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An ecological investigation on lichens and other lithobionts colonizing rock art in Valle Camonica (UNESCO WHS n. 94) addresses preventive conservation strategies

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Abstract

Environmental control strategies are commonly practised to limit biodeterioration issues threatening indoor cultural heritage objects, while they are still poorly exploited for the conservation of outdoor stone heritage surfaces, including rock art. In this study, we evaluated the environmental factors driving the diversity and abundance of lithobiontic communities in the Rock Engravings National Park of Naquane (UNESCO WHS n. 94, Italy). The survey considered 23 rocks that had been cleaned in the last three (3YC) or twelve (12YC) years or more than 40 years ago (NRC). A cyanobacteria-dominated biofilm and lichens (37 taxa) were the most widespread and abundant lithobiontic components, prevailing on 3YC–12YC and NRC rocks, respectively. On the latter, a turnover of xerophytic and meso-hygrophytic lichen communities was observed. On 3YC–12YC rocks lichen colonization, if present, was limited to nitrophytic species, including common epiphytes from surrounding trees and a small number of meso-hygrophytic species, with a prevalence of asexual reproductive strategies. Multivariate analyses including environmental parameters (canonical correspondence analyses) indicated that tree cover and the presence of bare or vegetated ground upstream of the rocks, probably prolonging wetness and providing nutrients by water transport, are the factors mostly related to the microbial and lichen recolonization of 3YC–12YC surfaces. On this basis, an experiment on preventive conservation was conducted, consisting of a new cleaning of a strongly recolonized 3YC surface combined with the building of a small wall to protect part of the rock from prolonged water fluxes. The fluorimetric and colorimetric monitoring of the rock surface, carried out 40 months after this new cleaning intervention, displayed recolonization on the unprotected area only, indicating the potential of preventive conservation strategies in outdoor environments.

Keywords: biodeterioration; biofilm; cultural heritage; nitrophytic community; recolonization

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Introduction

Saxicolous lichens, as well as other lithobionts, are a major threat to stone heritage conservation because of their physical and chemical interactions with mineral substrata, promoting weathering processes and thus affecting surface durability (Seaward 2015; Favero-Longo & Viles 2020). On the other hand, at least for some combinations of species, lithologies and climate conditions, bioprotective rather than biodeteriorative effects of lichens have been reported (Pinna (2021), and references therein). Besides these negative and/or positive impacts on material properties, lichen colonization influences the aesthetics and legibility of heritage surfaces, with critical consequences when thalli mask meaningful details, such as inscriptions or art reliefs (Pinna 2017). In a broader sense, any lithobiontic cover distances the heritage surface appearance from the original conception. Therefore, curators of outdoor stone heritage, particularly in the Latin cultural area, consider as a priority the maintenance of any stone heritage surface in a clean state (i.e. free of lichens and other lithobionts), and manage conservation plans accordingly. Devitalization and mechanical removal of lichen thalli and microbial biofilms are thus routinely included in restoration interventions (Pinna 2017). However, the wide use of synthetic chemicals such as biocides, practised for decades, is now increasingly considered environmentally unsustainable, and new alternative products and/or chemical-free approaches to control lithobionts are continually searched for (Cappitelli *et al.* 2020).

Lichenologists, and potentially others, may have different priorities to heritage site curators with regard to the conservation of heritage stone surfaces or of lichens and biodiversity in general

(Seaward 2004). Different perceptions of biodeterioration issues generally depend on the type of heritage surfaces affected (a statue, a grave, a church façade, a castle wall, an archaeological ruin) and the local cultural tradition (Favero-Longo & Viles 2020). Moreover, different evaluations may derive from the 'environmental scenery' of each artwork, with the lithobiontic colonization, although distancing the stone appearance from its original state, sometimes contributing to its positive integration with the surrounding natural context. Nimis and colleagues (1992) early on invoked the possibility of considering lichens to have an additional cultural value in certain heritage sites, such as archaeological areas, worth being preserved and brought to the attention of visitors.

Lithobiontic colonization and biodeterioration effects deserve particular attention when affecting rock art, since biological growths and the artworks may display a rather similar dimensional extent (i.e. (sub-)millimetric thickness), therefore particularly affecting conservation issues (Darvill & Batarda-Fernandes 2014; Zerboni *et al.* 2022). Lichens, in particular, can partially mask or fully cover engravings (Tratebas 2004), and were shown to induce physical and chemical deterioration processes on different lithologies bearing rock art, although negative effects on the surface durability were not always recognizable (e.g. Chiari & Cossio 2004; Marques *et al.* 2016). The impact on surface legibility, however, is sufficient to make lichens generally undesirable on engraved stone surfaces, even though their colonization is an obvious and unavoidable phenomenon on every rock outcrop (Jung & Büdel 2021) and just lichens are often a prominent and valuable biodiversity component of the environments hosting rock art (Tansem & Storemyr 2021). Treatments with synthetic chemical biocides, in combination with mechanical actions and other restoration products such as consolidants and water-repellents, have thus been routinely practised in rock art sites to 1) periodically remove lichens and other lithobionts from engraved surfaces, and 2) try to prolong the maintenance of the clean state (Tratebas 2004; Paz-Bermúdez *et al.* 2023). Only recently, in order to reduce the spread of chemicals into the environment, have alternative approaches for the control of lithobionts on engraved rocks been undertaken, including laser and microwave applications. However, the former seems less effective than traditional biocides and may even increase rock bioreceptivity (Paz-Bermúdez *et al.* 2023), and the latter needs technical improvements to allow outcrop-scale applications (Favero-Longo *et al.* 2021). On the other hand, approaches to prevent recolonization dynamics following cleaning interventions by controlling (micro-)environmental parameters, which is a usual and regulated practice (e.g. in Italy, Ministerial Decree DM 10/05/2001: MIBAC 2001) to limit biodeterioration in indoor environments (Caneva *et al.* 2008), still appear poorly considered in the case of outdoor stone heritage, and for rock art in particular.

In the Rock Engravings National Park of Naquane, the heart of the UNESCO site 'Rock Drawings in Valle Camonica' (WHS n. 94, Italy), outcrops hosting the most remarkable engravings have undergone a long series of cleaning interventions (including the application of biocides), which have been registered since the 1980s but started long before (www.irweb.it; Ruggiero & Poggiani-Keller 2014). In recent decades, recolonization dynamics on certain rocks, mostly related to fast spreading of cyanobacterial biofilms, made cleaning every few (2–3) years necessary. This makes the management unsustainable in terms of time and costs, but also with regard to the environmental pressure of the repeated biocide application and the potential stress on rock surfaces due to the repeated mechanical treatments. Therefore, a research project was started in 2016 to assess critical features of the adopted conservation strategies (e.g. the efficacy of adopted protocols of biocide applications; Favero-Longo *et al.* 2021), and to explore alternative approaches to better combine cultural and environmental heritage conservation (Ruggiero *et al.* 2021). Within this framework, the present work aims to characterize lithobiontic colonization on the engraved sedimentary rocks of the National Park of Naquane, focusing on the diversity and abundance of lichens on outcrops with different conservation histories and environmental conditions. It also gives an insight into their physical interaction with the sandstone substratum. The results were used to address a preventive strategy to limit lithobiontic recolonization after cleaning interventions, which was experimentally tested on a selected engraved outcrop. In particular, we tested the hypotheses that: 1) some environmental factors are the main drivers of diversity and abundance of lichens and other lithobionts on recently cleaned surfaces, 2) lichens and other lithobionts penetrate within the sandstone substratum, and 3) interventions limiting favourable environmental conditions for lichens may generally hinder the fast lithobiontic recolonization following cleaning interventions.

Material and Methods

Study site

The Rock Engravings National Park of Naquane is located in the middle part of Valle Camonica (Capo di Ponte, Brescia, Italy: UTM WGS84: 32T 604400 m E, 5097700 m N), where it was established in 1955 as the first national archaeological park. It extends between 400 and 600 m above sea level (a.s.l.) on *c.* 14 000 m^2 of the eastern side of the valley, and hosts the most important groups of prehistoric and protohistorical engravings of Valle Camonica. The engravings are distributed on 104 numbered surfaces of sedimentary rock outcrops, dimensionally ranging from a few to *c.* 250 m^2 (e.g. Rock 1, named the 'Great Rock of Naquane', with 65 m^2 of engraved surface; Liborio *et al.* 2011). Engravings are carved primarily in terrigenous sedimentary rocks (Verrucano Lombardo, Upper Permian; Brack *et al.* 2008), mainly consisting of sandstones/graywackes rich in quartz, feldspars and fragments of volcanic rocks, micro-conglomerates, and mudrocks. Sediments of the Verrucano Lombardo suffered a relatively high overburden (several kilometres) during burial which caused a high degree of compaction (documented by the prevalence of long contacts among grains in sandstones) and recrystallization of the clay matrix. The strong diagenetic imprint, in addition to the mineralogical composition of the sand, resulted in a great compactness and hardness, and very low porosity of the rock (Supplementary Material Fig. S1, available online). This in turn affected the landscape modelling by fluvial and glacial erosion during Quaternary glaciations, giving rise to a remarkable smoothness of rock surfaces.

The Park is located in the Cfb zone (C = temperate, f = no dry season, b = warm summer, according to the Köppen Geiger climate classification; Kottek *et al.* 2006), with average temperatures of 2 °C in winter and 21 °C in summer, with 1000 mm rainfall yr^{-1} (Ceriani & Carelli 2000; data monitored in the Capo di Ponte monitoring station no. 129, the closest to the Park, in the period 2003–2016, available at www.arpalombardia.it/Pages/Meteorologia/Richiesta-dati-misurati.aspx). In terms of land use and forest types, the site is characterized by the occurrence of abandoned chestnut stands (of meso-xeric soils), variously evolved to a mixed broadleaf forest (*Betula pendula* Roth, *Fraxinus ornus* L., *Populus tremula* L., *Salix caprea* L. and

Prunus avium (L.) L.), although natural (*Pinus sylvestris* L., as a relic of past submontane pine forests, preceding chestnut cultivation) and planted conifers (*Larix decidua* Mill., *Picea abies* (L.) Karst and some exotic species) also widely occur, together with sparse, xerophytic and acidophytic grassland stands (Ducoli 2012).

Diversity survey

Lithobiontic communities, and saxicolous lichen diversity in particular, were surveyed in the period between November 2017 and July 2018 on 23 engraved rocks with a different conservation history (information available at www.irweb.it). In total, 54 plots, 50×50 cm, were distributed on the surfaces of: 1) six rocks which were last cleaned in the period 2014–2015 (3YC; Rocks 1, 35, 50, 70, 73, 99; $n = 19$ plots), 2) four rocks which were last cleaned in the period 2005–2008 (12YC; Rocks 6, 7, 14, 57; $n = 8$ plots), and 3) nine rocks (or groups of neighbouring rocks) for which cleaning interventions are not documented in archives registering the conservation history of engravings since the early 1980s (Not Recently Cleaned, NRC; Rocks 2, 4, 8–9, 11, 17–18, 49, 58, 36–69–96, 74; $n = 27$ plots). Interventions performed in the period 2005–2008 included mainly mechanical removal of thalli, cleaning with NeoDes 5% or 10%, application of the benzalkonium chloride-based product Preventol 3% as a preservative, and final application of the water-repellents Akeogard CO or Silo 111; interventions performed in the period 2014–2015 included surface washing with low-pressurized water and biocide application of benzalkonium chloride-based biocides. On each rock (or group of neighbouring rocks), three plots (with the exceptions of Rock 1, with six plots because of its much larger surface, and Rocks 7, 14 and 73, with one plot each because of technical constraints) were preferentially positioned in areas visually recognized as representative of the predominant biodeterioration condition(s) affecting the surface legibility, and thus requiring attention from the point of view of heritage conservation.

For each plot, the cover of different lithobiontic components (namely bryophytes, lichens, cyanobacteria-dominated biofilms, green algae-dominated biofilms, microcolonial black fungi (MCF)) was visually estimated in the field and checked in the laboratory with digital images. In the case of biofilms, the extent of microbial mats which caused a visible colour shift of the surface, with respect to the bare rock, was considered. Sampling and microscopic observations allowed the biofilm(s) of each plot to be characterized with respect to the dominance of the different microbial components. Cover values were assigned according to the following ordinal scale: 5 = > 75%, 4 = 51–75%, 3 = 26–50%, 2 = 2–25%, 1 = < 2% (or diffuse covering, but not masking the mineral surface), 0 = absence. Moreover, for each plot, lichen diversity was surveyed using a square grid divided into 25 quadrats (10×10 cm), calculating the frequency of each species as the sum of their occurrences within the grid quadrats and visually estimating their cover through the whole plot.

Samples of lichen thalli were collected from each plot, without affecting the rock substratum for conservation reasons, to check field identifications in the laboratory. Lichen identification was based on Wirth (1995), Smith *et al.* (2009) and the online keys published in ITALIC version 7 (see Nimis & Martellos 2020). Nomenclature follows Nimis (2022). Species vouchers are deposited in the lichen section of the Herbarium Universitatis Taurinensis (TO). Indicator values proposed by Nimis (2022) were considered as a reference to express specific ecological ranges with respect to pH of substratum (pH), solar irradiation (IR), aridity (AR) and eutrophication (EU).

The plots were also characterized with regard to environmental variables, quantified in the field (estimated in the case of surface micromorphology) and then referred to ordinal scales as follows: aspect (EXP: 3 = SW, 2 = W, 1 = NW, 0 = N), inclination (INC: 3 = 0–10°, 2 = 11–30°, 1 = 31–50°, 0 = > 50°), surface micromorphology (ROU: 3 = rough and/or highly fractured surface, 2 = slightly rough and/or moderately fractured surface; 1 = smooth surface with few fractures; 0 = smooth surface without fractures), tree cover (TRC: 2 = tree cover above the plot, 1 = ground projection of the crown at less than 2 m from the plot, 0 = ground projection of the crown at more than 2 m), and distance from bare or vegetated ground upstream of the plot, probably providing nutrients by water transport (GRP: 3 = < 1 m, 2 = 1.1–4.9 m, 1 = > 4.9 m, 0 = absence of bare or vegetated ground upstream of the plot).

Analysis of diversity data

The abundance of each lichen taxon was calculated in terms of presence through the plots (%) and of average and maxima values of cover (%) and frequency (%) per plot. The relative importance of components of γ-diversity (i.e. similarity (S), relativized richness difference (D), and relativized species replacement (R)) was evaluated for all plots (NRC + 12YC + 3YC), and for plots on rock surfaces with a different conservation history considered in combination (NRC + 12YC, NRC + 3YC, 12YC + 3YC) and separately (NRC, 12YC, 3YC). The analysis was performed on the matrix of species presence/absence with the SDR Simplex software using the Simplex method, as detailed elsewhere (SDR Simplex; Podani & Schmera 2011). An ordination of plots was performed on the basis of frequency data by Principal Co-ordinate Analysis (PCoA: symmetric scaling, centring samples by samples, centring species by species; ter Braak & Šmilauer 2002). Two Canonical Correspondence Analyses were carried out with the matrices of environmental parameters and the cover values estimated for the different lithobiontic components (CCA-I) and the frequencies of lichen taxa (CCA-II), in order to partition variation explained by each variable and construct a model of significant variables (biplot scaling for interspecies distances; Hill's scaling for inter-sample distances; forward selection of variables option; Monte Carlo permutation test on the first and all ordination axes) (ter Braak & Verdonschot 1995). The ordinations were performed using CANOCO v. 4.5 (ter Braak & Šmilauer 2002).

Microscopic observation of lithobionts-rock interactions

A set of centimetric to decimetric blocks of the site sandstone bedrock, already detached from the outcrops, free of engravings and colonized by lithobionts, were collected to run microscopic observations on the physical interactions of cyanobacterial-dominated biofilms and mature thalli of representative crustose (*Verrucaria nigrescens*) and foliose (*Xanthoparmelia conspersa*) lichens with their substrata. Rock fragments (c. $3-4 \times 2-3 \times 0.5$ cm; $n = 3-5$ per lithobiont) were cross-sectioned, embedded in a polyester resin (R44 Politex-P fast, ICR, Reggio Emilia, Italy), polished with silicon carbide paper, and stained with PAS (Periodic acid-Schiff's method; Whitlach & Johnson 1974) to highlight lithobiontic penetration. Sections were observed under reflected light microscopy (RLM) with an Olympus SZH10 microscope in order to quantify the penetration depth reached by the microbial biofilm and the hyphal penetration component of lichens.

Experiment on preventive conservation

The possibility of locally limiting environmental conditions recognized as favourable to lithobionts, and thus their rapid recolonization after cleaning, was assayed on Rock 70 (WGS84 32T 604380 m E, 5097935 m N), on which different restoration interventions have been conducted since the 1980s, the last in 2014 (details in the caption of Supplementary Material Fig. S2, available online). In 2017, after only three years, the whole rock surface was deeply affected by the presence of a cyanobacterial-dominated biofilm and the local occurrence of small lichen thalli (*Fuscidea lygaea, Pertusaria flavicans, Phlyctis argena*), with the exception of the perimeter of the main engravings that some unknown individual(s) had improperly tried to clean (Supplementary Material Fig. S2A).

In the framework of this work, Rock 70 was cleaned again in Summer 2019, with the mechanical removal of the microbial biofilms and the lichens preceded by their devitalization with a 4-h poultice application of the biocide Biotin T (N-octyl-isothiazolinone, 7–10%, and didecyl-dimethyl ammonium chloride, 40–60%, as active principles; CTS, Altavilla Vicentina, Italy). The effectiveness of this treatment had been verified by fluorimetric measurements on other outcrops of the park (Favero-Longo *et al.* 2021) and further checked on a small number of parcels on Rock 70 itself (see below). In Autumn 2019, a 10 cm tall and *c.* 3 m long wall of bricks, covered and fixed with mortar, was built 20–30 cm from the upper border of the rock, to limit water fluxes from upstream vegetated and bare ground following rain events. Only the right portion of the rock was left free from the wall protection. It is worth remarking that the wall was built to assay the effect of water control on recolonization dynamics and not as a permanent structure. Moreover, some of the trees bordering the rock outcrop were cut or pruned, to reduce their shading effect on the engraved surface.

Measurements of the vitality of the cyanobacterial-dominated biofilm were performed a few hours before and one day after the biocide application using a Handy-PEA fluorimeter (Hansatech Instruments Ltd, Norfolk, England; saturating light pulse of 1s, 1500 µmol m^{-2} s^{-1}, peak at 650 nm), as described elsewhere (e.g. Favero-Longo *et al.* 2021). Measurements were performed early in the morning, on pre-moistened and dark-adapted surfaces, and distributed on three parcels (*c.* 25 × 25 cm) on different parts of the rock outcrop (*n* > 70 at each measuring time point). Measurements on an additional untreated parcel were also collected as a control. The basal fluorescence (F_0), which is related to the chlorophyll *a* content, and the maximum quantum yield of PSII (F_v/F_m), which is informative on the functionality of the photosynthetic process, were monitored as indicators of the microbial viability (Tretiach *et al.* 2010; Favero-Longo *et al.* 2021). Potential recolonization after the cleaning intervention was monitored by fluorimetric measures 20 and 40 months after the cleaning (i.e. in March 2021, after the limitations due to the COVID-19 pandemic, and November 2022), on newly selected parcels, randomly distributed in areas protected by the wall (*n* = 6), out of the wall protection (*n* = 4) and on the uncleaned Rock 71, adjacent to Rock 70 (*n* = 3).

The fluorimetric monitoring was combined with spectrocolorimetric measures, in order to evaluate the potential deteriogenic effect of lithobiontic recolonization in terms of colour and aesthetic disfiguring. Measurements were performed with a portable spectrophotometer (Konica Minolta CM-23d) on target areas of 8 mm (diameter) in geometric condition d/8 specular component included as setting conditions, using the CIE D65 illuminant and 2° observer, and the CIELAB colour system to process and analyze the spectral data (ISO 2019). At least five measurements were collected for each of 10 parcels distributed in areas protected (*n* = 5) and not protected (*n* = 2) by the wall, and on the adjacent uncleaned Rock 71 (*n* = 3), corresponding or adjacent to the parcels used for fluorimetric measures. The L* parameter, an indicator of surface lightness, was considered as reference to recognize a different development of a dark lithobiontic biofilm (Gambino *et al.* 2019).

Results

Lithobiontic colonization of engraved rock surfaces

All plots displayed a visible lithobiontic colonization with two exceptions, involving rocks restored in 2015 and still largely maintaining a clean state after three years. However, total lithobiontic cover and abundance of its components were remarkably varied through the different plots, particularly with respect to the different conservation history of the rocks. On NRC rocks, a high total cover was a common feature (mean 81.6 ± 6.0% SE), while highly variable values were observed for 12YC (mean 55.9 ± 16.9% SE) and 3YC (mean 22.6 ± 9.45% SE) rocks. The NRC cover was statistically significantly higher than the 3YC cover (ANOVA, *P* < 0.05).

A dark, blackish to red-brownish biofilm was the most widespread and abundant component of lithobiontic communities (Supplementary Material Fig. S3A, available online), with thickness ranging from a few microns to millimetres and thus varying from simple 'dirtying' of mineral grains to remarkable masking effects of surface micromorphology and engravings. Microscopic observations showed cyanobacteria as dominant constituents, including filamentous (mostly *Stigonema* sp. and *Scytonema* sp.; Supplementary Material Fig. S3B, available online) and, less abundant, coccoid (as *Gloeocapsa* sp. and *Chroococcus* sp.) species. Black yeasts and meristematic fungi, as well as green algae and primordia of lichen thalli, were also occasionally observed. The dark biofilm (CyB in Fig. 1) was dominant on almost all surveyed surfaces (Fig. 1A), but covered significantly lower areas on 3YC rocks (Fig. 1B). On these latter rocks, in particular, lithobionts were absent in six out of 19 plots, and cover values higher than 25% characterized only one third of the plots (Fig. 1B). High covers were instead prevalent on 12YC rocks (Fig. 1C), displaying the maximum percentage of plots with values higher than 75%, and on NRC (Fig. 1D), where the dark biofilm generally covered the entire surface free of the other lithobiontic components.

Greenish biofilms (AlB) (Supplementary Material Fig. S3E, available online) also occurred on some rocks, including 12YC and 3YC, although they never displayed cover values higher than 50% (Fig. 1) and their thickness was generally limited, discolouring rather than having a masking effect. Microscopic observations showed filamentous green algae (frequently *Microspira* sp.) as dominant constituents, together with coccoid species, including free-living *Trebouxia* sp., while cyanobacteria occurred only minimally.

Circular colonies of meristematic fungi (MCF), of (sub-)millimetric size but sometimes merging to give crusts of several dm^2 (Supplementary Material Fig. S3C & D, available online), were an additional lithobiontic component on some engraved surfaces. Although their frequency was low as well as their cover values, they were evident on both 12YC and NRC rocks (Fig. 1).
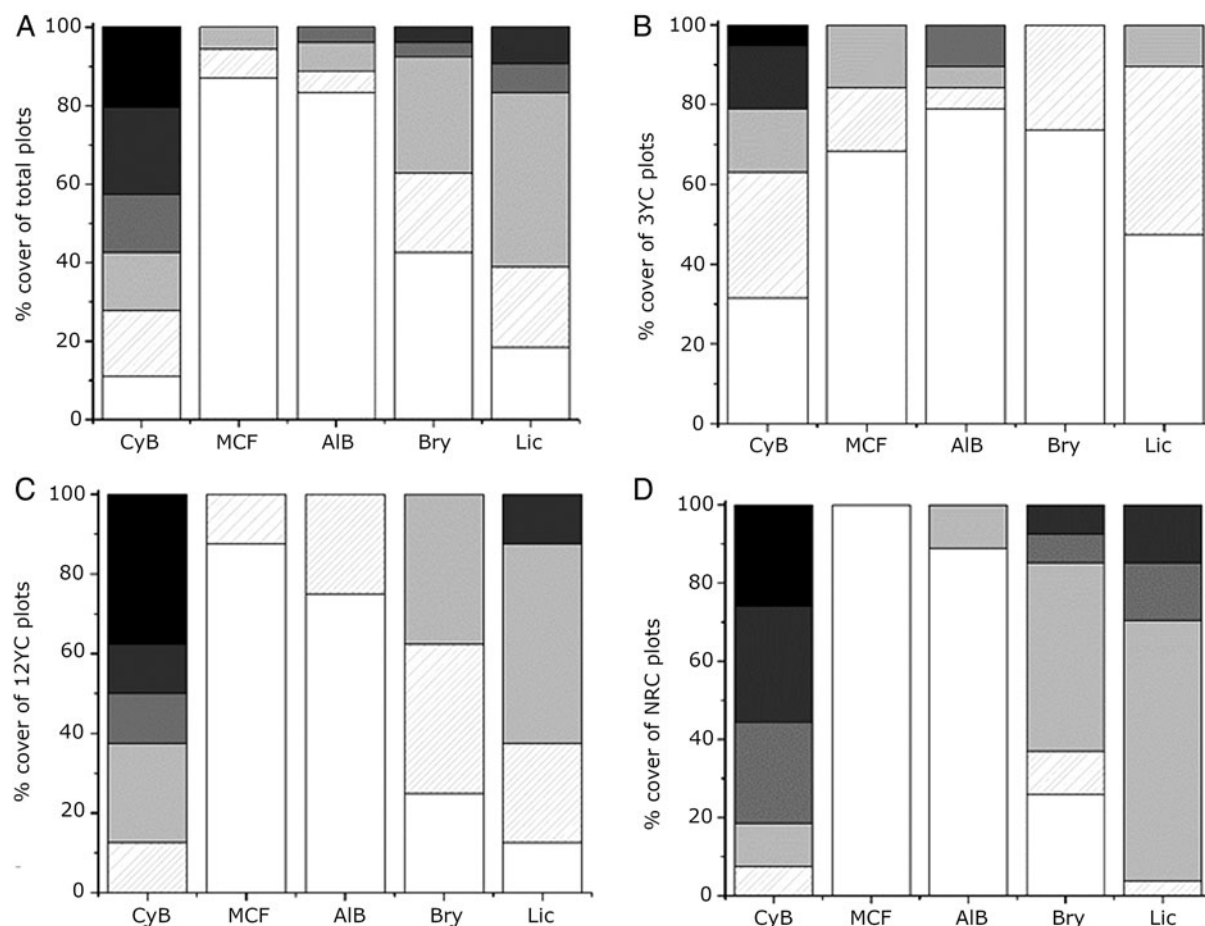
Figure 1. Abundance of different lithobiontic components (CyB = cyanobacterial-dominated biofilm, MCF = microcolonial fungi crusts, AlB = green algal-dominated biofilm, Bry = bryophytes, Lic = lichens) on the engraved rocks, considering the overall plots together (*n* = 54; A) and separately for rocks cleaned in the last three years (3YC, *n* = 19; B), 12 years (12YC, *n* = 8; C) or more than 40 years ago (NRC, *n* = 27; D). Data are expressed in terms of percentage of plots with cover values in the following ranges: > 75% (black), 51–75% (dark grey), 26–50% (grey), 2–25% (light grey), visible cover but < 2% (grey hatched), absence of visible cover (white).

Lichens (Lic) occurred in 10 out of 19 plots surveyed on 3YC rocks, but cover values were mostly lower than 2%; specific lichen diversity is considered below. On 12YC and NRC rocks, lichens were present in almost all the plots (except on one 12YC), and cover values were mostly in the 2–25% range (Fig. 1), although in some cases values higher than 50% were observed (Supplementary Material Fig. S3F, available online). Bryophytes (Bry), and particularly mosses, also occurred in most of the plots, often localized along cracks and fissures (Supplementary Material Fig. S3G, available online). Their cover values were rather negligible on 3YC rocks, and always lower than 25% on 12YC (Fig. 1). On some NRC rocks, they were instead the dominant component, with cover values higher than 50%.

Lichen diversity

A total of 37 saxicolous lichen taxa was recorded from the surveyed plots (Table 1), with a prevalence of crustose (59%) rather than foliose species (38%), although the latter showed higher cover values, and a rather high number of taxa with an asexual reproductive strategy (35%). In particular, a high diversity of yellow-green *Xanthoparmelia* spp. was recorded, including five isidiate and two non-isidiate species. However, due to the logistical constraints of identifying each individual, only isidiate and non-isidiate

Xanthoparmelia spp. were distinguished in the abundance analyses. For the same reason, other species groupings were considered, including *Circinaria caesiocinerea*/*Aspicilia cinerea* and *Rhizocarpon disporum*/*R. reductum*, reducing to 30 the final number of taxa considered for the subsequently described analyses.

All 30 taxa were found on NRC rocks, while diversity was lower on 12YC and 3YC (17 taxa). Accordingly, SDR analysis performed for the overall plots showed a very high beta diversity (81.2%), but with richness difference (43.8%) prevailing over species replacement (37.5%) (Table 2). Similarity showed a decreasing trend from plots on NRC rocks (28.2%) to those on 12YC (22.5%) and 3YC (17.5%) rocks, with richness difference appearing most important for 3YC (46.3%) and replacement more notable in 12YC (38.4%). Higher similarity and lower replacement were detected by considering plots on NRC and 12YC ($S_{NRC+12YC} = 25.5\%$; $R_{NRC+12YC} = 25.8$) together, compared to the combinations of plots on NRC and 3YC ($S_{NRC+3YC} = 19.4$; $R_{NRC+12YC} = 37.9$) and on 12YC and 3YC ($S_{12YC+3YC} = 12.3$; $R_{NRC+12YC} = 41.4$).

On NRC rocks, eight taxa displayed the highest occurrence in the plots (37– 81%; Table 1), including both heliophytic-xerophytic (*Circinaria caesiocinerea*, yellow-green *Xanthoparmelia* spp. with and without isidia, *Xanthoparmelia glabrans*, *Candelariella vitellina*, *Rhizocarpon disporum*) and mesophytic (*Caloplaca chlorina*, *Pertusaria flavicans*) species. They all showed high frequency values

Table 1. Lichens recorded on sandstone outcrops of the Rock Engravings National Park of Naquane (mean and max cover and frequency values are reported for the plots considered in total and separately for 3YC, 12YC and NRC outcrops, as well as the % specific occurrence through the plots; taxa with the highest occurrence through the plots are reported in bold). Growth forms (GF): Cr = crustose, Fo = foliose, Fr = fruticose. Prevailing reproduction strategy (Repr.): S = sexual, A = asexual. Ecological indicator values from Nimis (2022): pH = pH of the substratum, IR = irradiation, AR = aridity, EU = eutrophication.

Species	Code	GF	Repr.	pH	IR	AR	EU	Occurrence (plot %)	Cover Mean	Cover Max.	Freq. Mean	Freq. Max.	Occurrence (plot %)	Cover Mean	Cover Max.	Freq. Mean	Freq. Max.	Occurrence (plot %)	Cover Mean	Cover Max.	Freq. Mean	Freq. Max.	Occurrence (plot %)	Cover Mean	Cover Max.	Freq. Mean	Freq. Max.
								All plots (n = 27)					**3YC rocks (n = 19 plots)**					**12YC rocks (n = 8 plots)**					**NRC rocks (n = 27 plots)**				
Acarospora fuscata (Schrad.) Arnold	Ac.f	Cr	S	3-4	4-5	3-4	3-4	3.7	0.0	0.1	0.2	8.0	-	-	-	-	-	-	-	-	-	-	7.4	0.0	0.1	0.4	8.0
Buellia aethalea (Ach.) Th. Fr.	Bu.a	Cr	S	1-3	4-5	4-5	1-3	7.4	0.2	8.0	2.0	56.0	5.3	0.1	1.0	0.4	8.0	12.5	0.0	0.1	7.0	56.0	7.4	0.3	8.0	1.6	36.0
Buellia stellulata (Taylor) Mudd	Bu.s	Cr	S	3-4	4-5	4	1-2	1.9	0.0	0.1	0.1	4.0	-	-	-	-	-	-	-	-	-	-	3.7	0.0	0.1	0.1	4.0
***Caloplaca chlorina* (Flot.) H. Olivier**	Ca.c	Cr	A	2-3	3-4	3	3-4	**27.8**	1.1	30.0	11.1	96.0	-	-	-	-	-	37.5	5.1	30.0	28.5	96.0	44.4	0.6	6.0	13.8	60.0
Candelaria concolor (Dicks.) Stein	Cd.c	Cr	A	3-4	4-5	3-4	3-5	24.1	0.0	0.1	4.1	40.0	21.1	0.0	0.1	3.4	40.0	12.5	0.0	0.1	0.5	4.0	29.6	0.0	0.1	5.8	36.0
Candelariella coralliza (Nyl.) H. Magn.	Cn.c	Cr	S	2-3	4-5	4	4-5	1.9	0.0	0.1	0.1	4.0	-	-	-	-	-	-	-	-	-	-	3.7	0.0	0.1	0.1	4.0
***Candelariella vitellina* (Hoffm.) Müll. Arg.**	Cn.v	Cr	S	1-3	3-5	3-4	2-5	**33.3**	0.1	2.0	19.6	100.0	5.3	0.0	0.1	0.6	12.0	37.5	0.0	0.1	8.0	52.0	51.9	0.2	2.0	36.3	100.0
Chrysothrix sp.	Ch.s	Cr	A	1-2	2-4	1-3	1	13.0	0.0	0.1	0.9	12.0	-	-	-	-	-	37.5	0.0	0.1	3.5	12.0	14.8	0.0	0.1	0.7	8.0
***Circinaria caesiocinerea* (Malbr.) A. Nordin *et al.* (± *Aspicilia cinerea* (L.) Körb.)**	Ci.c	Cr	S	2-4	3-5	2-4	2-5	**50.0**	1.6	40.0	10.8	100.0	10.5	0.0	0.1	1.9	32.0	62.5	0.4	2.0	9.5	28.0	74.1	3.1	40.0	17.5	100.0
Cladonia sp.	Cl.s	Fr	S	4-5	4-5	4	1-3	5.6	0.1	3.0	1.5	32.0	-	-	-	-	-	-	-	-	-	-	11.1	0.2	3.0	3.0	32.0
Fuscidea lygaea (W. Mann) V. Wirth & Vězda	Fu.l	Cr	S	1-2	3-4	2-3	1	11.1	0.4	10.0	5.9	100.0	5.3	0.2	3.0	5.3	100.0	12.5	0.0	0.1	0.5	4.0	14.8	0.7	10.0	7.9	96.0
***Pertusaria flavicans* Lamy**	Pe.f	Cr	A	2-3	3-4	2-3	1	**25.9**	0.1	1.0	8.1	96.0	10.5	0.0	0.1	2.3	24.0	25.0	0.0	0.1	8.0	60.0	37.0	0.1	1.0	12.3	96.0
Phaeophyscia endococcina (Körb.) Moberg	Ph.e	Fo	S	2-3	3-4	1-3	2-3	1.9	0.0	0.1	0.2	12.0	-	-	-	-	-	-	-	-	-	-	3.7	0.0	0.1	0.4	12.0
Phaeophyscia orbicularis (Neck.) Moberg	Ph.o	Fo	A	2-5	3-5	3-4	4-5	5.6	0.0	1.0	2.5	96.0	10.5	0.0	0.1	5.5	96.0	-	-	-	-	-	3.7	0.0	1.0	1.2	32.0
Phlyctis argena (Spreng.) Flot.	Pl.a	Cr	A	1-2	2-3	2-3	1-2	22.2	0.5	5.0	5.8	84.0	26.3	0.3	4.0	2.5	12.0	-	-	-	-	-	25.9	0.7	5.0	9.8	84.0
Physcia adscendens H. Olivier	Py.a	Fo	A	2-5	4-5	3-4	3-5	7.4	0.0	2.0	1.6	60.0	-	-	-	-	-	-	-	-	-	-	14.8	0.1	2.0	3.3	60.0
Physcia aipolia (Humb.) Fürnr.	Py.i	Fo	S	2-3	4-5	3	3-4	3.7	0.0	0.1	0.4	20.0	-	-	-	-	-	-	-	-	-	-	7.4	0.0	0.1	0.9	20.0
Physcia magnussonii Frey	Py.m	Fo	S	3-4	4-5	4-5	3-4	1.9	0.0	0.1	0.1	4.0	-	-	-	-	-	-	-	-	-	-	3.7	0.0	0.1	0.1	4.0
Physconia grisea (Lam.) Poelt	Ps.g	Fo	A	3-4	3-5	3	4-5	1.9	0.0	0.1	0.7	40.0	-	-	-	-	-	-	-	-	-	-	3.7	0.0	0.1	1.5	40.0
Protoparmeliopsis muralis (Schreb.) M. Choisy s. lat.	Pr.m	Cr	S	2-4	3-5	3-4	3-5	14.8	0.4	18.0	4.7	96.0	-	-	-	-	-	25.0	0.0	0.1	2.0	12.0	22.2	0.9	18.0	8.7	96.0
***Rhizocarpon disporum* (Hepp) Müll. Arg. (± *Rhizocarpon reductum* Th. Fr.)**	Rh.d	Cr	S	1-3	3-5	2-4	1-3	**27.8**	0.4	6.0	5.9	56.0	5.3	0.0	0.1	0.2	4.0	25.0	0.9	6.0	10.0	56.0	44.4	0.5	3.0	8.6	40.0
Rhizocarpon geographicum (L.) DC. s. lat.	Rh.g	Cr	S	1-3	3-5	3-4	1-3	7.4	0.0	1.0	0.5	8.0	-	-	-	-	-	-	-	-	-	-	14.8	0.0	1.0	1.0	8.0
Rinodina occulta (Körb.) Sheard	Ri.o	Cr	S	1-2	3-4	2-3	1	5.6	0.0	1.0	0.7	28.0	-	-	-	-	-	-	-	-	-	-	11.1	0.0	1.0	1.5	28.0
Rufoplaca gr. *arenaria* (Pers.) Arup *et al.*	Ru.s	Cr	S	2-3	4-5	3-4	2-3	9.3	0.0	2.0	2.0	48.0	5.3	0.0	0.1	0.4	8.0	-	-	-	-	-	14.8	0.1	2.0	3.7	48.0
Rusavskia elegans (Link) S.Y. Kondr. & Kärnefelt	Rv.e	Fo	S	3-5	4-5	4	3-4	3.7	0.0	1.0	0.6	28.0	-	-	-	-	-	-	-	-	-	-	7.4	0.0	1.0	1.2	28.0
Scoliciosporum umbrinum (Ach.) Arnold	Sc.u	Cr	S	1-3	3-4	2-4	1-3	1.9	0.0	0.1	0.1	4.0	-	-	-	-	-	-	-	-	-	-	3.7	0.0	0.1	0.1	4.0

(*Continued*)

Table 1. (Continued)

Species	Ecological indicator values							All plots (n = 27)					3YC rocks (n = 19 plots)					12YC rocks (n = 8 plots)					NRC rocks (n = 27 plots)				
	Code	GF	Repr.	pH	IR	AR	EU	Occurrence (plot %)	Cover (%) Mean	Max.	Frequency (%) Mean	Max.	Occurrence (plot %)	Cover (%) Mean	Max.	Frequency (%) Mean	Max.	Occurrence (plot %)	Cover (%) Mean	Max.	Frequency (%) Mean	Max.	Occurrence (plot %)	Cover (%) Mean	Max.	Frequency (%) Mean	Max.
Verrucaria nigrescens f. *tectorum* (A. Massal.) Coppins & Aptroot	Ve.n	Cr	A	3-5	3-5	2-5	2-5	22.2	0.5	17.0	10.8	100.0	-	-	-	-	-	62.5	2.4	17.0	34.5	100.0	25.9	0.3	4.0	11.4	100.0
Xanthoparmelia with isidia*	X.is	Fo	A	2-3	3-5	3-4	2-4	29.6	3.6	50.0	13.7	100.0	-	-	-	-	-	12.5	0.6	5.0	7.0	56.0	55.6	7.0	50.0	25.3	100.0
Xanthoparmelia without isidia**	X.ni	Fo	S	2-3	3-5	3-4	2-3	46.3	3.0	45.0	21.6	100.0	5.3	0.0	0.0	1.3	24.0	25.0	1.5	12.0	9.5	72.0	81.5	5.5	45.0	39.6	100.0
Xanthoparmelia glabrans (Nyl.) O. Blanco et al.	Xa.g	Fo	S	2-3	4-5	3	2-3	33.3	0.5	10.0	5.3	80.0	-	-	-	-	-	12.5	0.3	2.0	2.5	20.0	63.0	0.9	10.0	9.8	80.0

* *X. conspersa* (Ehrh. ex Ach.) Hale more frequent, but also *X. mexicana* (Gyeln.) Hale, *X. plittii* (Gyeln.) Hale, *X. tinctina* (Maheu & A. Gillet) Hale and *X. verrucigera* (Nyl.) Hale present
** *X. angustiphylla* (Gyeln.) Hale more frequent, but also *X. stenophylla* (Ach.) Ahti & D. Hawksw. Present

per plot (mean 8.6–39.6%), but very different cover values related to the different growth form, with foliose and continuous crustose thalli (mean cover 0.5–7.0%, but maximum cover of 6.0–50.0%) recording higher cover values than discontinuous crustose thalli (e.g. *C. vitellina*, *P. flavicans*: mean cover < 0.2%, and maximum up to 2.0%). Other taxa also displayed rather high values of diffusion (i.e. occurrence in the plots; see Table 1) (15–30% of plots) and frequency, including a group of species commonly found on stone heritage surfaces even in urban environments, such as *Protoparmeliopsis muralis* and *Verrucaria nigrescens* f. *tectorum*, and others which are usually associated with bark rather than rock substrata, such as *Candelaria concolor*, *Phlyctis argena* and *Physcia adscendens*. These are all nitrophytic species, sharing a high tolerance to eutrophication and, with the exception of *P. muralis*, an asexual reproductive strategy. Remarkably, the group of commonly epiphytic species showed the highest diffusion on 3YC rocks, together with *C. caesiocinerea,* and also *Fuscidea lygaea* and *P. flavicans* which are meso-hygrophytic species, poorly tolerant to eutrophication. On 12YC rocks, lichen diversity was almost completely represented by the taxa dominating NRC rocks (*C. caesiocinerea* > green-yellow *Xanthoparmelia* spp., *C. vitellina* > *C. chlorina* > *R. disporum* > *X. glabrans*) and the nitrophytic saxicolous species *V. nigrescens* and *P. muralis*, which similarly showed high diffusion, frequency and cover values, while the presence of typically epiphytic species was limited to *C. concolor*.

The PCoA extracted four components which explained 65.4% of the total variance and ordinated the plots on the basis of specific frequency data (Fig. 2). Axis 1 (29.1% of total variance) showed a strongly positive correlation with *Xanthoparmelia* spp. without isidia (X.ni in Fig. 2) and *C. vitellina* (Cn.v), which displayed the highest frequency values, while axis 2 (15.4%) showed a distinct positive correlation with *V. nigrescens* (Ve.n) and *C. chlorina* (Ca.c), and a negative correlation with *Phlyctis argena* (Pl.a), and axis 3 (13.0%) a positive correlation with *Xanthoparmelia* spp. with isidia (X.is). Accordingly, plots on NRC rocks, with the highest abundances of these dominant species, mostly scattered on the right side of the diagram. Conversely, plots of 12YC and 3YC rocks scattered on the left side, probably driven by the relatively lower frequencies of dominant species more than the abundance of other subordinate species. It should be noted that the 10 plots without lichens are not represented in the ordination.

## Lithobiontic penetration within the sandstone substratum

RLM observations showed a limited penetration within the sandstone substratum for both the cyanobacterial-dominated biofilm and the lichens. The microbial biomass developed only epilithically, with the exception of very limited chasmoendolithic growths, down to *c.* 500 µm, where slight fractures occurred (Fig. 3A). The hyphal penetration component of *Verrucaria nigrescens* was also poorly pervasive, with a discontinuous occurrence of thin hyphal bundles down to 500 µm within the substratum (Fig. 3C & D). The penetration of *Xanthoparmelia conspersa* was even poorer, with only a couple of hyphal bundles observed down to 1 mm beneath one of the observed thalli (Fig. 3B).

Factors conditioning lithobiontic and lichen colonization

The analysis of cover values estimated for the different lithobiontic groups and environmental variables (CCA-I) extracted four axes which accounted for 100% of species-environmental

Table 2. Percentage contribution from the SDR simplex analyses (Podani & Schmera 2011) of lichen communities through the surveyed plots, considered in total, in combination and separately for NRC, 12YC and 3YC rock surfaces.

	Plots (n)	Similarity (S)	Richness difference (D)	Replacement (R)	R+D (Beta diversity)	S+R (Richness agreement)	S+D -Anti-nest. - Rich. Id. (Nestedness)
All plots	54	18.8	43.8	37.5	81.2	65.5	38.5
NRC+3YC	46	19.4	42.7	37.9	80.6	62.1	39.3
NRC+12YC	35	25.5	48.6	25.8	74.5	74.2	43.5
12YC+3YC	27	12.3	46.4	41.4	87.7	58.6	25.9
NRC	27	28.2	50.5	21.4	71.8	78.6	43.9
12YC	8	22.5	39.2	38.4	77.5	61.6	51.7
3YC	19	17.7	46.3	36.0	82.3	64.0	27.1
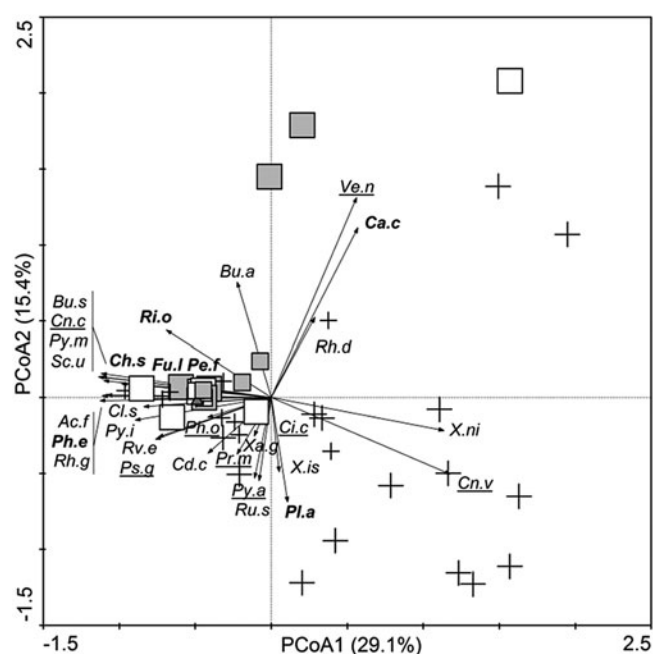
Figure 2. Ordination of plots on the basis of the specific lichen frequencies (PCoA). Plots are marked according to the different conservation history of the surveyed rocks (NRC = crosses, 12YC = grey squares, 3YC = white squares). The half of the plots with the highest lichen abundance for the NRC and 12YC/3YC categories (in terms of total specific frequencies) display a larger symbol size. Species abbreviations follow Table 1 (nitrophytic species underlined, meso-hygrophytic species in bold).

relationships (Fig. 4A). All canonical axes were significant (Monte Carlo test, $P = 0.002$). The first axis (60.9% of correlation) was positively correlated with surface roughness (ROU, weighted correlation (w.c.) = 0.89) and negatively with the distance from bare and vegetated ground upstream of the plot (GRP, w.c. = −0.32), while the second axis (30.4%) was positively correlated with rock inclination (INC, w.c. = 0.80) and negatively with tree cover (TRC, w.c. = −0.23) and GRP (w.c. = −0.41). Only ROU and INC were significant conditional factors ($P = 0.002$). Plots on NRC rocks scattered in the upper and right part of the diagram, positively correlated with lichens and mosses, respectively. 12YC and 3YC plots scattered through the whole diagram, including the lower left quadrant, correlated with cyanobacterial and green-algal biofilms.

The analysis of lichen frequency data and environmental variables (CCA-II) extracted four axes which accounted for 93% of species-environmental relationships (Fig. 4B and Supplementary Material Fig. S4, available online). All canonical axes were significant (Monte Carlo test, $P = 0.002$). The first axis (36.9% of correlation) was positively correlated with rock inclination (INC; w.c. = 0.65) and negatively with the distance from bare and vegetated ground upstream of the plot (GRP, w.c. = −0.70). The second axis (32.7%) was positively correlated with tree cover (TRC, w.c. = 0.75) and surface micromorphology (ROU, w.c. = 0.44) and negatively with surface aspect (w.c. = −0.45). All factors, except surface aspect, showed significant conditional effects according to forward selection, with tree cover displaying the highest value (F = 2.48, $P = 0.002$), followed by inclination (F = 2.39, $P = 0.004$), surface micromorphology (F = 2.28, $P = 0.006$) and distance from the ground (F = 1.71, $P = 0.036$).

Given that uncolonized plots do not appear in the factorial map, most of the colonized plots on 3YC and 12YC rocks, including those with the highest lichen abundance (in terms of total lichen frequencies), showed a positive correlation with tree cover and/or distance from the ground, in the space characterized by the most abundant meso-hygrophytic species *Fuscidea lygaea* and *Pertusaria flavicans* and the typically epiphytic species. Plots on NRC showing the highest lichen abundance mostly scattered in the right lower part of the diagram, in the space characterized by the dominant xerophytic species, namely the *Xanthoparmelia* spp. with (X.is) and without isidia (X.ni), *Candelariella vitellina* (Cn.v) and *Rhizocarpon disporum* (Rh.d), and the mesophytic *Caloplaca chlorina* (Ca.c) (Supplementary Material Fig. S4).

Control of lithobiontic recolonization by preventive microenvironmental conditioning

Assays of the efficacy of Biotin T against lithobionts on Rock 70, and the cyanobacterial biofilm in particular, showed a significant decrease of F_0 values in the treated parcels (decrease > 80%) with respect to measures performed before the biocide application, and the zeroing of F_v/F_m (Fig. 5A & B). Twenty months after the cleaning intervention, and after two winter seasons, F_0 values quantified on the rock surface protected by the wall were zeroed, while slightly higher values were detected in the unprotected area, suggesting that recolonization was possibly starting. Accordingly, after 20 months more, F_0 and
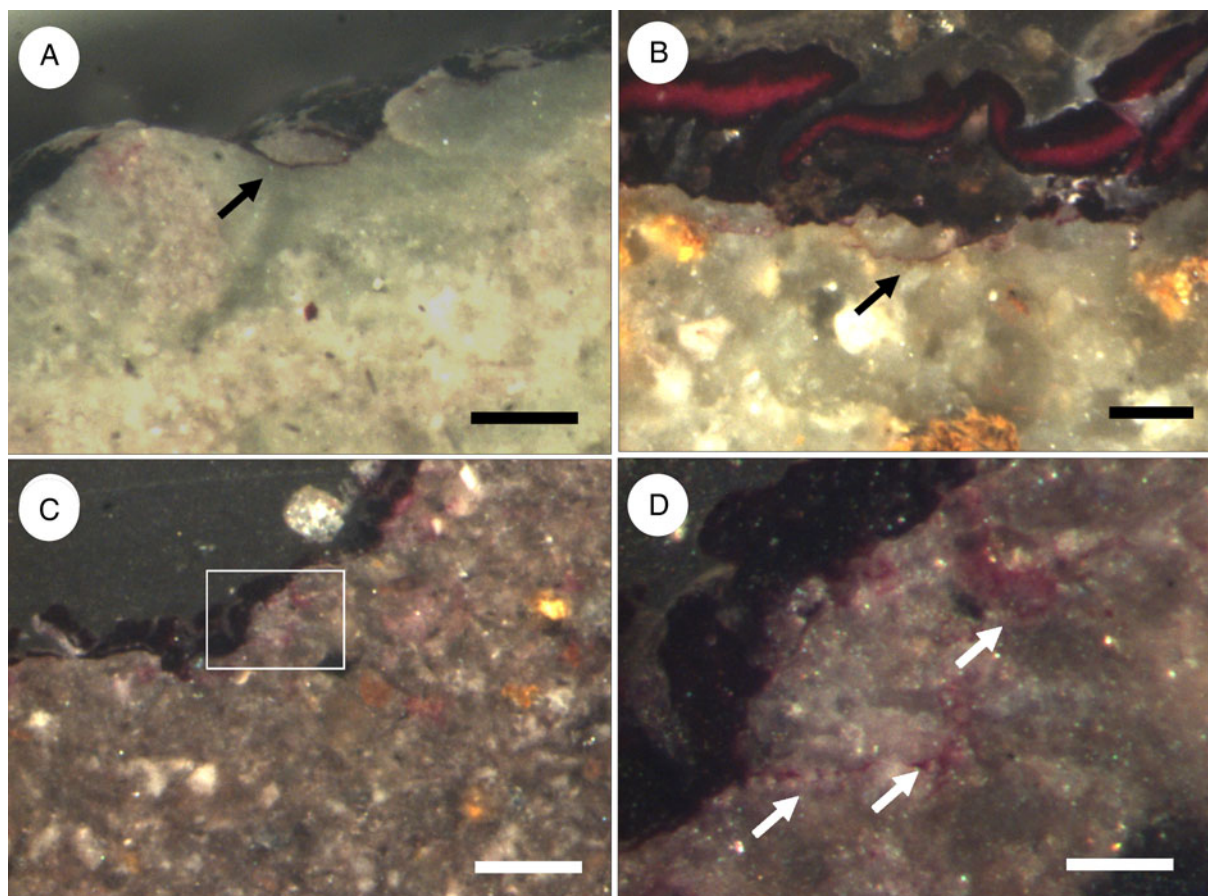
Figure 3. Lithobiontic penetration within the sandstone substratum. A, cyanobacterial biofilm. B, *Xanthoparmelia conspersa*. C & D (inset), *Verrucaria nigrescens*. Arrows indicate cyanobacterial penetration within a fracture (A) and the hyphal penetration component of lichens (B & D). Scales: A = 1.0 mm; B & C = 1.5 mm; D = 350 μm. In colour online.
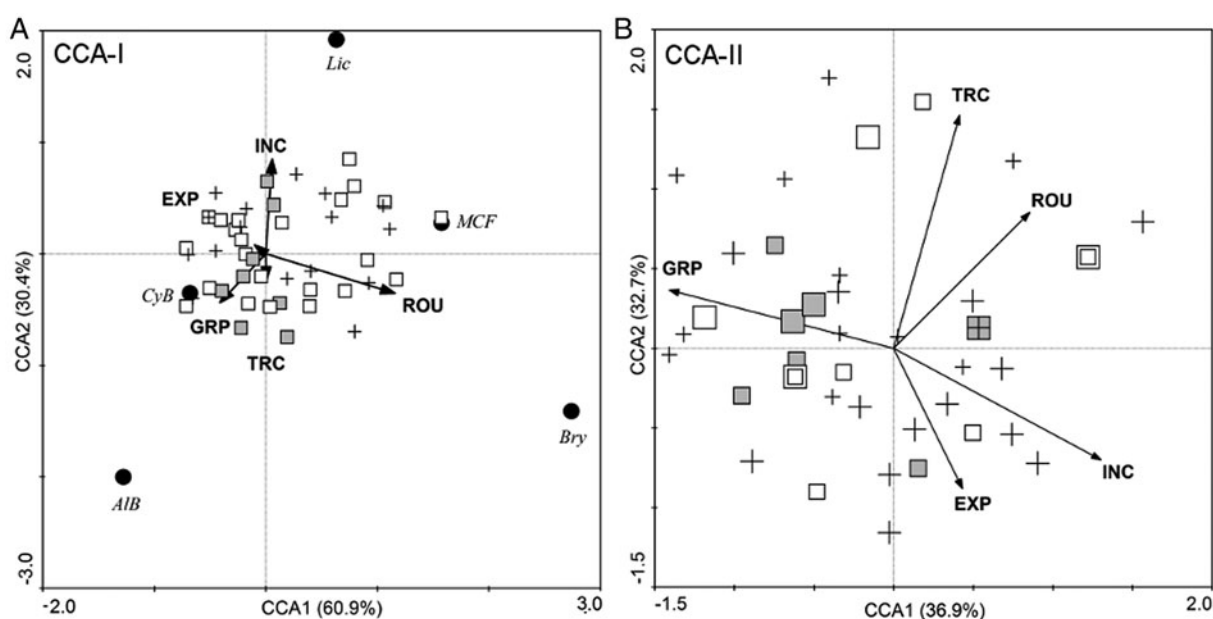

Figure 4. Factorial map in the canonical correspondence analysis showing the position of plots having a different conservation history with the contributions of lithobiontic covers (A, CCA-I) and specific lichen frequencies (B, CCA-II), together with environmental factors (TRC = tree cover, ROU = surface micromorphology, INC = inclination, GRP = distance from bare or vegetated ground upstream, EXP = aspect). Symbols indicate different lithobionts (black circles): Lic = lichens, Bry = bryophytes, CyB = cyanobacterial biofilm, AlB = green-algal biofilm, MCF = meristematic fungi); and NRC (crosses), 12YC (grey squares) and 3YC (white squares) rocks. In CCA-II (B), the half of the plots with highest lichen abundance for the NRC and 12YC-3YC categories (in terms of total specific frequencies) display a larger symbol size; contributions of the different species are shown separately in Supplementary Material Fig. S4 (available online).
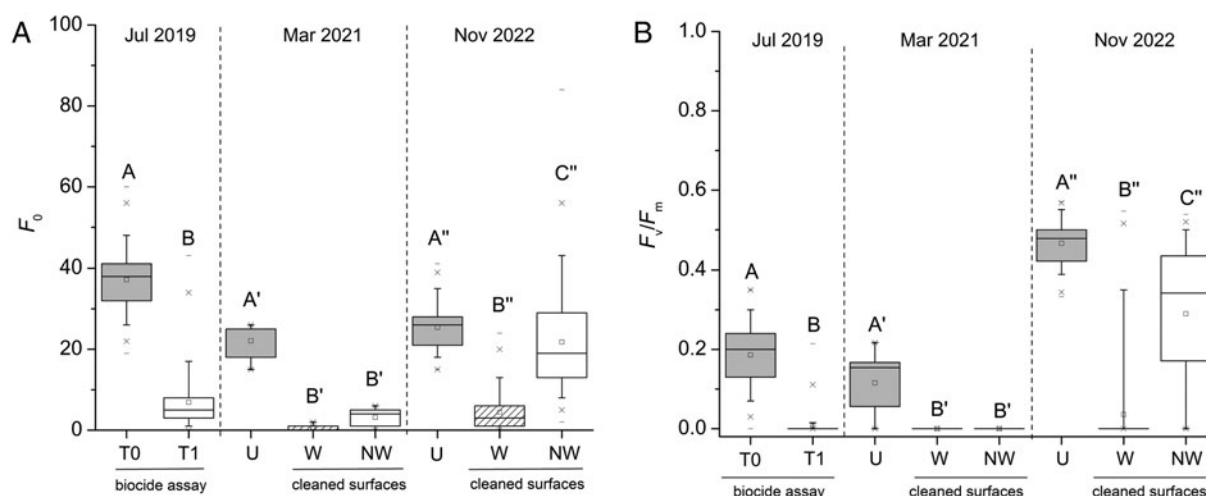
Figure 5. Basal fluorescence (F_0; A) and maximum quantum efficiency of Photosystem II photochemistry (F_v/F_m; B) quantified on Rock 70 during preliminary biocide assays (July 2019; T0 = one day before biocide application, T1 = one day after biocide application), and 20 (March 2021) and 40 (November 2022) months after the cleaning, in areas of the outcrop protected (W) and non-protected (NW) by the wall, and on uncleaned areas as control (U). Box-plots show 95th percentile (upper whisker), 75th percentile (top box), median (transversal line), mean (small square), 25th percentile (bottom box), 5th percentile (lower whisker). At each measuring time point, box-plots which do not share at least one letter are statistically different (ANOVA, Tukey's test, $P < 0.05$).

F_v/F_m values quantified on the unprotected surface indicated the recovery of the lithobiontic colonization, while values were still zeroed in the area protected by the wall (with the exception of a single parcel, close to the ground at the foot of the rock). Lichen recolonization was not observed either in the protected or in the unprotected areas of Rock 70.

At 20 months after the cleaning, cleaned surfaces protected and unprotected by the wall did not show significant differences in lightness (L*), while uncleaned and unprotected surfaces had lower L* values (Fig. 6). Twenty months later, the rock surfaces unprotected by the wall were significantly darkened (low L* in Fig. 6), with different levels of darkening depending on the proximity to the vegetated ground upwards and the prevalent direction of water fluxes. Conversely, rock surfaces well protected by the

Figure 6. Lightness of the surface (L*) of Rock 70 quantified 20 (March 2021) and 40 (November 2022) months after the cleaning in areas of the outcrop protected (W) and non-protected (NW) by the wall, and on uncleaned areas as control (U). Box plots as in Fig. 5. At each measuring time point, box-plots which do not share at least one letter are statistically different (ANOVA, Tukey's test, $P < 0.05$).

wall showed no or just perceivable differences in L*, and uncleaned control surfaces (Rock 71) displayed a smooth darkening (because they were already dark).

Discussion

Approaches to hinder recolonization dynamics following cleaning interventions are still mostly related to the application of products directly on the heritage surfaces in order to reduce their bioreceptivity (e.g. Pinna *et al.* 2012; Sasso *et al.* 2016; Domínguez *et al.* 2021), and to the regulation of artificial light regimes (Sanmartín 2021). In the case of rock art, hypotheses and suggestions on a potential conservation effect of reducing the shade created by trees, and redirecting water flow, were formulated (Tratebas 2004) but have been poorly experimentally verified and put into practice (e.g. in the case of Norwegian sites; Bjelland & Kjeldsen 2020). In this work, we show that the characterization of lithobiontic communities in a rock art site and the recognition of environmental factors favouring (re-)colonization dynamics may address preventive strategies based on local (micro-)environmental conditioning, successfully prolonging the clean state of heritage surfaces. The characterization of lichen diversity particularly supported the recognition of factors responsible for lithobiontic colonization patterns, confirming the role of lichens as useful indicators in various fields of application, including the conservation of Cultural Heritage (Aptroot & James 2002).

Lichens and other lithobionts on rocks with different conservation histories

The lack of detailed knowledge on the conservation history of each outcrop in the Naquane site before the 1980s (further details in the caption of Supplementary Material Fig. S3, available online) prevents a full reconstruction of (re-)colonization patterns in the investigated site. Nevertheless, the abundances of lithobiontic components within the plots are significantly explained by their different colonization rates following recent cleaning interventions and some heterogeneity in available niches.
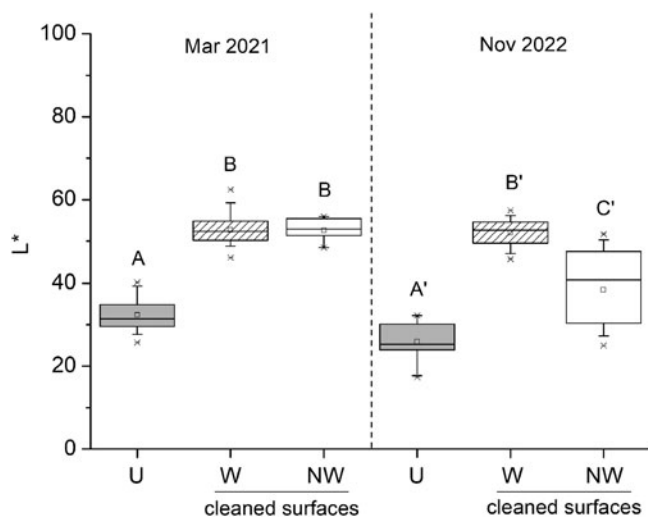
Microbial biofilms, including cyanobacterial ones, were reported as the main lithobiontic component in several rock art sites. Their presence was variously associated with biodeterioration or bioprotection processes, which depend on the lithology and the environmental conditions (Villa *et al.* 2016), and even in some cases with the past formation of surface crusts which coat the stones and were carved by the engraving activities (Rabacchin *et al.* 2022; Zerboni *et al.* 2022). In the case of Naquane, the low porosity and high cohesion of the substratum seem to limit a diffuse endolithic, and more deteriogenic, behaviour of cyanobacteria, which find enough suitable conditions for a rich epilithic growth in the local temperate climate with no dry season (Rubel *et al.* 2017). The prevalence of cyanobacterial and algal patinas on 3YC surfaces agrees with their ability to colonize rocks faster than lichens (e.g. Lázaro *et al.* 2008), which are in turn widespread on 12YC and prominent on several NRC outcrops. In agreement with the succession proposed by Caneva *et al.* (2008), mosses are also negligible on 3YC and 12YC surfaces, while they are dominant on some NRC outcrops. Such different levels of pioneer activity combine with a preference in mosses and lichens for rougher and less steep surfaces with respect to the biofilms, as displayed in CCA-I (Fig. 4A).

Levels of direct irradiation and shading were shown to influence the distribution (and deteriogenic impact) of lithobiontic components on building surfaces, with epilithic cyanobacteria and green algae dominating shaded sides and lichens prevailing on sunny dry ones (Ariño & Saiz-Jimenez 1996). Moreover, for each component, the different (micro-)environments host different species assemblages, as shown in the cases of the Roman Amphitheater of Italica (Spain; Nimis *et al.* 1998) and the engraved schists of the Côa Valley Archaeological Park (UNESCO, Portugal; Marques *et al.* 2014), where different lichen communities characterized surfaces with different aspects. In the case of Naquane, the aspect (EXP) factor was not a significant conditional factor, either with respect to the distribution of the different lithobiontic components or for the different lichen taxa. This is probably because the effect of the local surface aspect was masked by the general NW exposure of the side of the valley occupied by the park. However, different lichen communities were observed in Naquane, with the high beta-diversity values obtained in SDR analysis mostly associated with the turnover of xerophytic and mesophytic-hygrophytic species, as shown by the PCoA. Such patterns of lithobiontic distribution on heritage stone surfaces were generally related to different orientations and aspects (Aubry *et al.* 2012; Adamson *et al.* 2013; Marques *et al.* 2014). In the case of Naquane, each outcrop was differently shaded by tree cover and exposed to water run-off after rain events (see below).

Lichen communities on 12YC and 3YC plots mostly showed very low cover values and appear as subsets of the richer communities on NRC outcrops. Nevertheless, the higher similarity of 12YC and NRC with respect to the NRC-3YC and 12YC-3YC combinations (SDR analysis) indicates that the earliest pioneer phase of recolonization is already concluded in less than 12 years after the cleaning interventions. Species commonly found in synanthropic environments prevail, although some species usually associated with undisturbed conditions persist, such as *Fuscidea lygaea* and *Pertusaria flavicans*. Such a pattern reflects the shift observed on several heritage surfaces after cleaning interventions, with nitrophytic, fast-growing species becoming more prevalent than originally dominant species (Nascimbene *et al.* 2009). Persistence of original species and, in general, fast recolonization in not many years is probably related to the ineffective application of biocides by brush, which generally showed poor effectiveness in the devitalization of crustose species and particularly in dedicated assays recently performed in Naquane (Favero-Longo *et al.* 2021). Such results show the importance of performing effective devitalization treatments to avoid losing the original lichen biodiversity value without obtaining a durable cleaning result. Remarkably, most species on 12YC and 3YC plots show a prevalence of asexual reproductive modes (mostly soredia) and/or produce small, highly dispersive ascospores (species of the genera *Caloplaca* s. lat., *Candelariella* s. lat. and *Lecanora* s. lat.), indicating their potential for rapid recolonization and their potential threat to heritage surfaces (Scheidegger & Werth 2009; Morando *et al.* 2019). It is worth noting that the total diversity of 37 taxa is rather low for the surveyed area, and mostly includes common species of silicate substrata. This result may reflect the fact that the communities on NRC rocks are also the product of long-term recolonization processes over several decades following the early and, unfortunately, poorly documented cleaning interventions in the area. However, the comparison with outcrops outside the boundaries of the park was beyond the aims of this project and, surprisingly, it may be very difficult to find outcrops in the mid Valle Camonica which do not host engravings and therefore have not suffered any human disturbance in recent times.

Physical interaction of lichens and other lithobionts with the sandstone substratum

Lichen colonization of engraved outcrops and the resulting deteriogenic impact have already been investigated in several sites, including the Côa Valley, in the Mediterranean area, where deep hyphal penetration and physical bioweathering were recorded on schists (Marques *et al.* 2016). Lichens are also dominant on engraved sandstones from the subarctic zone, where their biogeochemical activity was associated with the waning of an original surface red colour (e.g. Alta, Norway; Tansem & Storemyr 2021), to the dry semi-arid zone, where physical and chemical degradation processes have been microscopically documented (e.g. El Morro National Monument, New Mexico; Knight *et al.* 2004). Although the observations were limited to a small number of cross-sections for conservation reasons, the physical interaction of lichens with the examined sandstones appears rather mild, as we observed poor hyphal penetration even for *Verrucaria nigrescens*. This common colonizer of heritage surfaces has often been reported as a deeply penetrating and impacting species on different lithologies, including other sandstones (Tonon *et al.* (2022), with references therein), although with different intergranular matrices and lower level of compaction. The hyphal penetration beneath the points of attachment of *Xanthoparmelia* rhizinae was also negligible, in this case as usually observed on other lithologies (e.g. on gneiss; Favero-Longo *et al.* 2015). The cyanobacterial biofilm also displayed an epilithic behaviour, differing from observations on other sandstone substrata, in which the endolithic growth was prominent (e.g. Büdel *et al.* 2004; Zerboni *et al.* 2022). Accordingly, the lithobiontic colonization in Naquane appears as a deteriogenic phenomenon mostly because of surface masking and chromatic disfiguring, while interactions with the substratum responsible for a decreased surface durability seem less important than in other cases. However, we observed a higher hyphal penetration on the same lithology but on the opposite, ESE-facing, side of the valley (Favero-Longo *et al.* 2017), in agreement with the findings that different microenvironmental conditions related to a different surface aspect can result in different bioweathering impacts on stone durability (Marques *et al.* 2016).

Tree cover and water flow as driving factors and their potential conditioning for preventive conservation

A long period of wetness, due to slow drying or prevailing wind directions, has been demonstrated to support lithobiontic colonization on stone materials. Investigations in wet Northern Ireland showed that green algae and lichens colonized north-facing stone blocks (including sandstones) faster and more abundantly than those facing south (Adamson *et al.* 2013). In Pompeii, surfaces exposed to the prevailing winds during rain events showed richer lithobiontic communities than differently oriented ones (Traversetti *et al.* 2018). In the case of Naquane, in a similar way, tree shading (TRC) and the presence of bare or vegetated ground above the engraved outcrops (GRP) are factors favouring lithobiontic recolonization after cleaning, according to CCAs. Their significant effect on water and moisture availability, and the consequent biological dynamics, is confirmed by the prevalent regrowth of meso-/hygro-phytic lichen species on 12YC and 3YC surfaces (PCoA). By contrast, recolonization by xerophytic species on directly exposed rock outcrops seems to require longer. The abundance of usually epiphytic species as pioneer colonizers on the 3YC and 12YC surfaces further indicates the threats related to tree proximity, even beyond the shading effect.

The recognition of the environmental factors that favour lithobiontic (re-)colonization was considered with success in the experiment of preventive conservation conducted on Rock 70, combining some reduction of tree cover with the altering of water flow on an engraved rock outcrop. The development of a phototrophic biofilm and the darkening of the rock surface, quantified by fluorimetric and colorimetric measures, respectively, was significantly related to the absence of the wall protection by prolonged and nutrient-enriched water fluxes. Thus, preventive approaches and the (micro-)environmental conditioning by water flow regulation seem particularly promising to limit lithobiontic communities and related biodeterioration effects on surfaces and thus preserve the legibility of engravings. On other surfaces, the lithobiontic presence may instead be accepted, and possibly exhibited as an additional value of the cultural heritage site.

On the other hand, the change of water flows may imply some community shift in the long term, in particular favouring lichens rather than cyanobacterial biofilms (Bjelland & Helberg 2006), although lichens have still not (re-)appeared 40 months after cleaning the whole outcrop. More generally, the drainage of water or, simply, the altering of water flows imply the addition of non-natural elements in the archaeological natural scenario, such as the considered brick wall or other kinds of barriers (Bjelland & Helberg 2006). With this regard, it must be noted that the wall considered here is an experimental structure to evaluate benefits obtainable through the control of water fluxes. The development of further strategies to obtain similar results without touching the engraved surface is necessary. In any case, although barriers to water flows may be visually unappealing, the traditional application of synthetic biocides to periodically devitalize and remove established lithobiontic communities may have an even greater impact by affecting the environmental equilibria (Cappitelli *et al.* 2020).

Conclusions

This work characterized the diversity and abundance of lithobiontic communities in the Rock Engravings National Park of Naquane (UNESCO WHS n. 94, Italy), highlighting cyanobacterial biofilms and lichens as the dominant constituents. Both

displayed poor penetration within the sandstone substratum, probably because of its high level of compaction and very low porosity, but they were responsible for chromatic disfiguring and limited the legibility of rock art. Tree cover and the presence of bare and vegetated ground upstream of the rocks were determined as the main drivers of recolonization on surfaces cleaned in the last 12 years, probably prolonging surface wetness after rain events and increasing nutrient availability. Nitrophytic species, including epiphytes from surrounding trees, and a small number of meso-hygrophytic species, mostly producing soredia, were mainly responsible for the rapid lichen recolonization. An experiment on preventive conservation performed on a critical rock, including an effective devitalization of lithobionts before cleaning combined with a reduction of tree cover and surface protection from prolonged water fluxes from vegetated ground, prevented recolonization by lichens and other lithobionts for a monitored period of 40 months. By contrast, cleaned surfaces unprotected from prolonged water fluxes showed recolonization, demonstrating the suitability of microenvironmental control strategies to limit and delay biodeterioration on outdoor stone cultural heritage. To make similar preventive approaches practicable, ecological investigations of environmental factors favouring lithobiontic colonization are crucial and, thanks to advanced knowledge on their specific ecological requirements, lichens in particular appear to be suitable indicators.

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