

Lichen impact on sandstone hardness is species-specific

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Abstract

The balance between lichen biodeterioration and bioprotection processes on stone surfaces depends on many variables and is crucial to understanding the role of lichens in biogeomorphology and their threat to stone heritage conservation. However, stones colonized by lichens have still been mostly examined in terms of affected volumes and physico-chemical modes of interactions, overlooking the overall effects on properties related to surface durability. In this study, the impact of lichen colonization patterns on Cortemilia sandstone was examined beneath thalli of three lichen species. Rock hardness, a proxy for rock durability, was measured at different depths from the surface using an Equotip hardness tester and compared to that of freshly cut surfaces and exposed surfaces uncolonized by lichens. Mineralogical analyses were performed by X-ray powder diffraction on rock beneath lichen colonization, in comparison with unweathered rock. Equotip analyses quantified a differential, species-specific decrease in stone hardness. This variability was related to differences in hyphal penetration patterns and, possibly, calcite (re-)precipitation. In particular, in the case of the species most impacting rock hardness, X-ray diffraction patterns of calcite showed a remarkable stability of crystallographic plane (01–12), known to be enhanced in the presence of organic chelants. These results confirm that decisions on lichen removal from stone surfaces should consider species-specific behaviour. Moreover, the innovative approach of measuring stone hardness variation in association with the analysis of biomineralization processes contributes to unveil the extension of the sphere of lichen interaction within the stone substrate beyond the limit of the hyphal penetration.

KEYWORDS

biodeterioration, biomineralization, lichens, sandstone, stone hardness

1 | INTRODUCTION

Lichens are self-sustaining ecosystems formed by the interaction of an exhabitant fungus (mycobiont) and an extracellular arrangement of one or more photosynthetic partners (photobiont[s]) and an indeterminate number of other microscopic organisms (Hawksworth & Grube, 2020). Saxicolous lichens represent a remarkable component of lithobiontic communities in many different environments, ranging from hot and cold deserts to tropical and temperate areas (Feuerer & Hawksworth, 2007; Wierchoś et al., 2012). The thallus of epilithic lichens develops above the surface of stones, which is penetrated by

mycobiont hyphal structures only, while endolithic lichens live embedded in the mineral substrate, including the photobiont partner, with only the fruiting structures protruding (Smith et al., 2009). Their colonization of rock surfaces contributes to weathering processes, supporting pedogenesis and geomorphic transformation (Asplund & Wardle, 2016; Jones, 1988), but it is also of relevance for conservation of outdoor stone cultural heritage (Seaward, 2015). Such proven influence on shaping environment, along with their ubiquity, resulted in the last few decades in a wide interest in characterizing and modeling processes of interaction between lichens and lithic substrates (McIlroy de la Rosa et al., 2013).

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Lichen colonization is generally associated with biodeterioration processes, which are carefully considered in the case of cultural heritage because of their negative implications for conservation (Favero-Longo & Viles, 2020). Adhesion and penetration of lichen structures, together with the release of acidic and chelating metabolites, have physical and chemical impacts, exerting mechanical stress and causing dissolution and/or neof ormation of minerals (Salvadori & Casanova-Municchia, 2016). However, at least in some cases, lichen bioprotection has been also documented, due to their umbrella-like action against other weathering forces (Carter & Viles, 2004; McIlroy de la Rosa et al., 2014) or the sealing of rock discontinuities due to biomineralization (Lee & Parsons, 1999).

In this regard, patterns of interactions depend on colonized lithology and lichen species involved, with their different growth form, penetration, and metabolome relating to different physical and chemical deteriorogenic activities (Gazzano et al., 2009). Moreover, for the same species and lithologies, the balance between biodeterioration and bioprotection may change depending on (micro-)environmental conditions (Carter & Viles, 2004). However, possibly due to this wide range of variables involved and, in the case of cultural heritage, the limitations in sampling, stones colonized by lichens have still been mostly examined in terms of affected volumes and physico-chemical modes of interactions, overlooking the overall effects on properties related to durability. Such information is needed to understand the contribution of saxicolous lichens to biogeomorphology (Viles, 2012, 2020) and their threats to heritage conservation, addressing choices of removing or preserving their colonization (Casanova-Municchia et al., 2018).

In the case of limestones, lichen colonization has been associated with counterposed patterns of surface hardening and porosity increase, with their balance depending on the limestone and the species involved (Morando et al., 2017). Lichen interactions with sandstones – a lithology easily targeted by microbial colonization in nature and widely used in cultural heritage – have been documented with regard to mycobiont penetration, metabolite release at the interface and changes in mineral composition (Ariño et al., 1995; Bjelland et al., 2002; Edwards et al., 2002), but investigations have mostly neglected the consequent influence on rock physico-mechanical properties and potential divergence from abiotic and other biotic weathering (Jain et al., 2009; Wang et al., 2020).

The ability to measure rock hardness, which is considered to be a proxy for weathering state and durability, developed greatly in the last decade (Kamh & Koltuk, 2020; Wilhelm et al., 2016). In particular, Equotip devices are widely recognized as valuable, portable instruments to measure hardness without the need for destructive sampling. Their application has been thoroughly studied and calibrated for surface deterioration on a range of materials including limestone and sandstone (Desarnaud et al., 2019; Kovler et al., 2018; Viles et al., 2011; Wang et al., 2020; Wilhelm et al., 2016). Uncertainty about possible limits to the reliability of measures taken in the proximity of edges, however, has discouraged the application of Equotip to evaluate hardness on rock cross-sections, although some studies have ruled out an edge effect, particularly in the case of sandstone (Desarnaud et al., 2019; Viles et al., 2011). Such a cross-sectional approach may be particularly fruitful in evaluating the effect on hardness of lithobiontic communities including lichens, for which measurements on the colonized surfaces, ‘from the top’, pose the challenge of removing the biomass without disturbing the underlying

substrate and make it impossible to assay the bioweathering effects at different depths.

This research aimed to examine the impact of lichens on the physico-mechanical and mineralogical properties of sandstones related to durability. In particular, we tested the hypotheses that (a) the hardness of sandstones can be reliably quantified in proximity to block edges, (b) the hardness of sandstones beneath lichen thalli is lower than that detected without lichen colonization, and that (c) sandstone hardness at different depths beneath lichens can vary depending on their growth forms, possibly related to structural and mineralogical features of the lichen–rock interface. The investigation was carried out on blocks of Cortemilia sandstone, a lithology of interest for cultural heritage in northwest Italy, colonized by epilithic, endolithic, and epi-endolithic lichens. Equotip analyses were performed at different depths on cross-sectioned blocks to evaluate variations in rock hardness. These data were associated with microscopic characterization of lichen penetration patterns and lichen–rock interface mineralogical profiles evaluated by X-ray powder diffraction (XRPD), to explore possible biogenic interactions responsible for stone modification.

2 | METHODOLOGY AND METHODS

2.1 | Stone and lichens

Cortemilia sandstone is quarried in southern Piedmont, Italy, and is employed extensively in local historical and modern buildings. It is a poorly sorted sandstone with very fine to medium sized grains, composed of quartz, feldspar, mica flakes, lithic grains of metamorphic rocks, and carbonate grains locally consisting of bioclasts (Gelati et al., 2010). It originated in middle-late Burdigalian (early Miocene) (Ghibaudo et al., 2019) from mechanical and chemical compaction, and displays a limited amount of carbonate cement.

Rock samples were collected from natural outcrops located at the top of a sunny and xeric hill with sparse trees (upstream from the road SP47, Cortemilia, northwest Italy; WSG84: N 44°33′31.8″–E 8°14′45.3″), choosing or detaching blocks ($n = 15$) of minimal volume of 1 dm³. Selected blocks were colonized by three crustose lichen species displaying continuous thalli, namely the epilithic *Verrucaria nigrescens* Pers., the intermediate epi-endolithic *Verrucaria muralis* Ach. and the endolithic *Protoblastenia incrustans* (DC.) J. Steiner. Surfaces colonized by scarcely penetrating microbial biofilms, mostly consisting of cyanobacteria, green algae, and subordinate black fungi, were considered as weathered controls not exposed to lichen colonization (Morando et al., 2017). Lichen identification and nomenclature follow Nimis (2016).

2.2 | Equotip hardness testing

Sandstone surface hardness was measured using a Proceq Equotip Piccolo 2 (Proceq, Schwerzenbach, Switzerland) equipped with DL probe, which is very similar to D probe in terms of impact body diameter (D: 3 mm; DL: 2.78 mm) and energy (D: 11.5 N mm; DL: 11.1 N mm), but shows a better correlation with open porosity. Moreover, due to its geometry, DL is less prone to contamination by dust and debris which is an issue of relevance when dealing with weathered substrates (Wilhelm et al., 2016).

Hardness was measured on cross-sectioned surfaces cut with a diamond saw perpendicularly to lichen thalli, independently of any sandstone stratification, to expose the lichen–rock interface and the underlying block core. Three out of the 15 blocks (namely F, J, and Z) were further cut to obtain unweathered, right-angled surfaces, to evaluate the consistency of hardness measures collected close to block edges (Figure 1a). Cross-sectioned blocks maintained a volume > 90 cm³ and a thickness > 5 cm, in accord with technical studies and calibrations reported for Equotip probe D and specifically carried out on sandstone materials (Corkum et al., 2018; Desarnaud et al., 2019). To avoid interference due to the presence of moisture contained in the rock (Desarnaud et al., 2019), the blocks were left to dry for 3 weeks at room temperature to allow them to reach equilibrium. For the Equotip hardness tests, the blocks were tightly held in a bench vice, with the section face-up, to ensure surface stability and reduce as much as possible vibrations during the test.

Equotip measurements were carried out (a) at 0.2–0.3 cm from the unweathered, right-angle surface realized in the block cores ($n = 3$ measurements on independent cross-sections); (b) at 0.2–0.3 cm beneath each thallus (*V. nigrescens*, $n = 12$; *V. muralis*, $n = 7$;

P. incrustans, $n = 8$) and biofilm control ($n = 12$), to evaluate rock hardness as close as possible to the surface; (c) at 1 cm beneath the same thalli and biofilms, to evaluate biomodifications affecting stone hardness at greater depth; (d) at the core of each block, established as the point of the cross-section as far as possible, and always at a minimum distance of 3 cm, from the original block weathered surfaces. Each measurement consisted of a series of 11 readings, which was the maximum number of readings obtainable beneath the thalli due to their limited dimensions and thus consistently adopted for all measurements. The readings were taken in a row, parallel to unweathered edges, lichen thalli and biofilms (a, b, and c) or sparsely in small areas of ~2.5 cm² (d) (Figure 1b,c).

For each series of readings from the surface layers (a, b, and c measurements), a relative surface hardness (RSH) value (*sensu* Aye et al., 2010, Kamh & Koltuk, 2020) was obtained, that is the ratio between the median value and the median value of the series of readings from the core of the same block (d measurement). The value, reported as a percentage (RSH%), represents the variation of stone hardness due to edge effects (a) and bioweathering (b and c) compared with unweathered stone hardness (d):

$$\text{RSH}\% = \left(\frac{\text{median}_{\text{surface (a,b,c)}}}{\text{median}_{\text{core (d)}}} \right) \times 100 - 100.$$

This adjustment avoided the possibility of incorrect interpretations which might be caused by variability in hardness between different blocks.

In order to validate the method of impacting close to an edge (0.2–0.3 cm) with Equotip Piccolo 2 (probe DL) in the case of Cortemilia sandstone, variation between hardness measurements close to unweathered edges and their respective block cores was analysed by Kruskal–Wallis non-parametric test (Wilhelm et al., 2016), using PAST 4.05 (Hammer et al., 2001). Relative standard deviation (RSD% = standard deviation/average × 100, equivalent to ‘coefficient of variation’) for each series of measurements, excluding the two extreme values to avoid potential outliers, was also considered.

Significant differences in RSH% between the three lichen species and the control biofilms were tested using non-parametric statistics Kruskal–Wallis and Mann–Whitney U Test, using PAST 4.05. Data are visualized as box-plots obtained using Origin (Pro), Version 2021 (OriginLab Corporation, Northampton, MA, USA).

2.3 | Reflected light microscopy

Verrucaria nigrescens and *V. muralis* were selected for microscopy observations and the subsequently described XRPD analysis (see next sub-section) as representative of different lichen impacts on stone hardness. Small fragments of the sandstone blocks from the lichen–rock interface were examined to characterize their hyphal penetration within the substrate. The fragments were cross-sectioned with a diamond saw, embedded in a polyester resin (R44 Politex-P fast, ICR S.p. A, Reggio Emilia, Italy) and polished with silicon carbide paper (up to P1200 grit). Hyphal penetration within the substrate was stained with periodic acid–Schiff’s (PAS) reagent method (Whitlatch & Johnson, 1974) and examined under reflected light microscopy (RLM) with an Olympus SZH10 microscope. For each section, the depth of hyphal penetration was quantified, and the thickness and density of

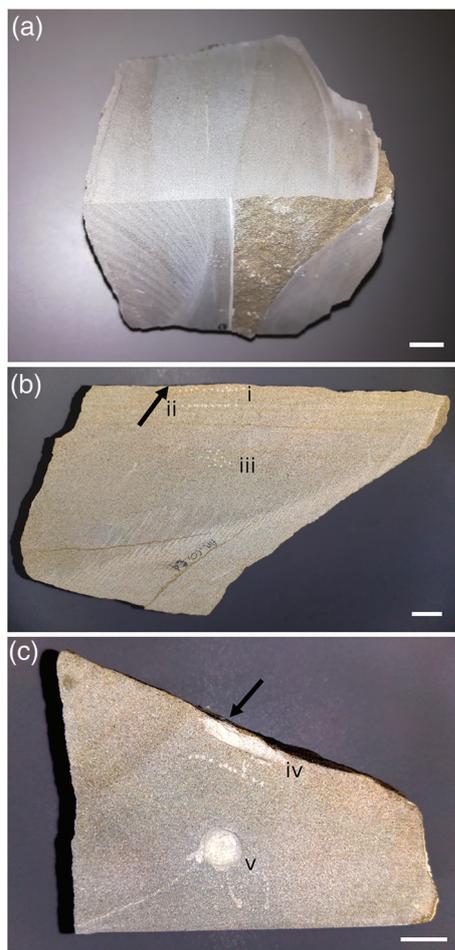


FIGURE 1 Cross-sectioned Cortemilia sandstone blocks with visible impacts and sampling marks. (A) Right-angled surface obtained by cutting a block to expose its core as a model of the unweathered surface. (B) Series of Equotip measurements beneath a lichen thallus, close to the surface ((i) 0.2–0.3 cm from the surface), at higher depth ((ii) 1.0 cm from the surface), and in the block core ((iii)). (C) Powder sampling for XRPD analysis beneath lichen thallus ((iv) 0.1–0.5 cm from the surface) and from the block core (v). Scale bar: 1 cm

hyphal structures were also observed. Depth data are reported as box-plots obtained using Origin (Pro), version 2021.

2.4 | X-ray powder diffraction

XRPD analyses investigated differences in mineralogical composition beneath *V. muralis* and *V. nigrescens*, compared with corresponding unweathered core samples. The contents of calcite and quartz, which are respectively prone and resistant minerals to (bio-)weathering induced-dissolution (Bjelland & Thorseth, 2002), were evaluated for potential relationships with sandstone hardness. Moreover, calcite was structurally examined, focusing on the contribution of different crystallographic planes which may be informative of different crystallization and stabilization conditions (Klug & Alexander, 1974; Leoni, 2019).

Analyses were carried out on four series of samples obtained from the sections of three of the blocks used for Equotip hardness tests, selecting blocks colonized by both *V. nigrescens* and *V. muralis* and sampling in the areas of hardness measurements.

For each series, three powder samples were collected from the rock immediately beneath *V. nigrescens* and *V. muralis* thalli (0.1–0.5 cm from the surface) and from the block core (at least 3 cm from the surface), respectively. Sampling was performed using a drill/grinder (Micromot 50/E, Proxxon, Niersdorf, Germany) equipped with diamond-coated grinding bits, and the powder was manually ground in an agate mortar. The XRPD patterns were acquired with a Miniflex 600 diffractometer (Rigaku, Tokyo, Japan) operating at 40 kV and 15 mA, using Cu-K α radiation ($\lambda = 1.5406$ Å), in the 2θ range of 3° to 70° , scan speed $2^\circ/\text{min}$ with step 0.02. Qualitative and semi-quantitative analyses were performed with SmartLab Studio II version 4.3 (Rigaku), using database PDF-4/Minerals 2020, to recognize main phases and peak heights referring to their different crystallographic planes.

For each XRPD pattern, the peak height (I) and the full-width at half maximum (FWHM) were calculated for the main peak (10–11) of

quartz and the main (10–14) and six less intense, namely (01–12), (11–20), (11–23), (20–22), (01–18), (11–26), peaks of calcite. The I/FWHM , which can be used as an indicator of crystallinity and related to the crystallite size by the Scherrer equation (Brindley & Brown, 1980), was calculated for each listed peak. The ratio between the I/FWHM of calcite and quartz was also calculated as follows:

$$R_{\text{Cc/Qz}} = \left(\frac{I_{\text{Cc}(10-14)}/\text{FWHM}_{\text{Cc}(10-14)}}{I_{\text{Qz}(10-11)}/\text{FWHM}_{\text{Qz}(10-11)}} \right)$$

The relationship between FWHM and material hardness is a standard approach in material science (Fu et al., 2018; Rai et al., 1999; Vashista & Paul, 2012) and was assessed using the $R_{\text{Cc/Qz}}$ ratio for *V. nigrescens*, *V. muralis* and block core samples by a Pearson correlation analysis (PAST 4.05). For each sample series, the I/FWHM percentage variation of each calcite peak obtained for *V. nigrescens* and *V. muralis* with respect to the core was calculated and visualized using a principal coordinate analysis (PCoA) plot (symmetric scaling, centring samples by samples, centring species by species, performed using CANOCO 4.5; Ter Braak & Smilauer, 2002).

3 | RESULTS

3.1 | Consistency of close-to-edge Equotip impacts on Cortemilia sandstone

Three independent series of measurements from block cores and unweathered right-angled surfaces were collected to validate the method of impacting with Equotip device close to the edge (0.2–0.3 cm) in the case of Cortemilia sandstone sections.

Each block, as expected for sedimentary rocks, was characterized by a different median hardness value (e.g., variation up to 10.6% between medians). Within each block however, $\text{RSD}_{\%}$ of both core and edge series of readings was low (in the range 1.2–3.5%). Median rock hardness measured close to the edges was lower than that measured in the core, but the differences were non-significant and lower than 1.2% (Table 1).

3.2 | Rock hardness variation beneath lichens

Hardness variation measured at two depths beneath lichens and the biofilm control ($\text{RSH}_{\%}$) indicated a softening of between -1.8% and -17.6% with respect to the core (Figure 2). $\text{RSH}_{\%}$ differed significantly beneath the different lichen species. In particular, at 0.2–0.3 cm, *V. nigrescens* and *P. incrustans* showed similarly high negative $\text{RSH}_{\%}$ (approximately -17.6%), while *V. muralis* displayed a significantly lower value (-2.4%). Biofilm control showed intermediate $\text{RSH}_{\%}$ and a wider range of variation, but the median was closer to *V. muralis*.

At higher depth (1 cm), $\text{RSH}_{\%}$ was generally lower, and was always lower than closer to the surface (0.2–0.3 cm) for the same species, with the exception of *V. muralis* (-4.2%). In particular, $\text{RSH}_{\%}$ was particularly low for biofilm control (-1.8%), while higher for all lichen species, with more evident hardness variation for *V. nigrescens* (-10.7%) and *P. incrustans* (-8.7%).

TABLE 1 Stone hardness variation measured on three Cortemilia sandstone blocks under fresh, unweathered surfaces, to validate the method of impacting close to the edge (0.2–0.3 cm) of the stone with Equotip piccolo 2 (DL probe). Percentage relative stone hardness ($\text{RSH}_{\%}$) values represent hardness variation measured as ratio between median edge hardness and core hardness, and is always negative and inferior to 1.2%. Average \pm standard deviation and percentage relative standard deviation ($\text{RSD}_{\%}$) are shown as informative measures of variance

	Block F	Block J	Block Z
Core median (Leeb units)	722	683	764
Fresh edge median (Leeb units)	717	681	755
$\text{RSH}_{\%}$	-0.69%	-0.29%	-1.18%
Core average (Leeb units)	725.6	676.0	764.1
Core standard deviation	13.10	20.59	9.46
Core $\text{RSD}_{\%}$	1.8%	3.0%	1.2%
Fresh edge average (Leeb units)	723.1	683.5	752.8
Fresh edge standard deviation	13.60	24.25	10.66
Fresh edge $\text{RSD}_{\%}$	1.9%	3.5%	1.4%

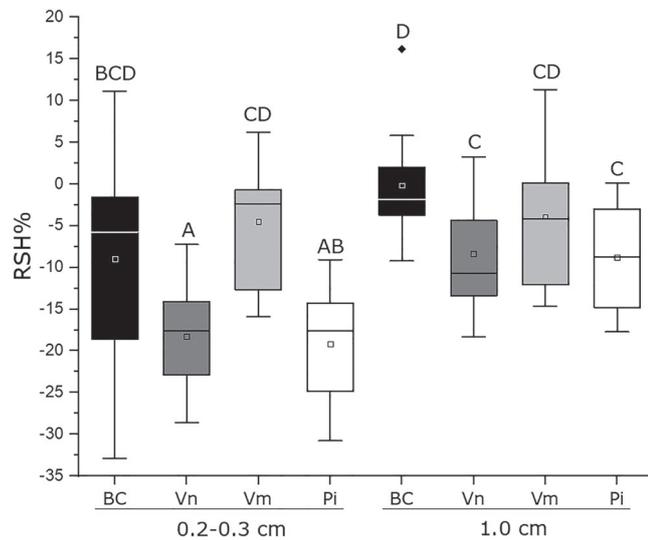


FIGURE 2 Relative stone hardness variation (RSH%) beneath lichens (*Verrucaria nigrescens*, Vn; *V. muralis*, Vm; *Protoblastenia incrustans*, Pi) and biofilm control (BC), at different distances from the surface (0.2–0.3 cm, on the left; 1.0 cm, on the right). Box-plots which do not share at least one letter are statistically different (Kruskal–Wallis with Mann–Whitney U *post hoc* test; $P < 0.05$)

3.3 | Hyphal penetration and mineralogical characterization at the lichen–rock interface

PAS stained cross-sections observed under RLM showed hyphal penetration within Cortemilia sandstone beneath the structurally different *V. nigrescens* and *V. muralis* thalli, but with different patterns and depths (Figure 3).

Verrucaria nigrescens showed a continuous crustose thallus with typical epilithic growth (Figure 3b), that is, with thallus completely above stone surface including photobiont layer and reproductive structures (perithecia). The mycobiont extensively penetrated within the stone down to 1.0–1.5 mm, with hyphal network exploiting intergranular porosity and locally organized as thick bundles (up to 50 μm). Sporadic, thin hyphae were occasionally observed at greater depths. The continuous, crustose thallus of the epi-endolithic *V. muralis* was poorly developed above the surface, showing photobiont clusters aligned at the rock surface and perithecia partially immersed in the substrate (Figure 3c). Its mycobiont penetrated extensively down to 1.2–1.3 mm and occasionally to greater depths, as observed for *V. nigrescens*, but with less dense and thinner hyphae, only rarely organized as bundles. In a couple of cases, the early 200–300 μm beneath the algal layer appeared quite free of hyphae and seemingly more compact, while mycobiont diffusely penetrated in the millimetre below.

XRPD analyses displayed similar $R_{\text{Cc}/\text{Qz}}$ values for all the samples, ranging between 0.33 and 0.72, with the exception of two core samples with $R_{\text{Cc}/\text{Qz}}$ higher than 1.75. No significant differences between *V. nigrescens* and *V. muralis* were observed. The relationship among $R_{\text{Cc}/\text{Qz}}$ and stone hardness is shown in Figure 4(a). A slight, non-significant, negative correlation was observed for both the species ($R = -0.2$; $P \geq 0.05$).

The PCoA (Figure 4b) extracted three components which explained 100% of total variance and ordinated samples collected beneath lichens on the basis of the percentage variation of I/FWHM

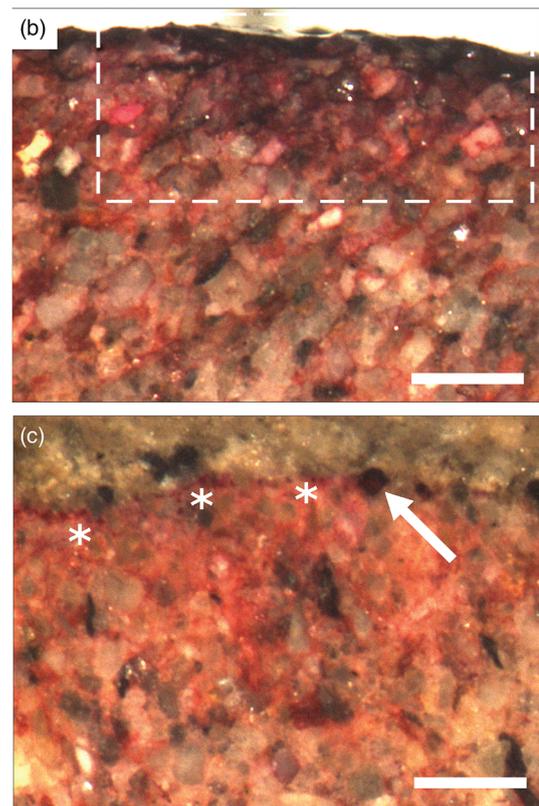
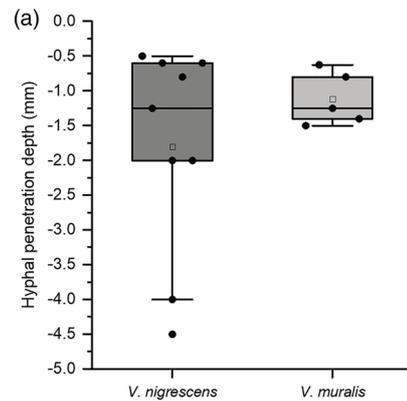


FIGURE 3 Penetration depth of *Verrucaria nigrescens* and *V. muralis* fungal component within stone surface. (A) Difference among *V. nigrescens* and *V. muralis* penetration depth. (B) *Verrucaria nigrescens* penetration patterns, with evident massive penetration of mycobiont hyphae red-stained by PAS coloration (within the dashed line). (C) *Verrucaria muralis* penetration patterns, with algal clusters (asterisks) and perithecia (arrows) partially immersed in stone. Scale bars: 500 μm

ratio of each calcite peak with respect to the relative core sample. The first axis (69.9% of total variance) displayed its highest positive correlation with (01–12) crystallographic plane and with samples collected beneath *V. nigrescens* showing lowest hardness values. Oppositely, samples with highest negative correlation with (01–12) showed the highest hardness.

4 | DISCUSSION

Analysis of lichen–rock interaction patterns may not fully clarify the balance between lichen bioweathering and bioprotection, particularly

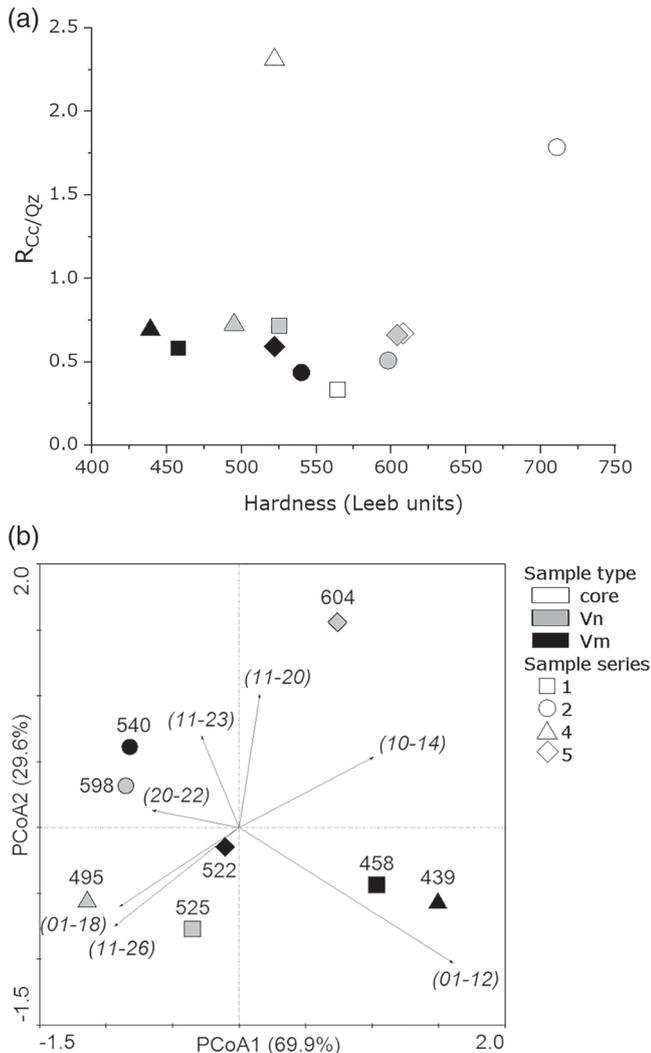


FIGURE 4 XRPD analyses. (A) Correlation of $R_{Cc/Qz}$ with stone hardness measured with Equotip for each sample. (B) Ordination of samples on the basis of the percentage variation of I//FWHM ratio of each calcite peak referred to a crystallographic plane (PCoA vectors) with respect to the relative core sample. Median hardness (Leeb units) is annotated for each sample

at the species-specific level, which determines their biogeomorphological role (McIlroy de la Rosa et al., 2013) and should contribute to decisions about whether to remove or preserve lichens on heritage surfaces (Casanova-Municchia et al., 2018). Measurements of rock hardness, as a proxy of surface durability versus erodibility (Wilhelm et al., 2016), have recently been applied to studies of sandstone weathering, encompassing lichen colonization as a general phenomenon rather than focusing on the complexity of lichen-rock interaction and species-specific patterns (Kamh & Koltuk, 2020). In our investigation, we proved the efficacy of a protocol of hardness measurements at the lichen-rock interface, particularly verifying the hypothesis of (a) a non-significant influence of measuring in proximity to block edges for the examined sandstone. With such an approach, we verified the hypotheses of (b) a significant hardness variation at different depths beneath lichen thalli with respect to a biofilm control, and of (c) the differential hardness variation as a species-specific phenomenon, which cannot be directly related to epilithic or endolithic growth forms. In the following sub-sections, we discuss the adopted

method to measure hardness variation with respect to most recent protocols calibrated for analyses of sandstone weathering, also taking into consideration limitations due to sampled material, variability in sandstones, and lichen thalli dimensions (next sub-section). Thereafter, we disentangle the patterns and species-specificity of lichen impact on a calcareous sandstone hardness (see later), and we address insights on the relationship between species-specific physico-chemical modes of lichen interaction, that is, hyphal penetration and biomineralization, and their effect on durability (see later).

4.1 | Suitability of measuring hardness along sandstone cross-sections

The method of measuring hardness on cross-sections of lichen colonized sandstone, including measurements close to block edges, is here proposed following previous protocols applied on fresh cut, unweathered sandstones (Desarnaud et al., 2019) and on the surface of lichen colonized rocks (e.g., limestone, Morando et al., 2017; sandstone, Kamh & Koltuk, 2020). Such previous protocols involving Equotip readings directly on colonized surfaces, however, reflected a cushion-like effect of the lichen biomass when thalli were not preliminarily removed. Alternatively, they required careful removal of a thallus (Morando et al., 2017), which for some lithologies, as sandstone, cannot be performed without affecting the substrate. Such limitations are overcome with our implemented method of collecting measurements directly from beneath lichen thalli or other lithobionts. This confers advantages related to the possibility of obtaining innovative information of biological influence on stone properties at different depths, including the first millimetres from stone-atmosphere interface. In particular, we showed that 0.2–0.3 cm from edges obtained by sectioning blocks is a suitable distance for the Equotip probe geometry, which still ensures a reliable measurement not significantly different from (almost equivalent to) that collected at the block core. Similarly, Viles et al. (2011) did not observe any edge-effect for Equotip applications on sandstones, although these have been detected for other lithologies, such as granite, concrete, and limestone (Coombes et al., 2013), suggesting the necessity of validating the cross-section approach on each lithology of interest. Sampling of different sandstone blocks in a natural environment implied issues related to a high variability due to different sedimentary layers, orientation, aspect, time of surface exposure, and other uncontrolled factors (e.g., Yun et al., 2013). Moreover, Equotip measurements on different blocks were not always taken perpendicularly to sedimentary layers (Desarnaud et al., 2019), but depending on the surface colonized by lichens and, consequently, its cross-section orientation. To balance out this variability, measurements were normalized to the core of each block, used as internal comparison. It was indeed not possible to obtain measurements of unweathered rock at the same 0.2–0.3 cm distance from the surface due to general and variable surface weathering independent of lichens.

4.2 | Stone hardness variation beneath lichens

Hardness variation – with respect to the core – beneath lichens and the biofilm control always showed higher negative values

(from -2 to -17%) than that detected beneath unweathered, right-angle edges ($< -1.2\%$). Accordingly, the closeness to the atmosphere-rock interface and the consequent exposure to biotic and abiotic weathering agents (Gorbushina & Broughton, 2009), rather than the geometry of the surfaces related to cross-sectioning, accounts for hardness variation. More remarkably, the highest hardness variation is detected beneath two of the assayed lichen species, confirming and quantifying a prominent role of lichens in rock deterioration with respect to other lithobionts (Morando et al., 2017; Salvadori & Casanova-Municchia, 2016; St. Clair & Seaward, 2004).

The crustose thalli of the investigated lichen species share the absence of secreted compounds, as oxalic acid and acidic and/or chelating secondary metabolites known for their deteriorogenic activity, while they are characterized by a different structural organization (Nimis, 2016). Noteworthy, hardness variation was similar beneath the epilithic *V. nigrescens* and the endolithic *P. incrustans*, rejecting the hypothesized higher biodeterioration by endolithic lichens due to their life completely embedded in the substrate (Caneva et al., 2008), but also their direct correlation with bioprotective effects (Gadd & Dyer, 2017). In detail, the hardness variation beneath these lichens is similarly high at 0.2–0.3 cm (-17.6%) and still remarkable (approximately -10%) at 1 cm, suggesting an equivalent impact for the two species on the investigated sandstone.

The detectable hardness variation at 1 cm beneath lichens is particularly remarkable with respect to the null variation driven by biofilm control with respect to the unweathered core, highlighting the deep impact of certain lichen species. Instead, at 0.2–0.3 cm, some negative hardness variation was also detected beneath the biofilm control (-6%), with higher variability possibly related to the heterogeneous biofilm composition on the different blocks. Nevertheless, a general influence of surface processes, including abiotic weathering, cannot be ruled out, as they can also significantly impact sandstone hardness (Kamh & Koltuk, 2020), but their effect is more superficial than that induced by lichens.

In a very different manner, a significantly lower variation in hardness was detected beneath the epi-endolithic *V. muralis*. This was remarkably significant at 0.2–0.3 cm depth, while the variation slightly increased and reached values more similar to those of the other species at 1 cm depth. This phenomenon may suggest not a simple lower impact of *V. muralis* on stone hardness, but even some form of hardening process, which likely compensate, at least in part, stone softening caused by hyphal penetration (see later). Accordingly, hardening processes were shown for some lichen species on limestone, and in the case of Botticino limestone the epi-endolithic *Xanthocarpia ochracea* was associated with unmodified hardness with respect to fresh rock where *V. nigrescens* determined hardness lowering (Morando et al., 2017).

4.3 | Insights on the basis of species-specific lichen impact

Different patterns of hardness variation were evaluated by comparing aspects of physico-chemical interactions of the epi-endolithic *V. muralis* with the Cortemilia sandstone with respect to the genetically related, but epilithic *V. nigrescens*.

Hyphal penetration down to millimetric depths was often reported for different sandstone lithologies (Chen et al., 2000). The highest penetration values here observed for both species agreed with similar previous reports, but the massive penetration mostly affected the first millimetre only. In this regard, textural features, including porosity, have been recognized as first determinants of the rock susceptibility to colonization and, particularly, to hyphal penetration (Camara et al., 2008). The absence of significant interspecific variability in hyphal penetration between *V. nigrescens* and *V. muralis* likely reflects the availability of passageways in the first upper millimetre, although it is always difficult to ascertain whether hyphae exploit existing discontinuities and actively contribute to produce new fissures (Ascaso & Wierchoś, 1995). Surface layers of sandstones beneath lichens displayed a porosity due to dissolution of calcite and other poorly stable minerals (Bjelland & Thorseth, 2002). Accordingly, XRPD analyses showed strongly higher $R_{Cc/Qz}$ for two core samples with respect to the related volumes beneath both the *Verrucaria* species, indicating some calcite dissolution. The absence of surfaces free of lichen or microbial colonization, however, prevented the possibility of verifying if such pattern is directly related to biological activity or is related to abiotic weathering factors, pre-dating lichen colonization (Turkington & Paradise, 2005). For the other two sample series, the low $R_{Cc/Qz}$ characterized for core samples, equal to that obtained for volumes beneath lichens, likely reflects an initial lower content of calcite, whose amount is known to vary in the Cortemilia sandstone (Gnaccolini & Rossi, 1994). In all cases, however, $R_{Cc/Qz}$ neither showed significant correlation with stone hardness nor explained different hardness beneath the two lichen species, excluding that calcite dissolution alone accounts for the hardness variation with respect to the core. Moreover, a partial dissolution, rather than the complete absence of calcite reported by Bjelland and Thorseth (2002), was detected through the whole set of samples collected in the 5 mm deep layer beneath the lichen thalli. Although a bioprotective umbrella-effect of lichen thalli on calcite-rich lithologies was experimentally demonstrated (McIlroy de la Rosa et al., 2014), such heterogeneity explains that many other factors, dealing with the overall history of stone surfaces, may account for their currently observable physico-chemical properties and the consequent conservation condition.

It is worth noting that maximum values of massive penetration were observed for *V. nigrescens*, which also displayed a denser hyphal presence in the penetrated layer, with hyphal bundles and thicker hyphal network. Such penetration patterns are consistent with pervasive penetration observed for *V. nigrescens* within limestone (Favero-Longo et al., 2009), which was related to its high negative impact on limestone hardness (Morando et al., 2017). Anyway, these slight differences between *V. nigrescens* and *V. muralis* are unlikely to explain different hardness variations beneath the two species, mostly because hyphal penetration only rarely affected the stone deeper than 2 mm, while close-to-surface hardness measurements were collected at 0.2–0.3 cm from the surface. The detected penetration patterns seem thus to reject the hypothesis of an exclusive role of the mechanical action of hyphae in determining stone hardness modification.

Higher heating of rock surfaces beneath *V. nigrescens* with respect to white lichen thalli was also indicated as responsible for the high stress rate induced on rock stability (Carter & Viles, 2004). However, XRPD analyses added further insights on the biogeochemical

side of lichens–sandstone interaction. As expected, oxalates were absent (or below the XRPD detection limit) beneath both the epilithic and epi-endolithic *Verrucaria* thalli. Accordingly, oxalic acid – recognized as a factor responsible for bioweathering by other lichen species on sandstone (Edwards et al., 2002) – is not a driver of the biodeterioration induced by Verrucariales, as already ascertained for endolithic species of the order (Pinna et al., 1998) (Pinna et al., 1998) with the exception of *Verrucaria rubrocincta* (Bungartz et al., 2004). The different peaks of calcite are instead informative on the stability of different crystallographic planes, which are known to be differently enhanced in presence of organic substance (Klug & Alexander, 1974; Leoni, 2019). In particular, calcite form (01–12) is stabilized by organic chelants (Pastero et al., 2003). Accordingly, the correlation between the lowest hardness values observed beneath *V. nigrescens* and (01–12) may be explained by exposure of the assayed stone volumes to organic chelants, indicating that lichen impact on the stone extended beyond the hyphal penetrated volume through metabolite release. Although the production of lichen secondary metabolites is not a trait of Verrucariales, the release of chelating compounds has been already observed for endolithic species (Favero-Longo et al., 2011) and may be a more widely shared feature, which does not leave prominent traces as oxalates. In this sense, we cannot exclude the possibility that *V. muralis* also releases metabolites affecting the rock stability, but the phenomenon is not reflected in the observed calcite crystallization and, if it exists, may be more limited in line with the poorly developed biomass, above and within the substrate.

Dissolution and re-precipitation of calcite associated with lichen colonization have been already characterized for endolithic species and associated with a respiration-induced acidification pathway (Weber et al., 2011). Lichen biomineralization of micrite has been recognized as a bioprotection factor, counterbalancing the deterioration induced by hyphal penetration (Bungartz et al., 2004). The same presence of organic matter may be the cause of hardening of upper rock layers, as already demonstrated for microbial biofilms (Slavik et al., 2017), but still poorly explored for lichens (Morando et al., 2017). Our analyses did not allow us to exclude the possibility that the low impact of *V. muralis* on rock hardness may result from similar re-precipitation and hardening processes, as suggested by the observation of a layer appearing more compact just beneath the algal clusters, whose investigation will be the object of a subsequent contribution.

5 | CONCLUSIONS

This investigation showed that each lichen species may have a different impact on physico-mechanical properties of sandstones, as measured by surface hardness, a proxy for durability. Accordingly, a reliable evaluation of biogeomorphological processes affecting sandstone cannot generalize lichen contributions as biodeteriorative or bioprotective, or univocally associate a certain effect with the epilithic and endolithic growth forms, but rather needs to disentangle and summarize the heterogeneous contributions of different species. Such species-specific patterns are known – and here confirmed – to depend on the balance between several mechanisms of physico-mechanical and chemical impact which positively or negatively impact

substrate durability. However, chemical processes may not always leave prominent evidence of their occurrence and extension at depth, as in the case of the investigated species which do not produce oxalate deposits. Our analyses suggested that microscopy observations may be integrated with mineralogical investigations, to unveil the extension of the sphere of lichen interaction within the rock substrate beyond the limit of hyphal penetration, by highlighting deeper traces of biomineralization processes. Such findings are also of relevance in the field of cultural heritage conservation, indicating that decisions on the preservation or removal of lichens, as agents of biodeterioration or bioprotection, cannot be generalized, but the behaviour of each species should be carefully considered, at least focusing on dominant ones.

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AUTHOR CONTRIBUTIONS

Conceptualization: CT, SEF-L; methodology (including methodological development): CT, LP, SEF-L, HV; investigation (data collection): CT, DB; (analysis of data): CT, SEF-L, LM, LP; supervision: SEF-L, HV; writing: CT, SEF-L, HV.

DATA AVAILABILITY STATEMENT

Data are available on request (chiara.tonon@unito.it; sergio.favero@unito.it).

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