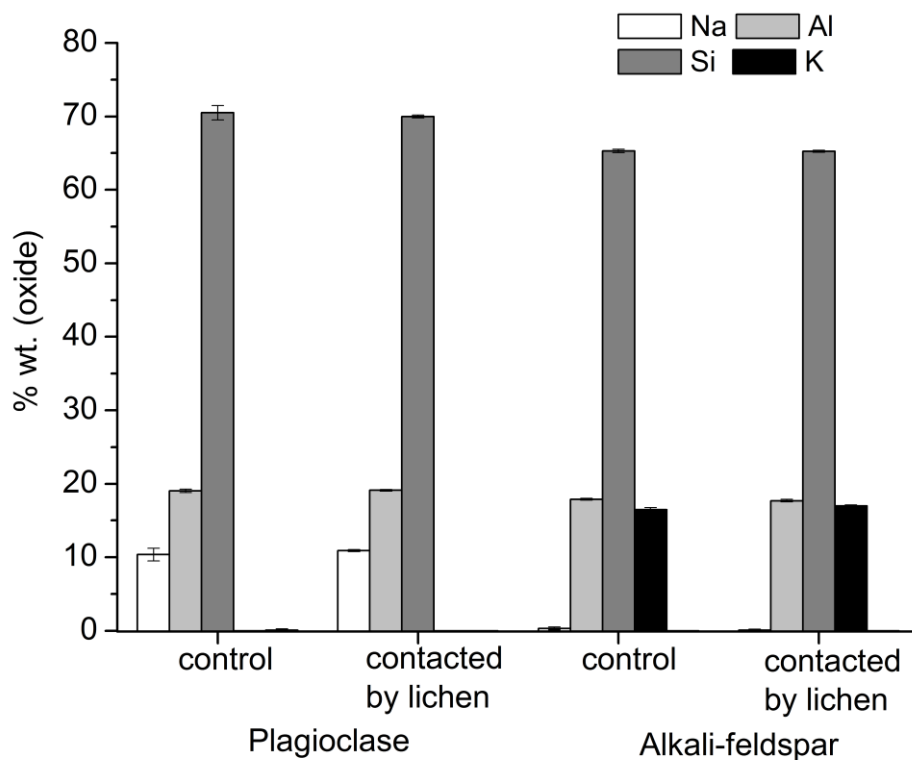


Fig. 5. Chemical analyses (av.  $\pm$  st. dev. of measures from the humid surfaces of both the active and historical quarries) of plagioclase and alkali feldspar contacted by the endolithic thalli with respect to control analyses performed far from the rock surface: no significant differences were detected.

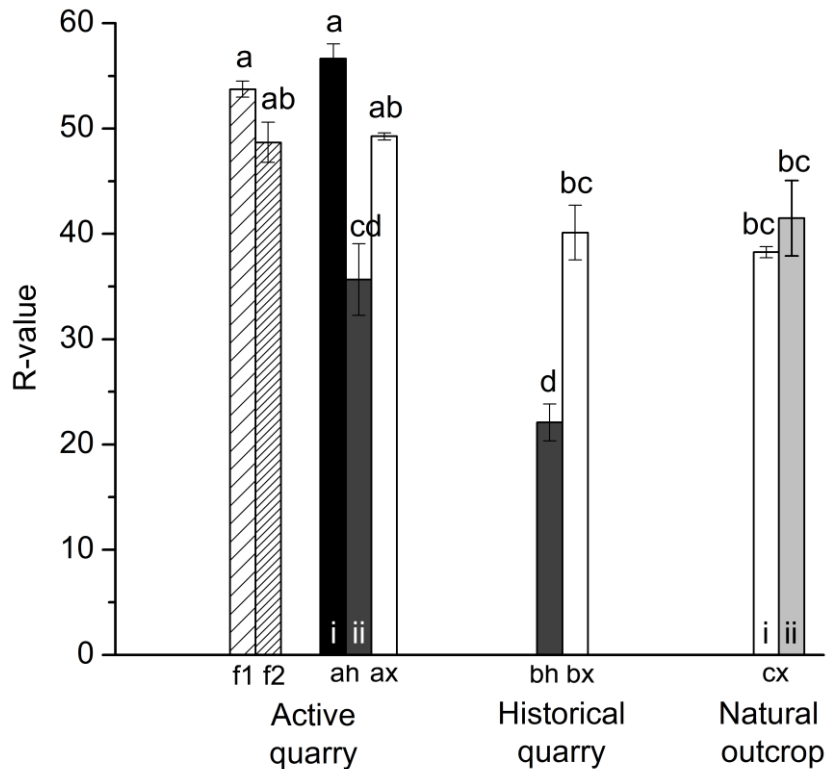


Fig. 6. Hardness of the Villarfochiardo Gneiss exposed to different microbial colonization. Active quarry: fresh control surfaces of the tourmaline rich (f1) and white-mica rich (f2) gneiss varieties; humid surfaces, abandoned since 25 years, colonized by an epilithic black biofilm (ah/i) and an epilithic black biofilm associated with endolithic lichen thalli (ah/ii); xeric surfaces, abandoned since 25 years, colonized by *Candelariella vitellina* and endolithic lichen thalli (ax). Historical quarry: humid surfaces, abandoned since 75 years, colonized by epilithic black biofilm associated with endolithic lichen thalli (bh); xeric surfaces, abandoned since 75 years, colonized by *Candelariella vitellina* and endolithic lichen thalli (bx). Natural outcrop: xeric surfaces colonized by *Candelariella vitellina* (cx/i) and *Xanthoparmelia gr. stenophylla* (cx/ii). The different lithobiontic communities are detailed in Table 1. According to Tukey's test, columns that do not share at least one letter are statistically different ( $p < 0.05$ ).