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(Article begins on next page)

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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 practiced to limit biodeterioration issues threatening indoor cultural heritage objects, while they are still poorly exploited for the conservation of the 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 which had been cleaned in the last three (3YC) or twelve (12YC) years or from more than 40 years (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 these 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 few meso-hygrophytic species, with prevalence of asexual reproductive strategies. Multivariate analyses including environmental parameters (canonical correspondence analyses) indicated the tree cover and the presence of bare or vegetated ground upstream of the rocks, likely prolonging wetness and providing nutrients by water transport, as 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, done 40 months after this new cleaning intervention, displayed recolonization on the unprotected area only, indicating the potential of preventive conservation strategies also in outdoor environments.

Keywords

biodeterioration, biofilm, cultural heritage, recolonization, nitrophytic community

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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 substrates, 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 were 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, as inscriptions or art reliefs (Pinna 2017). In a broader sense, any lithobiontic cover distances the heritage surface appearance from the original author's conception. Therefore, curators of the 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, a wide use of synthetic chemicals as biocides, practiced for decades, is now increasingly considered environmentally unsustainable, and new alternative products and/or chemical-free approaches to control lithobionts are incessantly searched for (Cappitelli et al. 2020).

Lichenologists, and potentially others, may have different priorities than 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

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lithobiontic colonization, although distancing the stone appearance from its original one, sometimes contributing to its positive integration with the surrounding natural context. With this regard, Nimis and colleagues (1992) early invoked the possibility of considering lichens as an additional cultural value in certain heritage sites, such as archaeological areas, worth to be preserved and brought to the attention of visitors.

Lithobiontic colonization and biodeterioration effects deserve particular attention when affecting rock art, as biological growths and the artworks may display a rather similar dimensional extent (i.e. (sub-)millimetric thickness), thus particularly implying 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 as consolidants and water-repellents, have been thus routinely practiced in rock art sites to (i) periodically remove lichens and other lithobionts from engraved surfaces, and (ii) 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, alternative approaches to control lithobionts on engraved rocks were assayed, 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

Manuscript For Review

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, DM 10/05/2001; MIBAC 2001) to limit biodeterioration in indoor environments (Caneva et al. 2008), still appear poorly considered in the case of the outdoor stone heritage, and for rock art in particular.

In the Rock Engravings National Park of Naquane, 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 were registered since 1980s but started long before (www.irweb.it; Ruggiero & Poggiani-Keller 2014). In the last decades, recolonization dynamics on certain rocks, mostly related to fast spreading of cyanobacterial biofilms, even renewed the necessity of cleaning every few (2-3) years. 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 a potential stress on rock surface due to the repeated mechanical treatments. Therefore, a research project 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). In 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 history and environmental conditions. It also gives an insight into their physical interaction with the sandstone substrate. 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

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hypotheses that: (a) some environmental factors are main drivers of diversity and abundance of lichens and other lithobionts on recently cleaned surfaces, (b) lichens and other lithobionts penetrate within the sandstone substrate, and (c) 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 approx. 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 few to approx. 250 square meters (e.g. Rock 1, named the “Great Rock of Naquane”, with 65 m^2 of engraved surface; Liborio et al. 2011). In particular, engravings are carved 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 quite high overburden (several kilometres) during burial which determined 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). 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 av. 2 °C in winter, 21 °C in summer, and 1000 mm rainfall yr^{-1} (Ceriani & Carelli 2000; data monitored in the Capo di Ponte monitoring station n. 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., *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, as well as 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 having a different conservation history (information available at www.irweb.it). In particular, 54 plots, 50 × 50 cm, were distributed on the surfaces of: (i) six rocks which were last cleaned in the period 2014-2015 (3YC; Rocks 1, 35, 50, 70, 73, 99; n= 19 plots), (ii) four rocks which were last cleaned in the period 2005-2008 (12YC; Rocks 6, 7, 14, 57; n = 8 plots), and (iii) 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). In particular, interventions performed in the period 2005-2008 included mechanical removal of thalli, cleaning with NeoDes 5% or 10%, application of the benzalkonium chloride based product Preventol 3% as preservative, 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 strongly larger surface, and of 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 lab on digital images. In the case of biofilms, the extent of microbial mats which determined a visible colour shift of the surface, with respect to the bare rock, was considered. Sampling and microscopic observations allowed to characterize the biofilm(s) of each plot 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 substrate for conservative reasons, to check field identifications in the lab. Lichen identification was based

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on Wirth (1995), Smith et al. (2009) and the online keys published in ITALIC, the Information System of the Italian Lichens, version 07 (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 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, likely 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 the 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,

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12YC, 3YC). The analysis was performed on the matrix of species presence/absence with the SDR Simplex software using the Simplex method, as elsewhere detailed (SDR Simplex; Podani and 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 and Verdonschot 1995). The ordinations were performed using CANOCO 4.5 (Ter Braak and 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 substrates. Rock fragments (ca. 3-4 × 2-3 × 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.

Manuscript For Review

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 were conducted since the 1980s, the last in 2014 (details in the caption of Supplementary Material Fig. S2). In 2017, after three years only, 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 four-hours poultice application of the biocide BiotinT (N-octyl-isothiazolinone, 7–10%, and didecyl-dimethyl ammonium chloride, 40–60%, as active principles; CTS, Altavilla Vicentina, Italy). Its effectiveness had been verified by fluorimetric measurements on other outcrops of the Park (Favero-Longo et al. 2021) and further checked on few parcels on Rock 70 itself (see below). In Autumn 2019, a 10 cm tall and approx. 3 m long wall of bricks, covered and fixed with mortar, was built at 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.

Manuscript For Review

Measurements of the vitality of the cyanobacterial-dominated biofilm were performed 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). Measures were performed early in the morning, on pre-moistened and dark-adapted surfaces. In particular, measures were distributed on three parcels (approx. 25 ×25 cm) on different parts of the rock outcrop (n>70 at each measuring time point). Measures on an additional untreated parcel were also collected as 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 twenty and forty months after the cleaning (i.e. in March 2021, after the limitations due to 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 spectro-colorimetric measures, in order to evaluate the potential deteriogenic effect of lithobiontic recolonization in terms of colour and aesthetic disfiguring. Measures were performed with a portable spectrophotometer (Konica Minolta CM-23d) on target areas of 8 mm (diameter) in geometrical 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 analyse the spectral data (ISO/CIE 2019). In particular, at least five measures were collected for each of ten parcels distributed in areas protected (n=5) and non-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*

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parameter, informative 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, dealing with rocks restored in 2015 and still largely maintaining a clean state after three years. However, total lithobiontic cover and abundance of its components remarkably varied through the different plots and, particularly, with respect to the different conservation history of the rocks. On NRC rocks, a high total cover was a common feature (av. 81.6±6.0% SE), while highly variable values were observed for 12YC (av. 55.9±16.9% SE) and 3YC (av. 22.6±9.45% SE) rocks. The NRC cover higher than the 3YC cover was statistically significant (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), with thickness ranging from 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) 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 was dominant on almost all surveyed surfaces (Fig. 1A), but covered significantly lower areas on 12YC and 3YC rocks (Fig. 1B-C). On these latter, in particular, lithobionts were absent in six out of 19 plots, and cover values higher than 25% only characterized one third of the plots (Fig. 1B). High covers were instead prevalent on 12YC

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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 (Supplementary Material Fig. S3E) 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, acting a discolouring rather than 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 only subordinately occurred.

Circular colonies of meristematic fungi, of (sub-)millimetric size, but sometimes merging to give crusts of several square decimetres (Supplementary Material Fig. S3C-D), 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).

Lichens occurred in ten out of 19 plots surveyed on 3YC rocks, but cover values were mostly lower than 2% - specific lichen diversity is considered in the next sub-chapter. On 12YC and NRC rocks, lichens were present in almost all the plots (out of one on 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). Bryophytes, and particularly mosses, also occurred in most of the plots, often localized along cracks and fissures (Supplementary Material Fig. S3G). 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

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A total of 37 saxicolous lichen *taxa* was recorded through the surveyed plots (Table 1), with prevalence of crustose (59%) with respect to foliose species (38%), although these latter showed higher cover values, and a rather high number of *taxa* showing asexual reproductive strategy (35%). In particular, a high diversity of yellow-green *Xanthoparmelia* spp. was found, including five isidiate and two non-isidiate species. However, due to the logistic 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 these 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 on 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%), with richness difference appearing mostly important on 3YC (46.3%) and replacement more remarkable in 12YC (38.4%). Higher similarity and lower replacement were detected by considering together plots on NRC and 12YC ($S_{NRC+12YC}$=25.5%; $R_{NRC+12YC}$=25.8) with respect 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 through the plots (37- 81%), 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 per plot (av. 8.6- 39.6%), but very different

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cover values related to the different growth form, with foliose and continuous crustose thalli (av. cover 0.5- 7.0%, but maximum cover of 6.0- 50.0%) determining higher cover values than discontinuous crustose thalli (e.g. *C. vitellina, P. flavicans*: av. cover <0.2%, and maximum up to 2.0%). Other *taxa* also displayed rather high values of diffusion (15-30% of plots) and frequency, including a group of species commonly found on stone heritage surfaces even in urban environments, as *Protoparmeliopsis muralis* and *Verrucaria nigrescens* f. *tectorum*, and others which are usually associated to the bark rather than to rock substrates, 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*, asexual reproductive strategy. Remarkably, the group of usually epiphytic species showed the highest diffusion on 3YC rocks, together with *P. flavicans* and *Fuscidea lygaea*, which are meso-hygrophytic species, poorly tolerant to eutrophication, and *C. caesiocinerea*. 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 usually 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 and *C. vitellina*, which displayed the highest frequency values, while axis 2 (15.4%) showed a remarkable positive correlation with *V. nigrescens* and *C. chlorina*, and negative with *Phlyctis argena*, and axis 3 (13.0%) a positive correlation with *Xanthoparmelia* spp. with isidia. Accordingly, plots on NRC rocks, with highest abundances of these dominant species, mostly scattered on the right side of the diagram. Oppositely, plots of 12YC and 3YC rocks scattered

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in the left side, likely driven by the relatively lower frequencies of dominant species more than by the abundance of other subordinate species. It is worth noting that the ten plots without lichens are not represented in the ordination.

Lithobiontic penetration within the sandstone substrate

RLM observations showed a scarce penetration within the sandstone substrate for both the cyanobacterial-dominated biofilm and the considered lichens. The microbial biomass only developed epilithically, with the exception of very limited chasmoendolithic growths, down to approx. 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 substrate (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 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 related 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 related with lichens and mosses, respectively. 12YC and 3YC plots scattered through the whole diagram, including the lower left quadrant, related 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 S4). 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; weighted correlation, 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 related 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, out of surface aspect, showed significant conditional effect 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 colonized plots on 3YC and 12YC rocks, including those with highest lichen abundance (in terms of total lichen frequencies), showed positive correlation with tree cover and/or distance from the ground, in the space characterized by the most abundant meso-hygrophytic species *F. lygaea* and *P. flavicans* and the usually 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 and without isidia, *Candelariella vitellina* and *Rhizocarpon disporum*, and the mesophytic *Caloplaca chlorina*.

Control of lithobiontic recolonization by preventive microenvironmental conditioning

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Assays of the efficacy of BiotinT 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 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 downwards the rock). Lichen recolonization was not observed neither in the protected nor in the unprotected areas of Rock 70.

At twenty 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 wall showed not 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),

Manuscript For Review

and to the regulation of artificial light regimes (Sanmartín 2021). In the case of rock art, hypotheses and suggestions on a potential conservative 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 maintenance of heritage surfaces in a clean state. 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 history

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 Fig. S3), prevents a full reconstruction of (re-)colonization patterns in the investigated site. Nevertheless, the abundances of lithobiontic components through 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, and their presence was variously associated to 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

Manuscript For Review

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 substrate 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 on their 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 add up to the preference of 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 of the engraved schists of the Côa Valley Archaeological Park (UNESCO, Portugal; Marques et al. 2014), where different lichen communities characterized surfaces with different aspect. In the case of Naquane, the EXP factor was not a significant conditional factor neither with respect to the distribution of the different lithobiontic components nor for the different lichen taxa. This is likely because the effect of the punctual surface aspect was masked by the general NW exposition of the valley side occupied by the Park. However, different lichen communities were observed in Naquane, with the high beta-diversity values obtained in SDR analysis mostly associated to the turnover

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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 aspect (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 runoff after rain events (see next sub-chapter).

Lichen communities on 12YC and 3YC plots mostly show 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) indicate that the most pioneer phase of recolonization is already concluded in less than twelve years after the cleaning interventions. Species commonly found in synanthropic environments prevail, although some species usually associated to undisturbed conditions persist, as *P. flavicans* and *F. lygaea*. Such pattern reflects the shift observed on several heritage surfaces after cleaning interventions, with nitrophytic, fast-growing species becoming prevalent with respect to originally dominant species (Nascimbene et al. 2009). Persistence of original species and, in general, fast recolonization in few years likely relates with 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 prevalence of asexual reproductive modes (mostly soredia) and/or produce small, highly dispersive ascospores (species of genera *Caloplaca* s.l., *Candelariella* s.l., *Lecanora* s.l.), remarking 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, mostly including common species of

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silicate substrates. This result may depend on the fact that the communities on NRC rocks are also the product of recolonization processes on the long term of several decades following the early and, unfortunately, poorly documented cleaning interventions in the area. However, the comparison with outcrops out of the boundaries of the Park was beyond the aims of this project and, surprisingly, it may be really difficult to find outcrops in the mid Valle Camonica which do not host engravings and, thus, did not suffer any human disturbance in recent times.

Physical interaction of lichens and other lithobionts with the sandstone substrate

Lichen colonization of engraved outcrops was already deeply considered with respect to the deteriogenic impact 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 to the waning of an original surface red colour (*e.g.* Alta, Norway; Tansem & Storemyr 2020), to the dry semi-arid zone, where physical and chemical degradation processes were microscopically documented (*e.g.* el Morro National Monument, New Mexico; Knight et al. 2004). Although the observations were limited to few cross sections for conservative reasons, the physical interaction of lichens with the examined sandstones appears rather mild, as we observe a poor hyphal penetration even for *Verrucaria nigrescens*. This common colonizer of heritage surfaces was indeed often reported as a deeply penetrating and impacting species on different lithologies, including other sandstones (Tonon et al. 2021, with refs. therein), although with different intergranular matrices and lower compactness. 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

Manuscript For Review

displayed an epilithic behaviour, differing from observations on other sandstone substrates, 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 substrate 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 micro-environmental conditions related to a different surface aspect can imply 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, were demonstrated to support lithobiontic colonization on stone materials. Investigations in the wet N-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 the 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). Oppositely, recolonization by xerophytic species on directly exposed rock outcrops seems to require longer times. The

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abundance of usually epiphytic species as pioneer colonizers on the 3YC and 12YC surfaces further remarks the threats related to the tree proximity, even beyond the shading effect.

Such recognition of environmental factors favouring 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 circumscribe surfaces where lithobiontic communities and related biodeterioration effects are hindered and the legibility of engravings is preserved. 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 on the long term, in particular favouring lichens rather than cyanobacterial biofilms (Bjelland and Helberg 2006), although lichens have still not (re-)appeared 40 months after the cleaning through 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, as the considered brick wall or other kinds of barriers (Bjelland and Helberg 2006). With this regard, it has to be remarked that the wall considered here is an experimental structure to evaluate benefits obtainable with the control of water fluxes. The development of further strategies to obtain similar results without touching the engraved surface is needed. In any case, although barriers to water flows may be visually unpleasant, the traditional applications of synthetic biocides to periodically devitalize and remove established lithobiontic communities may imply even a higher impact by affecting the environmental equilibria (Cappitelli et al. 2020).

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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. They both displayed poor penetration within the sandstone substrate, likely because of its high compactness 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 resulted as the main drivers of recolonization on surfaces cleaned in the last twelve years, likely prolonging surface wetness after rain events and increasing nutrient availability. Nitrophytic species, including epiphytes from surrounding trees, and few meso-hygrophytic species, mostly producing soredia, were mainly responsible of the rapid lichen recolonization. An experiment of preventive conservation performed on a critical rock, including an effective devitalization of lithobionts before cleaning, combined with 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 issues on the 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 particularly appear as suitable indicators.

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Figure captions

Fig. 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 (A) and separately for rocks cleaned in the last three years (3YC, B), twelve years (12YC; C) or from more than 40 years (NRC; 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=2-25% (light grey), visible cover, but <2% (grey bands), absence of visible cover (white).

Fig. 2. Ordination of plots on the basis of the specific lichen frequencies (PCoA). Plots are differently marked according to the different conservation history of the surveyed rocks (NRC, crosses; 12YC, grey squares; 3YC, white squares). Half of plots with highest lichen abundance for the NRC and 12YC/3YC categories (in terms of total specific frequencies) display a higher symbol size. Species abbreviation in Table 1 (nitrophytic species underlined, meso-hygrophytic species in bold).

Fig. 3. Lithobiontic penetration within the sandstone substrate. 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). Scale bars: 1.0 mm (A), 1.5 mm (B, C), 350 µm (D).

Fig. 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 (tree cover, TRC; surface micromorphology, ROU; inclination, INC; distance from bare or vegetated ground upstream, GRP; exposition, EXP). Symbols indicate different lithobionts (black circles: lichens, Lich; bryophytes, Bry; cyanobacterial biofilm, CyB; green algal

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Fig. 5. Basal fluorescence (F_0, A) and maximum quantum efficiency of Photosystem II photochemistry (B, F_v/F_m) 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). At each measuring time point, box-plots which do not share at least one letter are statistically different (ANOVA, Tukey's test, $p < 0.05$).

Fig. 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). At each measuring time point, box-plots which do not share at least one letter are statistically different (ANOVA, Tukey's test, $p < 0.05$).

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Table 1. Lichens recorded on sandstone outcrops of the Rock Engravings National Park of Naquane [av. and max cover and frequency values are reported for the plots considered altogether and separately for 3YC, 12YC and NRC outcrops, as well as the % specific occurrence through the plots; growth form (GF): crustose (Cr), foliose (Fo), fruticose (Fr); prevailing reproduction strategy; sexual (S), asexual (A); ecological indicator values from Nimis (2022): pH of the substrate (pH), irradiation (IR), aridity (AR), eutrophication (EU); * *X. conspersa* more frequent, but also *X. tinctina, X. plittii, X. mexicana,* and *X. verrucigera* present; ***X. angustiphylla* more frequent, but also *X. stenophylla* present]

				Ecological indicator values				All the plots (n=27)					3YC rocks (n= 19 plots)					12YC (n=8 plots)					NRC (n= 27 plots)				
								Occurrence (plot %)	Cover (%)		Frequency(%)		Occurrence (plot %)	Cover (%)		Frequency(%)		Occurrence (plot %)	Cover (%)		Frequency(%)		Occurrence (plot %)	Cover (%)		Frequency(%)	
Species	Code	GF	Repr.	pH	IR	AR	EU		Av.	Max.	Av.	Max.		Av.	Max.	Av.	Max.		Av.	Max.	Av.	Max.		Av.	Max.	Av.	Max.
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, Savić & Tibell (± *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, Søchting & Frödén	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
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, A. Crespo, Elix, D. Hawksw. & Lumbsch	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

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Table 2. Percentage contribution from the SDR simplex analyses of lichen communities through the surveyed plots, considered altogether, in combination and separately for NRC, 12YC and 3YC rocks.

	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

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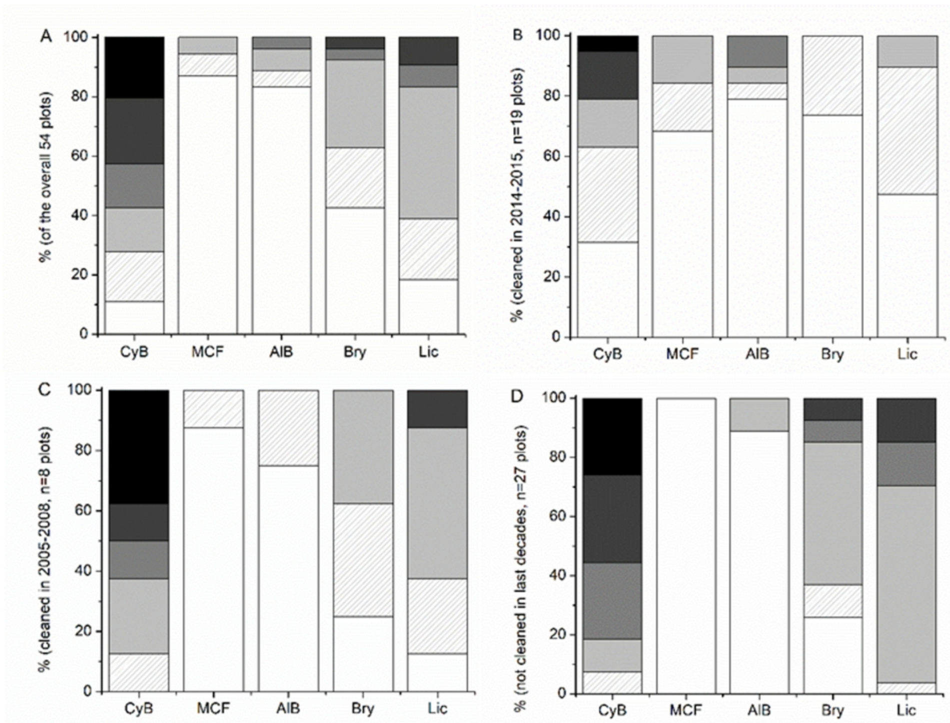

Fig. 1. Abundance of different lithobiontic components (CyB, cyanobacterial-dominated biofilm; MCF, microcolonial fungi crusts; AlB, green algal-dominated biofilm; Bry, bryophytes; Lic, lichens) on the engraved rocks, considering the overall plots together (A) and separately for rocks cleaned in the last three years (3YC, B), twelve years (12YC; C) or from more than 40 years (NRC; 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=2-25% (light grey), visible cover, but <2% (grey bands), absence of visible cover (white).

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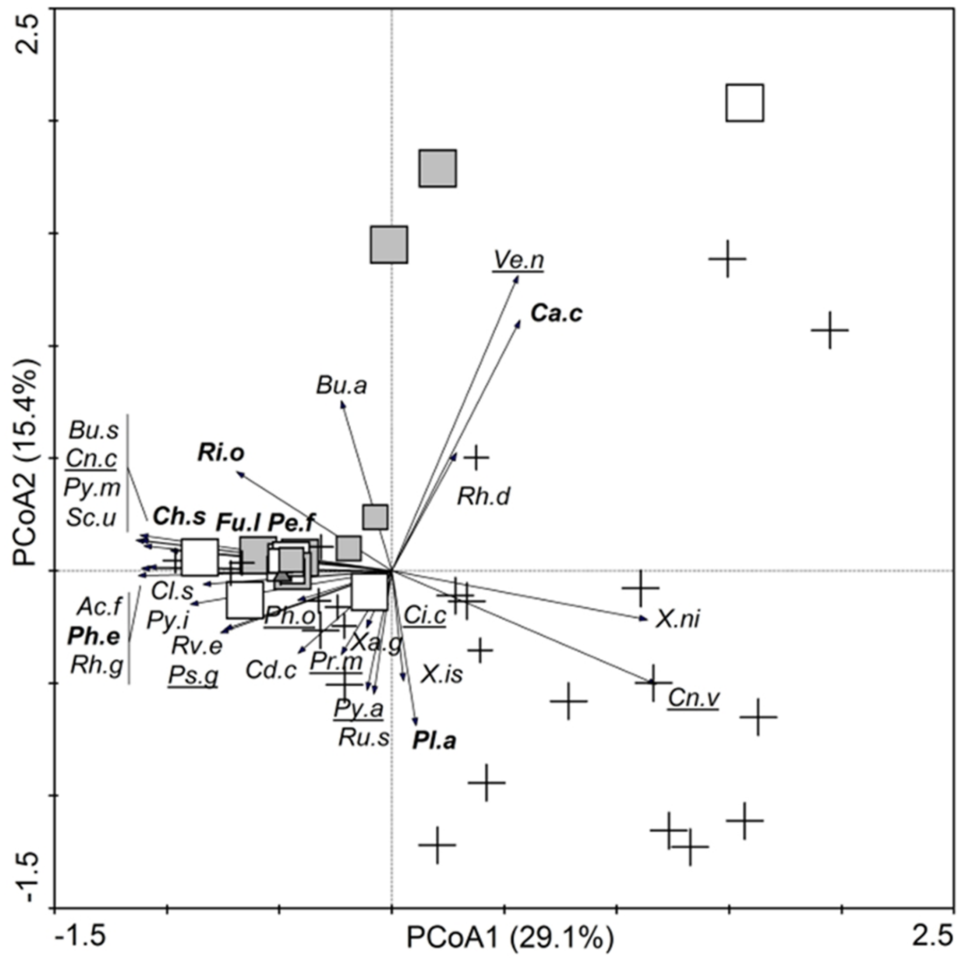

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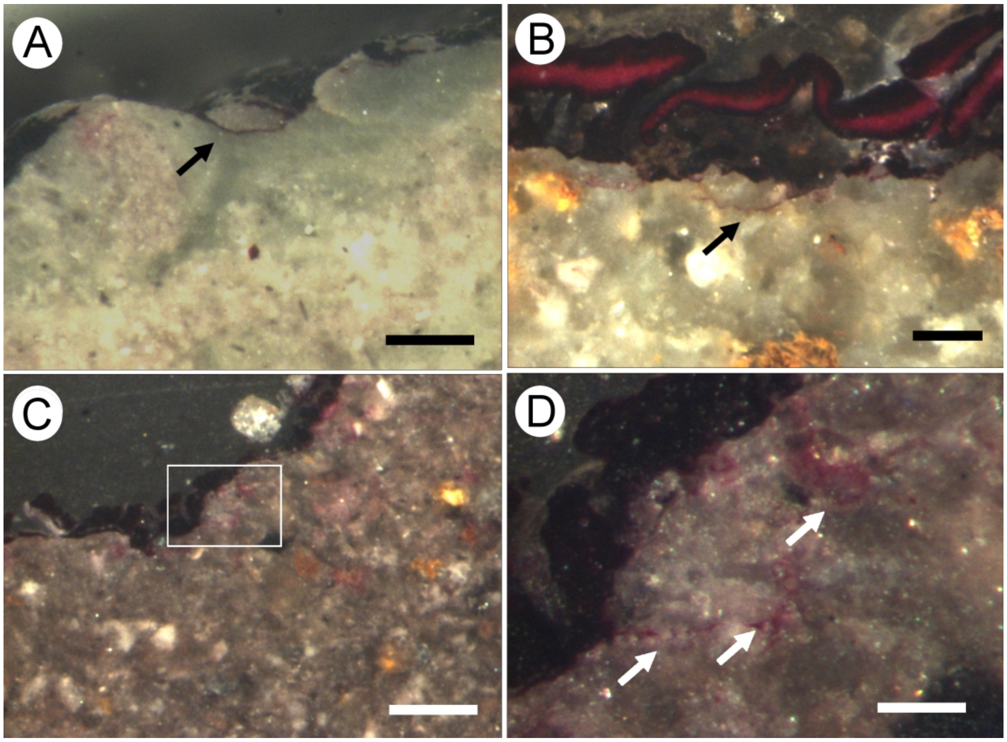

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137x101mm (500 x 500 DPI)

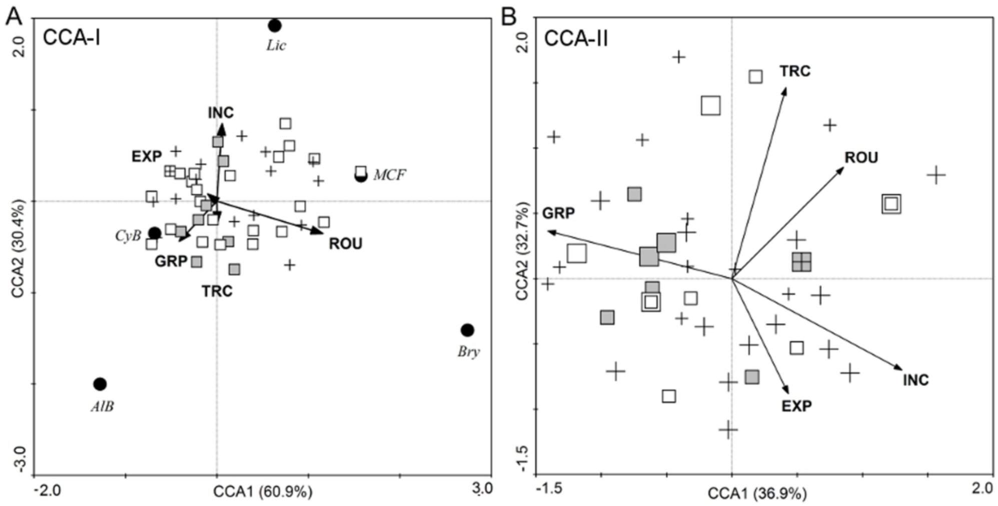
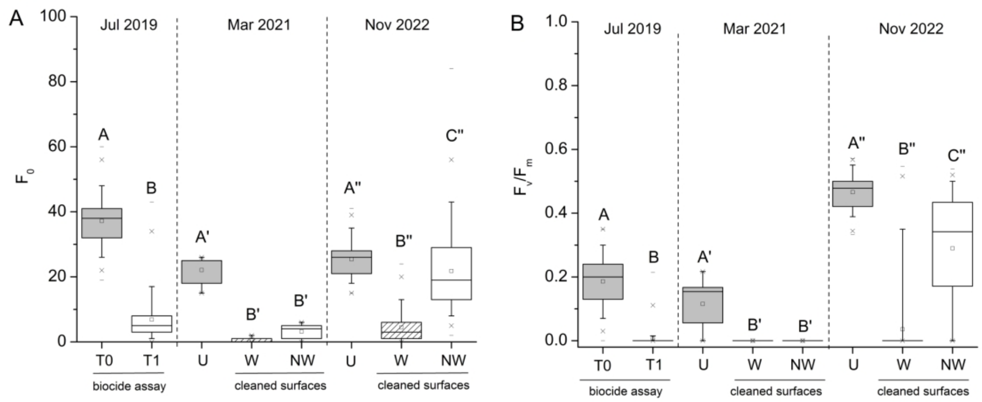


Fig. 4. Factorial map in the canonical correspondence analysis showing the position of plots having a different conservation history with the contributions of lithobiontic covers (A, CCA-I) and specific lichen frequencies (B, CCA-II), together with environmental factors (tree cover, TRC; surface micromorphology, ROU; inclination, INC; distance from bare or vegetated ground upstream, GRP; exposition, EXP). Symbols indicate different lithobionts (black circles: lichens, Lich; bryophytes, Bry; cyanobacterial biofilm, CyB; green algal biofilm, AlB; meristematic fungi, MCF), and NRC (crosses), 12YC (grey squares) and 3YC (white squares) rocks. In CCA-II (B), half of plots with highest lichen abundance for the NRC and 12YC-3YC categories (in terms of total specific frequencies) display a higher symbol size; contributions of the different species are separately shown in Fig. S4.

325x169mm (300 x 300 DPI)



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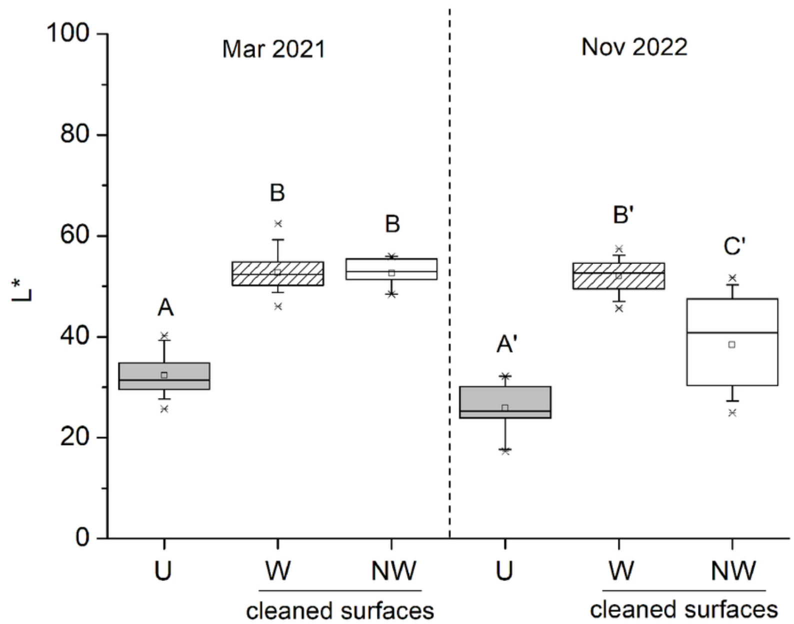
136x59mm (500 x 500 DPI)



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89x63mm (300 x 300 DPI)

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

Declaration of interests

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

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Torino, 23th February 2022

Faithfully

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Species	Code	GF
Acarospora fuscata (Schrad.) Arnold	Ac.f	Cr
Buellia aethalea (Ach.) Th. Fr.	Bu.a	Cr
Buellia stellulata (Taylor) Mudd	Bu.s	Cr
Caloplaca chlorina **(Flot.) H. Olivier**	Ca.c	Cr
Candelaria concolor (Dicks.) Stein	Cd.c	Cr
Candelariella coralliza (Nyl.) H. Magn.	Cn.c	Cr
Candelariella vitellina (Hoffm.) Müll. Arg.	Cn.v	Cr
Chrysothrix sp.	Ch.s	Cr
Circinaria caesiocinerea (Malbr.) A. Nordin, Savić & Tibell (± *Aspicilia cinerea* (L.) Körb.)	Ci.c	Cr
Cladonia sp.	Cl.s	Fr
Fuscidea lygaea (W. Mann) V. Wirth & Vězda	Fu.l	Cr
Pertusaria flavicans **Lamy**	Pe.f	Cr
Phaeophyscia endococcina (Körb.) Moberg	Ph.e	Fo
Phaeophyscia orbicularis (Neck.) Moberg	Ph.o	Fo
Phlyctis argena (Spreng.) Flot.	Pl.a	Cr
Physcia adscendens H. Olivier	Py.a	Fo
Physcia aipolia (Humb.) Fürnr.	Py.i	Fo
Physcia magnussonii Frey	Py.m	Fo
Physconia grisea (Lam.) Poelt	Ps.g	Fo
Protoparmeliopsis muralis (Schreb.) M. Choisy s.lat.	Pr.m	Cr
Rhizocarpon disporum **(Hepp) Müll. Arg.** (± *Rhizocarpon reductum* Th. Fr.)	Rh.d	Cr
Rhizocarpon geographicum (L.) DC. s.lat.	Rh.g	Cr
Rinodina occulta (Körb.) Sheard	Ri.o	Cr
Rufoplaca gr. *arenaria* (Pers.) Arup, Søchting & Frödén	Ru.s	Cr
Rusavskia elegans (Link) S.Y. Kondr. & Kärnefelt	Rv.e	Fo

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Scoliciosporum umbrinum (Ach.) Arnold	Sc.u	Cr
Verrucaria nigrescens f. *tectorum* (A. Massal.) Coppins & Aptroot	Ve.n	Cr
Xanthoparmelia* with isidia	X.is	Fo
Xanthoparmelia* without isidia*	X.ni	Fo
Xanthoparmelia glabrans (Nyl.) O. Blanco, A. Crespo, Elix, D. Hawksw. & Lumbsch	Xa.g	Fo

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	Ecological indicator values				All the plots (n=27)			
						Cover (%)		Freque
Repr.	pH	IR	AR	EU	Occurrence (plot %)	Av.	Max.	Av.
S	3-4	4-5	3-4	3-4	0.0	0.0	0.1	0.2
S	1-3	4-5	4-5	1-3	0.0	0.2	8.0	2.0
S	3-4	4-5	4	1-2	0.0	0.0	0.1	0.1
A	2-3	3-4	3	3-4	**0.0**	1.1	30.0	11.1
A	3-4	4-5	3-4	3-5	0.0	0.0	0.1	4.1
S	2-3	4-5	4	4-5	0.0	0.0	0.1	0.1
S	1-3	3-5	3-4	2-5	**0.0**	0.1	2.0	19.6
A	1-2	2-4	1-3	1	0.0	0.0	0.1	0.9
S	2-4	3-5	2-4	2-5	**0.0**	1.6	40.0	10.8
S	4-5	4-5	4	1-3	0.0	0.1	3.0	1.5
S	1-2	3-4	2-3	1	0.0	0.4	10.0	5.9
A	2-3	3-4	2-3	1	**0.0**	0.1	1.0	8.1
S	2-3	3-4	1-3	2-3	0.0	0.0	0.1	0.2
A	2-5	3-5	3-4	4-5	0.0	0.0	1.0	2.5
A	1-2	2-3	2-3	1-2	0.0	0.5	5.0	5.8
A	2-5	4-5	3-4	3-5	0.0	0.0	2.0	1.6
S	2-3	4-5	3	3-4	0.0	0.0	0.1	0.4
S	3-4	4-5	4-5	3-4	0.0	0.0	0.1	0.1
A	3-4	3-5	3	4-5	0.0	0.0	0.1	0.7
S	2-4	3-5	3-4	3-5	0.0	0.4	18.0	4.7
S	1-3	3-5	2-4	1-3	**0.0**	0.4	6.0	5.9
S	1-3	3-5	3-4	1-3	0.0	0.0	1.0	0.5
S	1-2	3-4	2-3	1	0.0	0.0	1.0	0.7
S	2-3	4-5	3-4	2-3	0.0	0.0	2.0	2.0
S	3-5	4-5	4	3-4	0.0	0.0	1.0	0.6

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S	1-3	3-4	2-4	1-3	0.0	0.0	0.1	0.1
A	3-5	3-5	2-5	2-5	0.0	0.5	17.0	10.8
A	2-3	3-5	3-4	2-4	**0.0**	3.6	50.0	13.7
S	2-3	3-5	3-4	2-3	**0.0**	3.0	45.0	21.6
S	2-3	4-5	3	2-3	**0.0**	0.5	10.0	5.3

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	3YC rocks (n= 19 plots)					
:ncy(%)		Cover (%)		Frequency(%)		
Max.	Occurrence (plot %)	Av.	Max.	Av.	Max.	Occurrence (plot %)
8.0	-	-	-	-	-	-
56.0	5.3	0.1	1.0	0.4	8.0	12.5
4.0	-	-	-	-	-	-
96.0	-	-	-	-	-	37.5
40.0	21.1	0.0	0.1	3.4	40.0	12.5
4.0	-	-	-	-	-	-
100.0	5.3	0.0	0.1	0.6	12.0	37.5
12.0	-	-	-	-	-	37.5
100.0	10.5	0.0	0.1	1.9	32.0	62.5
32.0	-	-	-	-	-	-
100.0	5.3	0.2	3.0	5.3	100.0	12.5
96.0	10.5	0.0	0.1	2.3	24.0	25.0
12.0	-	-	-	-	-	-
96.0	10.5	0.0	0.1	5.5	96.0	-
84.0	26.3	0.3	4.0	2.5	12.0	-
60.0	-	-	-	-	-	-
20.0	-	-	-	-	-	-
4.0	-	-	-	-	-	-
40.0	-	-	-	-	-	-
96.0	-	-	-	-	-	25.0
56.0	5.3	0.0	0.1	0.2	4.0	25.0
8.0	-	-	-	-	-	-
28.0	-	-	-	-	-	-
48.0	5.3	0.0	0.1	0.4	8.0	-
28.0	-	-	-	-	-	-

Manuscript For Review

4.0	-	-	-	-	-	-
100.0	-	-	-	-	-	62.5
100.0	-	-	-	-	-	12.5
100.0	5.3	0.0	0.0	1.3	24.0	25.0
80.0	-	-	-	-	-	12.5

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12YC rocks (n=8 plots)				NRC rocks (n= 27 plots)			
Cover (%)		Frequency(%)		Occurrence (plot %)	Cover (%)		Freque
Av.	Max.	Av.	Max.		Av.	Max.	Av.
-	-	-	-	7.4	0.0	0.1	0.4
0.0	0.1	7.0	56.0	7.4	0.3	8.0	1.6
-	-	-	-	3.7	0.0	0.1	0.1
5.1	30.0	28.5	96.0	44.4	0.6	6.0	13.8
0.0	0.1	0.5	4.0	29.6	0.0	0.1	5.8
-	-	-	-	3.7	0.0	0.1	0.1
0.0	0.1	8.0	52.0	51.9	0.2	2.0	36.3
0.0	0.1	3.5	12.0	14.8	0.0	0.1	0.7
0.4	2.0	9.5	28.0	74.1	3.1	40.0	17.5
-	-	-	-	11.1	0.2	3.0	3.0
0.0	0.1	0.5	4.0	14.8	0.7	10.0	7.9
0.0	0.1	8.0	60.0	37.0	0.1	1.0	12.3
-	-	-	-	3.7	0.0	0.1	0.4
-	-	-	-	3.7	0.0	1.0	1.2
-	-	-	-	25.9	0.7	5.0	9.8
-	-	-	-	14.8	0.1	2.0	3.3
-	-	-	-	7.4	0.0	0.1	0.9
-	-	-	-	3.7	0.0	0.1	0.1
-	-	-	-	3.7	0.0	0.1	1.5
0.0	0.1	2.0	12.0	22.2	0.9	18.0	8.7
0.9	6.0	10.0	56.0	44.4	0.5	3.0	8.6
-	-	-	-	14.8	0.0	1.0	1.0
-	-	-	-	11.1	0.0	1.0	1.5
-	-	-	-	14.8	0.1	2.0	3.7
-	-	-	-	7.4	0.0	1.0	1.2

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-	-	-	-	3.7	0.0	0.1	0.1
2.4	17.0	34.5	100.0	25.9	0.3	4.0	11.4
0.6	5.0	7.0	56.0	55.6	7.0	50.0	25.3
1.5	12.0	9.5	72.0	81.5	5.5	45.0	39.6
0.3	2.0	2.5	20.0	63.0	0.9	10.0	9.8

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ency(%)
Max.
8.0
36.0
4.0
60.0
36.0
4.0
100.0
8.0
100.0
32.0
96.0
96.0
12.0
32.0
84.0
60.0
20.0
4.0
40.0
96.0
40.0
8.0
28.0
48.0
28.0

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4.0
100.0
100.0
100.0
80.0

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	Plots (n)	Similarity (S)	Richness difference (D)	Replacement (R)
All plots	54	18.8	43.8	37.5
NRC+3YC	46	19.4	42.7	37.9
NRC+12YC	35	25.5	48.6	25.8
12YC+3YC	27	12.3	46.4	41.4
NRC	27	28.2	50.5	21.4
12YC	8	22.5	39.2	38.4
3YC	19	17.7	46.3	36.0

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R+D (Beta diversity)	S+R (Richness agreement)	S+D -Anti-nest. - Rich. Id. (Nestedness)
81.2	65.5	38.5
80.6	62.1	39.3
74.5	74.2	43.5
87.7	58.6	25.9
71.8	78.6	43.9
77.5	61.6	51.7
82.3	64.0	27.1

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