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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.



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

3
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16

17

18 **Abstract**

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20 threatening indoor cultural heritage objects, while they are still poorly exploited for the
21 conservation of the outdoor stone heritage surfaces, including rock art. In this study, we
22 evaluated the environmental factors driving the diversity and abundance of lithobiontic
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31 reproductive strategies. Multivariate analyses including environmental parameters (canonical

32 correspondence analyses) indicated the tree cover and the presence of bare or vegetated
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34 transport, as the factors mostly related to the microbial and lichen recolonization of 3YC-
35 12YC surfaces. On this basis, an experiment on preventive conservation was conducted,
36 consisting of a new cleaning of a strongly recolonized 3YC surface combined with the
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40 potential of preventive conservation strategies also in outdoor environments.

41

42 **Keywords**

43 biodeterioration, biofilm, cultural heritage, recolonization, nitrophytic community

44

45 **Introduction**

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

64 Lichenologists, and potentially others, may have different priorities than heritage site curators
65 with regard to the conservation of heritage stone surfaces or of lichens and biodiversity in
66 general (Seaward 2004). Different perceptions of biodeterioration issues generally depend on
67 the type of heritage surfaces affected (a statue, a grave, a church façade, a castle wall, an
68 archaeological ruin) and the local cultural tradition (Favero-Longo & Viles 2020). Moreover,
69 different evaluations may derive from the 'environmental scenery' of each artwork, with the

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

75 Lithobiontic colonization and biodeterioration effects deserve particular attention when
76 affecting rock art, as biological growths and the artworks may display a rather similar
77 dimensional extent (i.e. (sub-)millimetric thickness), thus particularly implying conservation
78 issues (Darvill & Batarda-Fernandes 2014; Zerboni et al. 2022). Lichens, in particular, can
79 partially mask or fully cover engravings (Tratebas 2004), and were shown to induce physical
80 and chemical deterioration processes on different lithologies bearing rock art, although
81 negative effects on the surface durability were not always recognizable (e.g. Chiari & Cossio
82 2004; Marques et al. 2016). The impact on surface legibility, however, is sufficient to make
83 lichens generally undesirable on engraved stone surfaces, even though their colonization is an
84 obvious and unavoidable phenomenon on every rock outcrop (Jung & Büdel 2021) and just
85 lichens are often a prominent and valuable biodiversity component of the environments
86 hosting rock art (Tansem & Storemyr 2021). Treatments with synthetic chemical biocides, in
87 combination with mechanical actions and other restoration products as consolidants and
88 water-repellents, have been thus routinely practiced in rock art sites to (i) periodically remove
89 lichens and other lithobionts from engraved surfaces, and (ii) try to prolong the maintenance
90 of the clean state (Tratebas 2004; Paz-Bermúdez et al. 2023). Only recently, in order to reduce
91 the spread of chemicals into the environment, alternative approaches to control lithobionts on
92 engraved rocks were assayed, including laser and microwave applications. However, the
93 former seems less effective than traditional biocides and may even increase rock
94 bioreceptivity (Paz-Bermúdez et al. 2023), and the latter needs technical improvements to

95 allow outcrop-scale applications (Favero-Longo et al. 2021). On the other hand, approaches to
96 prevent recolonization dynamics following cleaning interventions by controlling (micro-
97)environmental parameters, which is a usual and regulated practice (e.g., in Italy, DM
98 10/05/2001; MIBAC 2001) to limit biodeterioration in indoor environments (Caneva et al.
99 2008), still appear poorly considered in the case of the outdoor stone heritage, and for rock art
100 in particular.

101 In the Rock Engravings National Park of Naquane, heart of the UNESCO site ‘Rock
102 Drawings in Valle Camonica’ (WHS n. 94, Italy), outcrops hosting the most remarkable
103 engravings have undergone a long series of cleaning interventions (including the application
104 of biocides), which were registered since 1980s but started long before (www.irweb.it;
105 Ruggiero & Poggiani-Keller 2014). In the last decades, recolonization dynamics on certain
106 rocks, mostly related to fast spreading of cyanobacterial biofilms, even renewed the necessity
107 of cleaning every few (2-3) years. This makes the management unsustainable in terms of time
108 and costs, but also with regard to the environmental pressure of the repeated biocide
109 application and a potential stress on rock surface due to the repeated mechanical treatments.
110 Therefore, a research project started in 2016 to assess critical features of the adopted
111 conservation strategies (e.g., the efficacy of adopted protocols of biocide applications;
112 Favero-Longo et al. 2021), and to explore alternative approaches to better combine cultural
113 and environmental heritage conservation (Ruggiero et al. 2021). In this framework, the
114 present work aims to characterize lithobiontic colonization on the engraved sedimentary rocks
115 of the National Park of Naquane, focusing on the diversity and abundance of lichens on
116 outcrops with different conservation history and environmental conditions. It also gives an
117 insight into their physical interaction with the sandstone substrate. The results were used to
118 address a preventive strategy to limit lithobiontic recolonization after cleaning interventions,
119 which was experimentally tested on a selected engraved outcrop. In particular, we tested the

120 hypotheses that: (a) some environmental factors are main drivers of diversity and abundance
121 of lichens and other lithobionts on recently cleaned surfaces, (b) lichens and other lithobionts
122 penetrate within the sandstone substrate, and (c) interventions limiting favourable
123 environmental conditions for lichens may generally hinder the fast lithobiontic recolonization
124 following cleaning interventions.

125

126 **Material and methods**

127 *Study site*

128 The Rock Engravings National Park of Naquane is located in the middle part of Valle
129 Camonica [Capo di Ponte, Brescia, Italy: UTM WGS84: 32T 604400 m E, 5097700 m N],
130 where it was established in 1955 as the first national archaeological park. It extends between
131 400 and 600 m above sea level (a.s.l.) on approx. 14,000 m² of the eastern side of the valley,
132 and hosts the most important groups of prehistoric and protohistorical engravings of Valle
133 Camonica. The engravings are distributed on 104 numbered surfaces of sedimentary rock
134 outcrops, dimensionally ranging from few to approx. 250 square meters (e.g. Rock 1, named
135 the “Great Rock of Naquane”, with 65 m² of engraved surface; Liborio et al. 2011). In
136 particular, engravings are carved in terrigenous sedimentary rocks (Verrucano Lombardo,
137 Upper Permian; Brack et al. 2008) mainly consisting of sandstones/graywackes rich in quartz,
138 feldspars and fragments of volcanic rocks, micro-conglomerates, and mudrocks. Sediments of
139 the Verrucano Lombardo suffered a quite high overburden (several kilometres) during burial
140 which determined a high degree of compaction (documented by the prevalence of long
141 contacts among grains in sandstones) and recrystallization of the clay matrix. The strong
142 diagenetic imprint, in addition to the mineralogical composition of the sand, resulted in a
143 great compactness and hardness and very low porosity of the rock (Supplementary Material

144 Fig. S1). This in turn affected the landscape modelling by fluvial and glacial erosion during
145 Quaternary glaciations giving rise to a remarkable smoothness of rock surfaces.

146 The Park is located in the Cfb zone (C – temperate, f - no dry season b - warm summer,
147 according to the Köppen Geiger climate classification; Kottek et al. 2006), with av. 2 °C in
148 winter, 21 °C in summer, and 1000 mm rainfall yr⁻¹ (Ceriani & Carelli 2000; data monitored
149 in the Capo di Ponte monitoring station n. 129, the closest to the Park, in the period 2003-
150 2016, available at www.arpalombardia.it/Pages/Meteorologia/Richiesta-dati-misurati.aspx).

151 In terms of land use and forest types, the site is characterized by the occurrence of abandoned
152 chestnut stands (of meso-xeric soils), variously evolved to a mixed broadleaf forest [*Betula*
153 *pendula* Roth, *Fraxinus ornus* L., *Populus tremula* L., *Salix caprea* L., *Prunus avium* (L.) L.],
154 although natural (*Pinus sylvestris* L., as a relic of past submontane pine forests, preceding
155 chestnut cultivation) and planted conifers [*Larix decidua* Mill., *Picea abies* (L.) Karst and
156 some exotic species] also widely occur, as well as sparse, xerophytic and acidophytic
157 grassland stands (Ducoli 2012).

158

159 *Diversity survey*

160 Lithobiontic communities, and saxicolous lichen diversity in particular, were surveyed in the
161 period between November 2017 and July 2018 on 23 engraved rocks having a different
162 conservation history (information available at www.irweb.it). In particular, 54 plots, 50 × 50
163 cm, were distributed on the surfaces of: (i) six rocks which were last cleaned in the period
164 2014-2015 (3YC; Rocks 1, 35, 50, 70, 73, 99; n= 19 plots), (ii) four rocks which were last
165 cleaned in the period 2005-2008 (12YC; Rocks 6, 7, 14, 57; n = 8 plots), and (iii) nine rocks
166 (or groups of neighbouring rocks) for which cleaning interventions are not documented in
167 archives registering the conservation history of engravings since the early 1980s (Not

168 Recently Cleaned, NRC; Rocks 2, 4, 8-9, 11, 17-18, , 49, 58, 36-69-96, 74; n= 27 plots). In
169 particular, interventions performed in the period 2005-2008 included mechanical removal of
170 thalli, cleaning with NeoDes 5% or 10%, application of the benzalkonium chloride based
171 product Preventol 3% as preservative, final application of the water-repellents Akeogard CO
172 or Silo 111; interventions performed in the period 2014-2015 included surface washing with
173 low-pressurized water and biocide application of benzalkonium chloride-based biocides. On
174 each rock (or group of neighbouring rocks), three plots (with the exceptions of Rock 1, with
175 six plots because of its strongly larger surface, and of Rocks 7, 14 and 73, with one plot each
176 because of technical constraints) were preferentially positioned in areas visually recognized as
177 representative of the predominant biodeterioration condition(s) affecting the surface legibility,
178 and thus requiring attention from the point of view of heritage conservation.

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

191 Samples of lichen thalli were collected from each plot, without affecting the rock substrate for
192 conservative reasons, to check field identifications in the lab. Lichen identification was based

193 on Wirth (1995), Smith et al. (2009) and the online keys published in ITALIC, the
194 Information System of the Italian Lichens, version 07 (see Nimis & Martellos 2020).
195 Nomenclature follows Nimis (2022). Species vouchers are deposited in the Lichen section of
196 the Herbarium Universitatis Taurinensis (TO). Indicator values proposed by Nimis (2022)
197 were considered as reference to express specific ecological ranges with respect to pH of
198 substratum (pH), solar irradiation (IR), aridity (AR) and eutrophication (EU).

199 The plots were also characterized with regard to environmental variables, quantified in the
200 field (estimated in the case of surface micromorphology) and then referred to ordinal scales as
201 follows: aspect (EXP: 3= SW, 2= W, 1= NW, 0= N), inclination (INC: 3= 0-10°, 2= 11-30°,
202 1= 31-50°, 0= >50°), surface micromorphology (ROU: 3= rough and/or highly fractured
203 surface, 2= slightly rough and/or moderately fractured surface; 1= smooth surface with few
204 fractures; 0= smooth surface without fractures), tree cover (TRC: 2= tree cover above the plot,
205 1= ground projection of the crown at less than 2 m from the plot, 0= ground projection of the
206 crown at more than 2 m), and distance from bare or vegetated ground upstream of the plot,
207 likely providing nutrients by water transport (GRP: 3= <1 m, 2= 1.1-4.9 m, 1= > 4.9 m, 0=
208 absence of bare or vegetated ground upstream of the plot).

209

210 *Analysis of diversity data*

211 The abundance of each lichen *taxon* was calculated in terms of presence through the plots (%)
212 and of average and maxima values of cover (%) and frequency (%) per plot. The relative
213 importance of components of γ -diversity [i.e. similarity (S), relativized richness difference
214 (D), and relativized species replacement (R)] was evaluated for all the plots
215 (NRC+12YC+3YC), and for plots on rock surfaces with a different conservation history
216 considered in combination (NRC+12YC, NRC+3YC, 12YC+3YC) and separately (NRC,

217 12YC, 3YC). The analysis was performed on the matrix of species presence/absence with the
218 SDR Simplex software using the Simplex method, as elsewhere detailed (SDR Simplex;
219 Podani and Schmera 2011). An ordination of plots was performed on the basis of frequency
220 data by Principal Co-ordinate Analysis (PCoA: symmetric scaling, centring samples by
221 samples, centring species by species; Ter Braak & Šmilauer 2002). Two Canonical
222 Correspondence Analyses were carried out with the matrices of environmental parameters and
223 the cover values estimated for the different lithobiontic components (CCA-I) and the
224 frequencies of lichen *taxa* (CCA-II), in order to partition variation explained by each variable
225 and construct a model of significant variables (biplot scaling for interspecies distances, Hill's
226 scaling for inter-sample distances; forward selection of variables option; Monte Carlo
227 permutation test on the first and all ordination axes) (Ter Braak and Verdonschot 1995). The
228 ordinations were performed using CANOCO 4.5 (Ter Braak and Šmilauer 2002).

229

230 *Microscopic observation of lithobionts-rock interactions*

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

242

243 *Experiment on preventive conservation*

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

253 In the framework of this work, Rock 70 was cleaned again in Summer 2019, with the
254 mechanical removal of the microbial biofilms and the lichens preceded by their devitalization
255 with a four-hours poultice application of the biocide BiotinT (N-octyl-isothiazolinone, 7–
256 10%, and didecyl-dimethyl ammonium chloride, 40–60%, as active principles; CTS, Altavilla
257 Vicentina, Italy). Its effectiveness had been verified by fluorimetric measurements on other
258 outcrops of the Park (Favero-Longo et al. 2021) and further checked on few parcels on Rock
259 70 itself (see below). In Autumn 2019, a 10 cm tall and approx. 3 m long wall of bricks,
260 covered and fixed with mortar, was built at 20-30 cm from the upper border of the rock, to
261 limit water fluxes from upstream vegetated and bare ground following rain events. Only the
262 right portion of the rock was left free from the wall protection. It is worth remarking that the
263 wall was built to assay the effect of water control on recolonization dynamics and not as a
264 permanent structure. Moreover, some of the trees bordering the rock outcrop were cut or
265 pruned, to reduce their shading effect on the engraved surface.

266 Measurements of the vitality of the cyanobacterial-dominated biofilm were performed few
267 hours before and one day after the biocide application using a Handy-PEA fluorimeter
268 (Hansatech Instruments Ltd, Norfolk, England; saturating light pulse of 1s, 1500 $\mu\text{mol m}^{-2}\text{s}^{-1}$,
269 peak at 650 nm), as described elsewhere (e.g. Favero-Longo et al. 2021). Measures were
270 performed early in the morning, on pre-moistened and dark-adapted surfaces. In particular,
271 measures were distributed on three parcels (approx. 25 \times 25 cm) on different parts of the rock
272 outcrop ($n > 70$ at each measuring time point). Measures on an additional untreated parcel were
273 also collected as control. The basal fluorescence (F_0), which is related to the chlorophyll *a*
274 content, and the maximum quantum yield of PSII (F_v/F_m), which is informative on the
275 functionality of the photosynthetic process, were monitored as indicators of the microbial
276 viability (Tretiach et al. 2010; Favero-Longo et al. 2021). Potential recolonization after the
277 cleaning intervention was monitored by fluorimetric measures twenty and forty months after
278 the cleaning (i.e. in March 2021, after the limitations due to COVID-19 pandemic, and
279 November 2022), on newly selected parcels, randomly distributed in areas protected by the
280 wall ($n=6$), out of the wall protection ($n=4$) and on the uncleaned Rock 71, adjacent to Rock
281 70 ($n=3$).

282 The fluorimetric monitoring was combined with spectro-colorimetric measures, in order to
283 evaluate the potential deteriogenic effect of lithobiontic recolonization in terms of colour and
284 aesthetic disfiguring. Measures were performed with a portable spectrophotometer (Konica
285 Minolta CM-23d) on target areas of 8 mm (diameter) in geometrical condition d/8 specular
286 component included as setting conditions, using the CIE D65 illuminant and 2° observer, and
287 the CIELAB colour system to process and analyse the spectral data (ISO/CIE 2019). In
288 particular, at least five measures were collected for each of ten parcels distributed in areas
289 protected ($n=5$) and non-protected ($n=2$) by the wall, and on the adjacent uncleaned Rock 71
290 ($n=3$), corresponding or adjacent to the parcels used for fluorimetric measures. The L^*

291 parameter, informative of surface lightness, was considered as reference to recognize a
292 different development of a dark lithobiontic biofilm (Gambino et al. 2019).

293

294 **Results**

295 *Lithobiontic colonization of engraved rock surfaces*

296 All plots displayed a visible lithobiontic colonization with two exceptions, dealing with rocks
297 restored in 2015 and still largely maintaining a clean state after three years. However, total
298 lithobiontic cover and abundance of its components remarkably varied through the different
299 plots and, particularly, with respect to the different conservation history of the rocks. On NRC
300 rocks, a high total cover was a common feature (av. $81.6 \pm 6.0\%$ SE), while highly variable
301 values were observed for 12YC (av. $55.9 \pm 16.9\%$ SE) and 3YC (av. $22.6 \pm 9.45\%$ SE) rocks.
302 The NRC cover higher than the 3YC cover was statistically significant (ANOVA, $p < 0.05$).

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

315 rocks (Fig. 1C), displaying the maximum percentage of plots with values higher than 75%,
316 and on NRC (Fig. 1D), where the dark biofilm generally covered the entire surface free of the
317 other lithobiontic components.

318 Greenish biofilms (Supplementary Material Fig. S3E) also occurred on some rocks, including
319 12YC and 3YC, although they never displayed cover values higher than 50% (Fig. 1) and
320 their thickness was generally limited, acting a discolouring rather than a masking effect.

321 Microscopic observations showed filamentous green algae (frequently *Microspira* sp.) as
322 dominant constituents, together with coccoid species, including free-living *Trebouxia* sp.,
323 while cyanobacteria only subordinately occurred.

324 Circular colonies of meristematic fungi, of (sub-)millimetric size, but sometimes merging to
325 give crusts of several square decimetres (Supplementary Material Fig. S3C-D), were an
326 additional lithobiontic component on some engraved surfaces. Although their frequency was
327 low as well as their cover values, they were evident on both 12YC and NRC rocks (Fig. 1).

328 Lichens occurred in ten out of 19 plots surveyed on 3YC rocks, but cover values were mostly
329 lower than 2% - specific lichen diversity is considered in the next sub-chapter. On 12YC and
330 NRC rocks, lichens were present in almost all the plots (out of one on 12YC), and cover
331 values were mostly in the 2-25% range (Fig. 1), although in some cases values higher than
332 50% were observed (Supplementary Material Fig. S3F). Bryophytes, and particularly mosses,
333 also occurred in most of the plots, often localized along cracks and fissures (Supplementary
334 Material Fig. S3G). Their cover values were rather negligible on 3YC rocks, and always
335 lower than 25% on 12YC (Fig. 1). On some NRC rocks, they were instead the dominant
336 component, with cover values higher than 50%.

337

338 *Lichen diversity*

339 A total of 37 saxicolous lichen *taxa* was recorded through the surveyed plots (Table 1), with
340 prevalence of crustose (59%) with respect to foliose species (38%), although these latter
341 showed higher cover values, and a rather high number of *taxa* showing asexual reproductive
342 strategy (35%). In particular, a high diversity of yellow-green *Xanthoparmelia* spp. was
343 found, including five isidiate and two non-isidiate species. However, due to the logistic
344 constraints of identifying each individual, only isidiate and non-isidiate *Xanthoparmelia* spp.
345 were distinguished in the abundance analyses. For the same reason, other species groupings
346 were considered, including *Circinaria caesiocinerea*/*Aspicilia cinerea* and *Rhizocarpon*
347 *disporum*/*R. reductum*, reducing to 30 the final number of *taxa* considered for the
348 subsequently described analyses.

349 All these 30 *taxa* were found on NRC rocks, while diversity was lower on 12YC and 3YC (17
350 *taxa*). Accordingly, SDR analysis performed for the overall plots showed a very high beta-
351 diversity (81.2%), but with richness difference (43.8%) prevailing on replacement (37.5%)
352 (Table 2). Similarity showed a decreasing trend from plots on NRC rocks (28.2%) to those on
353 12YC (22.5%) and 3YC (17.5%), with richness difference appearing mostly important on
354 3YC (46.3%) and replacement more remarkable in 12YC (38.4%). Higher similarity and
355 lower replacement were detected by considering together plots on NRC and 12YC
356 ($S_{\text{NRC}+12\text{YC}}=25.5\%$; $R_{\text{NRC}+12\text{YC}}=25.8$) with respect to the combinations of plots on NRC and
357 3YC ($S_{\text{NRC}+3\text{YC}} = 19.4$; $R_{\text{NRC}+12\text{YC}}=37.9$) and on 12YC and 3YC ($S_{12\text{YC}+3\text{YC}} = 12.3$;
358 $R_{\text{NRC}+12\text{YC}}=41.4$).

359 On NRC rocks, eight *taxa* displayed the highest occurrence through the plots (37- 81%),
360 including both heliophytic-xerophytic (*Circinaria caesiocinerea*, yellow green
361 *Xanthoparmelia* spp. with and without isidia, *Xanthoparmelia glabrans*, *Candelariella*
362 *vitellina*, *Rhizocarpon disporum*) and mesophytic (*Caloplaca chlorina*, *Pertusaria flavicans*)
363 species. They all showed high frequency values per plot (av. 8.6- 39.6%), but very different

364 cover values related to the different growth form, with foliose and continuous crustose thalli
365 (av. cover 0.5- 7.0%, but maximum cover of 6.0- 50.0%) determining higher cover values
366 than discontinuous crustose thalli (e.g. *C. vitellina*, *P. flavicans*: av. cover <0.2%, and
367 maximum up to 2.0%). Other *taxa* also displayed rather high values of diffusion (15-30% of
368 plots) and frequency, including a group of species commonly found on stone heritage surfaces
369 even in urban environments, as *Protoparmeliopsis muralis* and *Verrucaria nigrescens* f.
370 *tectorum*, and others which are usually associated to the bark rather than to rock substrates, as
371 *Candelaria concolor*, *Phlyctis argena* and *Physcia adscendens*. These are all nitrophytic
372 species, sharing a high tolerance to eutrophication and, with the exception of *P. muralis*,
373 asexual reproductive strategy. Remarkably, the group of usually epiphytic species showed the
374 highest diffusion on 3YC rocks, together with *P. flavicans* and *Fuscidea lygaea*, which are
375 meso-hygrophytic species, poorly tolerant to eutrophication, and *C. caesiocinerea*. On 12YC
376 rocks, lichen diversity was almost completely represented by the taxa dominating NRC rocks
377 (*C. caesiocinerea* > green-yellow *Xanthoparmelia* spp., *C. vitellina* > *C. chlorina* > *R.*
378 *disporum* > *X. glabrans*) and the nitrophytic saxicolous species *V. nigrescens* and *P. muralis*,
379 which similarly showed high diffusion, frequency and cover values, while the presence of
380 usually epiphytic species was limited to *C. concolor*.

381 The PCoA extracted four components which explained 65.4% of the total variance and
382 ordinated the plots on the basis of specific frequency data (Fig. 2). Axis 1 (29.1% of total
383 variance) showed a strongly positive correlation with *Xanthoparmelia* spp. without isidia and
384 *C. vitellina*, which displayed the highest frequency values, while axis 2 (15.4%) showed a
385 remarkable positive correlation with *V. nigrescens* and *C. chlorina*, and negative with *Phlyctis*
386 *argena*, and axis 3 (13.0%) a positive correlation with *Xanthoparmelia* spp. with isidia.
387 Accordingly, plots on NRC rocks, with highest abundances of these dominant species, mostly
388 scattered on the right side of the diagram. Oppositely, plots of 12YC and 3YC rocks scattered

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

392

393 *Lithobiontic penetration within the sandstone substrate*

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

402

403 *Factors conditioning lithobiontic and lichen colonization*

404 The analysis of cover values estimated for the different lithobiontic groups and environmental
405 variables (CCA-I) extracted four axes which accounted for 100% of species-environmental
406 relationships (Fig. 4A). All canonical axes were significant (Monte Carlo test, $P=0.002$). The
407 first axis (60.9% of correlation) was positively correlated with surface roughness (ROU,
408 weighted correlation, w.c., 0.89) and negatively with the distance from bare and vegetated
409 ground upstream of the plot (GRP, w.c. -0.32), while the second axis (30.4%) was positively
410 related with rock inclination (INC, w.c. 0.80) and negatively with tree cover (TRC, w.c. -
411 0.23) and GRP (w.c. -0.41). Only ROU and INC were significant conditional factors
412 ($P=0.002$). Plots on NRC rocks scattered in the upper and right part of the diagram, positively

413 related with lichens and mosses, respectively. 12YC and 3YC plots scattered through the
414 whole diagram, including the lower left quadrant, related with cyanobacterial and green algal
415 biofilms.

416 The analysis of lichen frequency data and environmental variables (CCA-II) extracted four
417 axes which accounted for 93% of species-environmental relationships (Fig. 4B and S4). All
418 canonical axes were significant (Monte Carlo test, $P=0.002$). The first axis (36.9% of
419 correlation) was positively correlated with rock inclination (INC; weighted correlation, w.c.,
420 0.65) and negatively with the distance from bare and vegetated ground upstream of the plot
421 (GRP, w.c. -0.70). The second axis (32.7%) was positively related with tree cover (TRC, w.c.
422 0.75) and surface micromorphology (ROU, w.c. 0.44) and negatively with surface aspect
423 (w.c. -0.45). All factors, out of surface aspect, showed significant conditional effect according
424 to forward selection, with tree cover displaying the highest value ($F = 2.48$, $P = 0.002$),
425 followed by inclination ($F = 2.39$, $P = 0.004$), surface micromorphology ($F = 2.28$, $P = 0.006$)
426 and distance from the ground ($F = 1.71$, $P = 0.036$).

427 Given that uncolonized plots do not appear in the factorial map, most of colonized plots on
428 3YC and 12YC rocks, including those with highest lichen abundance (in terms of total lichen
429 frequencies), showed positive correlation with tree cover and/or distance from the ground, in
430 the space characterized by the most abundant meso-hygrophytic species *F. lygaea* and *P.*
431 *flavicans* and the usually epiphytic species. Plots on NRC showing the highest lichen
432 abundance mostly scattered in the right lower part of the diagram, in the space characterized
433 by the dominant xerophytic species, namely the *Xanthoparmelia* spp. with and without isidia,
434 *Candelariella vitellina* and *Rhizocarpon disporum*, and the mesophytic *Caloplaca chlorina*.

435

436 *Control of lithobiontic recolonization by preventive microenvironmental conditioning*

437 Assays of the efficacy of BiotinT against lithobionts on Rock 70, and the cyanobacterial
438 biofilm in particular, showed a significant decrease of F_0 values in the treated parcels
439 (decrease > 80%) with respect to measures performed before the biocide application, and the
440 zeroing of F_v/F_m (Fig. 5A, B). Twenty months after the cleaning intervention, and after two
441 winter seasons, F_0 values quantified on the rock surface protected by the wall were zeroed,
442 while slightly higher values were detected in the unprotected area, suggesting that
443 recolonization was possibly starting. Accordingly, after 20 months more, F_0 and F_v/F_m values
444 quantified on the unprotected surface indicated the recovery of the lithobiontic colonization,
445 while values were still zeroed in the area protected by the wall (with the exception of a single
446 parcel, close to the ground downwards the rock). Lichen recolonization was not observed
447 neither in the protected nor in the unprotected areas of Rock 70.

448 At twenty months after the cleaning, cleaned surfaces protected and unprotected by the wall
449 did not show significant differences in lightness (L^*), while uncleaned and unprotected
450 surfaces had lower L^* values (Fig. 6). Twenty months later, the rock surfaces unprotected by
451 the wall were significantly darkened (low L^* in Fig. 6), with different levels of darkening
452 depending on the proximity to the vegetated ground upwards and the prevalent direction of
453 water fluxes. Conversely, rock surfaces well protected by the wall showed not or just
454 perceivable differences in L^* , and uncleaned control surfaces (Rock 71) displayed a smooth
455 darkening (because they were already dark).

456

457 **Discussion**

458 Approaches to hinder recolonization dynamics following cleaning interventions are still
459 mostly related to the application of products directly on the heritage surfaces in order to
460 reduce their bioreceptivity (*e.g.* Pinna et al. 2012; Sasso et al. 2016; Domínguez et al. 2021),

461 and to the regulation of artificial light regimes (Sanmartín 2021). In the case of rock art,
462 hypotheses and suggestions on a potential conservative effect of reducing the shade created
463 by trees, and redirecting water flow, were formulated (Tratebas 2004), but have been poorly
464 experimentally verified and put into practice (e.g. in the case of Norwegian sites; Bjelland &
465 Kjeldsen 2020). In this work, we show that the characterization of lithobiontic communities in
466 a rock art site and the recognition of environmental factors favouring (re-)colonization
467 dynamics may address preventive strategies based on local (micro-)environmental
468 conditioning, successfully prolonging the maintenance of heritage surfaces in a clean state.
469 The characterization of lichen diversity particularly supported the recognition of factors
470 responsible for lithobiontic colonization patterns, confirming the role of lichens as useful
471 indicators in various fields of application, including the conservation of Cultural Heritage
472 (Aptroot & James 2002).

473

474 *Lichens and other lithobionts on rocks with different conservation history*

475 The lack of detailed knowledge on the conservation history of each outcrop in the Naquane
476 site before the 1980s (further details in the caption of Fig. S3), prevents a full reconstruction
477 of (re-)colonization patterns in the investigated site. Nevertheless, the abundances of
478 lithobiontic components through the plots are significantly explained by their different
479 colonization rates following recent cleaning interventions and some heterogeneity in available
480 niches.

481 Microbial biofilms, including cyanobacterial ones, were reported as the main lithobiontic
482 component in several rock art sites, and their presence was variously associated to
483 biodeterioration or bioprotection processes -which depend on the lithology and the
484 environmental conditions (Villa et al. 2016)-, and even, in some cases, with the past formation

485 of surface crusts which coat the stones and were carved by the engraving activities
486 (Rabacchin et al. 2022; Zerboni et al. 2022). In the case of Naquane, the low porosity and
487 high cohesion of the substrate seem to limit a diffuse endolithic, and more deteriogenic,
488 behaviour of cyanobacteria, which find enough suitable conditions for a rich epilithic growth
489 in the local temperate climate with no dry season (Rubel et al. 2017). The prevalence of
490 cyanobacterial and algal patinas on 3YC surfaces agrees with their ability to colonize rocks
491 faster than lichens (e.g. Lázaro et al. 2008), which are on their turn widespread on 12YC and
492 prominent on several NRC outcrops. In agreement with the succession proposed by Caneva et
493 al. (2008), mosses are also negligible on 3YC and 12YC surfaces, while they are dominant on
494 some NRC outcrops. Such different levels of pioneer activity add up to the preference of
495 mosses and lichens for rougher and less steep surfaces with respect to the biofilms, as
496 displayed in CCA-I (Fig. 4A).

497 Levels of direct irradiation and shading were shown to influence the distribution (and
498 deteriogenic impact) of lithobiontic components on building surfaces, with epilithic
499 cyanobacteria and green algae dominating shaded sides and lichens prevailing on sunny dry
500 ones (Ariño & Saiz-Jimenez 1996). Moreover, for each component, the different (micro-
501)environments host different species assemblages, as shown in the cases of the Roman
502 Amphitheater of Italica (Spain; Nimis et al. 1998) and of the engraved schists of the Côa
503 Valley Archaeological Park (UNESCO, Portugal; Marques et al. 2014), where different lichen
504 communities characterized surfaces with different aspect. In the case of Naquane, the EXP
505 factor was not a significant conditional factor neither with respect to the distribution of the
506 different lithobiontic components nor for the different lichen taxa. This is likely because the
507 effect of the punctual surface aspect was masked by the general NW exposition of the valley
508 side occupied by the Park. However, different lichen communities were observed in Naquane,
509 with the high beta-diversity values obtained in SDR analysis mostly associated to the turnover

510 of xerophytic and mesophytic-hygrophytic species, as shown by the PCoA. Such patterns of
511 lithobiontic distribution on heritage stone surfaces were generally related to different
512 orientations and aspect (Aubry et al. 2012; Adamson et al. 2013; Marques et al. 2014). In the
513 case of Naquane, each outcrop was differently shaded by tree cover and exposed to water
514 runoff after rain events (see next sub-chapter).

515 Lichen communities on 12YC and 3YC plots mostly show very low cover values and appear
516 as subsets of the richer communities on NRC outcrops. Nevertheless, the higher similarity of
517 12YC and NRC with respect to the NRC-3YC and 12YC-3YC combinations (SDR analysis)
518 indicate that the most pioneer phase of recolonization is already concluded in less than twelve
519 years after the cleaning interventions. Species commonly found in synanthropic environments
520 prevail, although some species usually associated to undisturbed conditions persist, as *P.*
521 *flavicans* and *F. lygaea*. Such pattern reflects the shift observed on several heritage surfaces
522 after cleaning interventions, with nitrophytic, fast-growing species becoming prevalent with
523 respect to originally dominant species (Nascimbene et al. 2009). Persistence of original
524 species and, in general, fast recolonization in few years likely relates with the ineffective
525 application of biocides by brush, which generally showed poor effectiveness in the
526 devitalization of crustose species and particularly in dedicated assays recently performed in
527 Naquane (Favero-Longo et al. 2021). Such results show the importance of performing
528 effective devitalization treatments to avoid losing the original lichen biodiversity value
529 without obtaining a durable cleaning result. Remarkably, most species on 12YC and 3YC
530 plots show prevalence of asexual reproductive modes (mostly soredia) and/or produce small,
531 highly dispersive ascospores (species of genera *Caloplaca* s.l., *Candelariella* s.l., *Lecanora*
532 s.l.), remarking their potential for rapid recolonization and their potential threat to heritage
533 surfaces (Scheidegger & Werth 2009; Morando et al. 2019). It is worth noting that the total
534 diversity of 37 taxa is rather low for the surveyed area, mostly including common species of

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

541

542 *Physical interaction of lichens and other lithobionts with the sandstone substrate*

543 Lichen colonization of engraved outcrops was already deeply considered with respect to the
544 deteriorative impact in several sites, including the Côa Valley, in the Mediterranean area,
545 where deep hyphal penetration and physical bioweathering were recorded on schists (Marques
546 et al. 2016). Lichens are also dominant on engraved sandstones from the subarctic zone,
547 where their biogeochemical activity was associated to the waning of an original surface red
548 colour (e.g. Alta, Norway; Tansem & Storemyr 2020), to the dry semi-arid zone, where
549 physical and chemical degradation processes were microscopically documented (e.g. el Morro
550 National Monument, New Mexico; Knight et al. 2004). Although the observations were
551 limited to few cross sections for conservative reasons, the physical interaction of lichens with
552 the examined sandstones appears rather mild, as we observe a poor hyphal penetration even
553 for *Verrucaria nigrescens*. This common colonizer of heritage surfaces was indeed often
554 reported as a deeply penetrating and impacting species on different lithologies, including
555 other sandstones (Tonon et al. 2021, with refs. therein), although with different intergranular
556 matrices and lower compactness. The hyphal penetration beneath the points of attachment of
557 *Xanthoparmelia rhizinae* was also negligible, in this case as usually observed on other
558 lithologies (e.g. on gneiss; Favero-Longo et al. 2015). The cyanobacterial biofilm also

559 displayed an epilithic behaviour, differing from observations on other sandstone substrates, in
560 which the endolithic growth was prominent (e.g. Büdel et al., 2004; Zerboni et al. 2022).
561 Accordingly, the lithobiontic colonization in Naquane appears as a deteriogenic phenomenon
562 mostly because of surface masking and chromatic disfiguring, while interactions with the
563 substrate responsible for a decreased surface durability seem less important than in other
564 cases. However, we observed a higher hyphal penetration on the same lithology, but on the
565 opposite, ESE-facing, side of the Valley (Favero-Longo et al. 2017), in agreement with the
566 findings that different micro-environmental conditions related to a different surface aspect can
567 imply different bioweathering impacts on stone durability (Marques et al. 2016).

568

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

571 A long period of wetness, due to slow drying or prevailing wind directions, were
572 demonstrated to support lithobiontic colonization on stone materials. Investigations in the wet
573 N-Ireland showed that green algae and lichens colonized north-facing stone blocks (including
574 sandstones) faster and more abundantly than those facing south (Adamson et al. 2013). In
575 Pompeii, surfaces exposed to the prevailing winds during rain events showed richer
576 lithobiontic communities than differently oriented ones (Traversetti et al. 2018). In the case of
577 Naquane, in a similar way, tree shading (TRC) and the presence of bare or vegetated ground
578 above the engraved outcrops (GRP) are factors favouring lithobiontic recolonization after
579 cleaning, according to CCAs. Their significant effect on the water and moisture availability,
580 and the consequent biological dynamics, is confirmed by the prevalent regrowth of meso-
581 /hygro-phytic lichen species on 12YC and 3YC surfaces (PCoA). Oppositely, recolonization
582 by xerophytic species on directly exposed rock outcrops seems to require longer times. The

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

585 Such recognition of environmental factors favouring lithobiontic (re-)colonization was
586 considered with success in the experiment of preventive conservation conducted on Rock 70,
587 combining some reduction of tree cover with the altering of water flow on an engraved rock
588 outcrop. The development of a phototrophic biofilm and the darkening of the rock surface,
589 quantified by fluorimetric and colorimetric measures, respectively, was significantly related to
590 the absence of the wall protection by prolonged and nutrient-enriched water fluxes. Thus,
591 preventive approaches and the (micro-)environmental conditioning by water flow regulation
592 seem particularly promising to circumscribe surfaces where lithobiontic communities and
593 related biodeterioration effects are hindered and the legibility of engravings is preserved. On
594 other surfaces, the lithobiontic presence may instead be accepted, and possibly exhibited as an
595 additional value of the cultural heritage site.

596 On the other hand, the change of water flows may imply some community shift on the long
597 term, in particular favouring lichens rather than cyanobacterial biofilms (Bjelland and Helberg
598 2006), although lichens have still not (re-)appeared 40 months after the cleaning through the
599 whole outcrop. More generally, the drainage of water or, simply, the altering of water flows
600 imply the addition of non-natural elements in the archaeological natural scenario, as the
601 considered brick wall or other kinds of barriers (Bjelland and Helberg 2006). With this regard,
602 it has to be remarked that the wall considered here is an experimental structure to evaluate
603 benefits obtainable with the control of water fluxes. The development of further strategies to
604 obtain similar results without touching the engraved surface is needed. In any case, although
605 barriers to water flows may be visually unpleasant, the traditional applications of synthetic
606 biocides to periodically devitalize and remove established lithobiontic communities may
607 imply even a higher impact by affecting the environmental equilibria (Cappitelli et al. 2020).

608

609 Conclusions

610 This work characterized the diversity and abundance of lithobiontic communities in the Rock
611 Engravings National Park of Naquane (UNESCO WHS n. 94, Italy), highlighting
612 cyanobacterial biofilms and lichens as the dominant constituents. They both displayed poor
613 penetration within the sandstone substrate, likely because of its high compactness and very low
614 porosity, but they were responsible for chromatic disfiguring and limited the legibility of rock
615 art. Tree cover and the presence of bare and vegetated ground upstream of the rocks resulted as
616 the main drivers of recolonization on surfaces cleaned in the last twelve years, likely prolonging
617 surface wetness after rain events and increasing nutrient availability. Nitrophytic species,
618 including epiphytes from surrounding trees, and few meso-hygrophytic species, mostly
619 producing soredia, were mainly responsible of the rapid lichen recolonization. An experiment
620 of preventive conservation performed on a critical rock, including an effective devitalization of
621 lithobionts before cleaning, combined with reduction of tree cover and surface protection from
622 prolonged water fluxes from vegetated ground, prevented recolonization by lichens and other
623 lithobionts for a monitored period of 40 months. By contrast, cleaned surfaces unprotected from
624 prolonged water fluxes showed recolonization, demonstrating the suitability of
625 microenvironmental control strategies to limit and delay biodeterioration issues on the outdoor
626 stone cultural heritage. To make similar preventive approaches practicable, ecological
627 investigations of environmental factors favouring lithobiontic colonization are crucial and,
628 thanks to advanced knowledge on their specific ecological requirements, lichens particularly
629 appear as suitable indicators.

630

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640

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811

812 **Figure captions**

813 Fig. 1. Abundance of different lithobiontic components (CyB, cyanobacterial-dominated
814 biofilm; MCF, microcolonial fungi crusts; AIB, green algal-dominated biofilm; Bry,
815 bryophytes; Lic, lichens) on the engraved rocks, considering the overall plots together (A) and
816 separately for rocks cleaned in the last three years (3YC, B), twelve years (12YC; C) or from
817 more than 40 years (NRC; D). , Data are expressed in terms of percentage of plots with cover
818 values in the following ranges: >75% (black), 51-75% (dark grey), 26-50% (grey), 2=2-25%
819 (light grey), visible cover, but <2% (grey bands), absence of visible cover (white).

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

826 Fig. 3. Lithobiontic penetration within the sandstone substrate. A, cyanobacterial biofilm; B,
827 *Xanthoparmelia conspersa*; C, D (inset), *Verrucaria nigrescens*. Arrows indicate
828 cyanobacterial penetration within a fracture (A) and the hyphal penetration component of
829 lichens (B, D). Scale bars: 1.0 mm (A), 1.5 mm (B, C), 350 μ m (D).

830 Fig. 4. Factorial map in the canonical correspondence analysis showing the position of plots
831 having a different conservation history with the contributions of lithobiontic covers (A, CCA-
832 I) and specific lichen frequencies (B, CCA-II), together with environmental factors (tree
833 cover, TRC; surface micromorphology, ROU; inclination, INC; distance from bare or
834 vegetated ground upstream, GRP; exposition, EXP). Symbols indicate different lithobionts
835 (black circles: lichens, Lich; bryophytes, Bry; cyanobacterial biofilm, CyB; green algal

836 biofilm, ALB; meristematic fungi, MCF), and NRC (crosses), 12YC (grey squares) and 3YC
837 (white squares) rocks. In CCA-II (B), half of plots with highest lichen abundance for the NRC
838 and 12YC-3YC categories (in terms of total specific frequencies) display a higher symbol
839 size; contributions of the different species are separately shown in Fig. S4.

840

841 Fig. 5. Basal fluorescence (F_0 , A) and maximum quantum efficiency of Photosystem II
842 photochemistry (B, F_v/F_m) quantified on Rock 70 during preliminary biocide assays (July
843 2019; T0, one day before biocide application, T1, one day after biocide application), and 20
844 (March 2021) and 40 (November 2022) months after the cleaning, in areas of the outcrop
845 protected (W) and non-protected (NW) by the wall, and on uncleaned areas as control (U). At
846 each measuring time point, box-plots which do not share at least one letter are statistically
847 different (ANOVA, Tukey's test, $p < 0.05$).

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

853

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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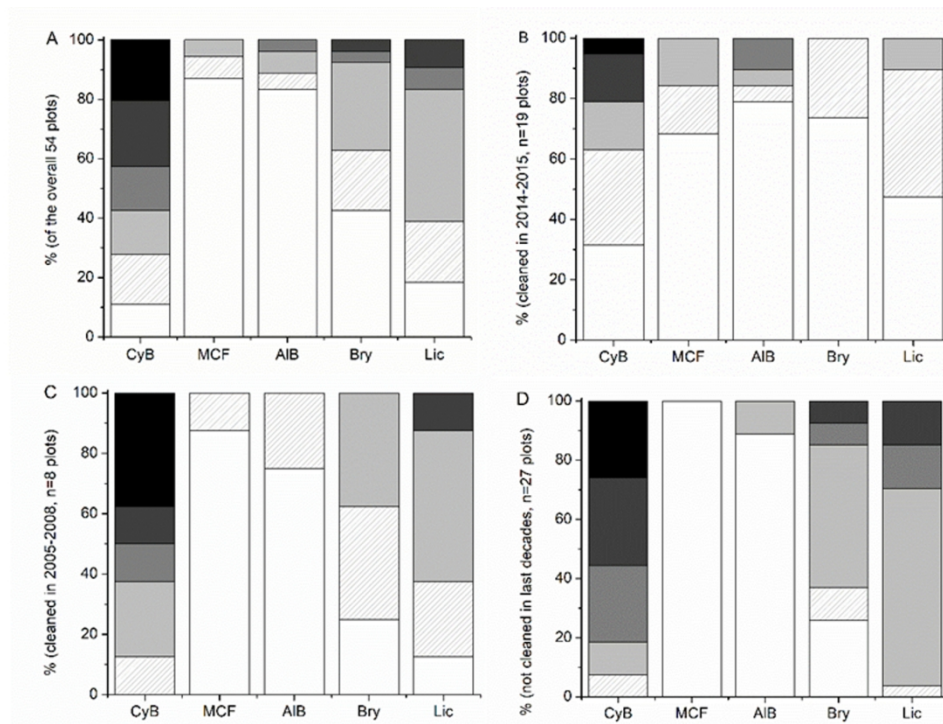


Fig. 1. Abundance of different lithobiontic components (CyB, cyanobacterial-dominated biofilm; MCF, microcolonial fungi crusts; AIB, green algal-dominated biofilm; Bry, bryophytes; Lic, lichens) on the engraved rocks, considering the overall plots together (A) and separately for rocks cleaned in the 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), 2-25% (light grey), visible cover, but <2% (grey bands), absence of visible cover (white).

169x125mm (300 x 300 DPI)

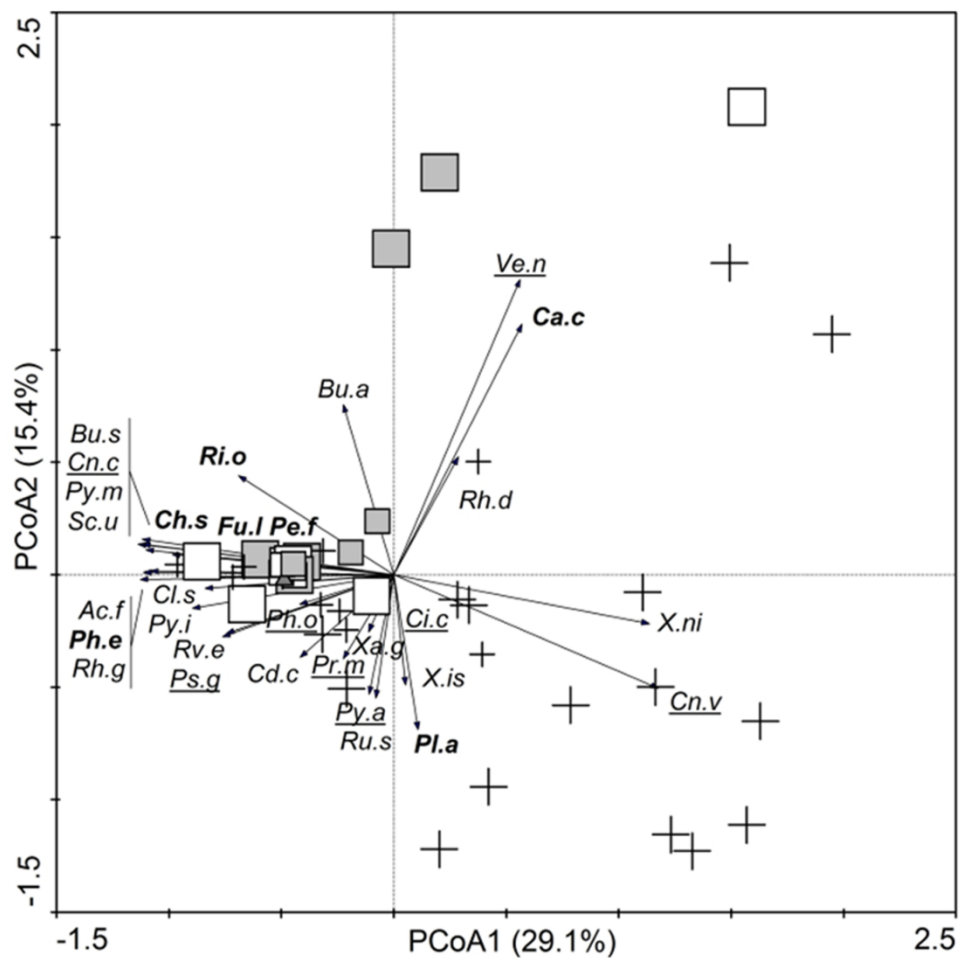


Fig. 2. Ordination of plots on the basis of the specific lichen frequencies (PCoA). Plots are differently marked according to the different conservation history of the surveyed rocks (NRC, crosses; 12YC, grey squares; 3YC, white squares). Half of plots with highest lichen abundance for the NRC and 12YC/3YC 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).

169x168mm (300 x 300 DPI)

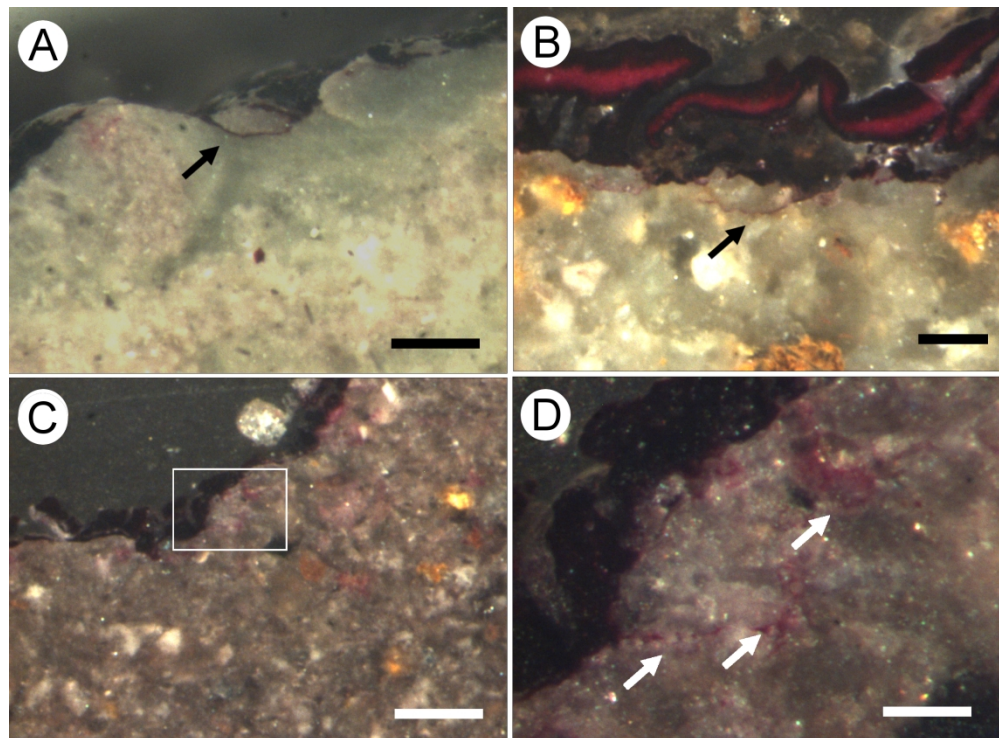


Fig. 3. Lithobiotic penetration within the sandstone substrate. A, cyanobacterial biofilm; B, *Xanthoparmelia conspersa*; C, D (inset), *Verrucaria nigrescens*. Arrows indicate cyanobacterial penetration within a fracture (A) and the hyphal penetration component of lichens (B, D). Scale bars: 1.0 mm (A), 1.5 mm (B, C), 350 μm (D).

137x101mm (500 x 500 DPI)

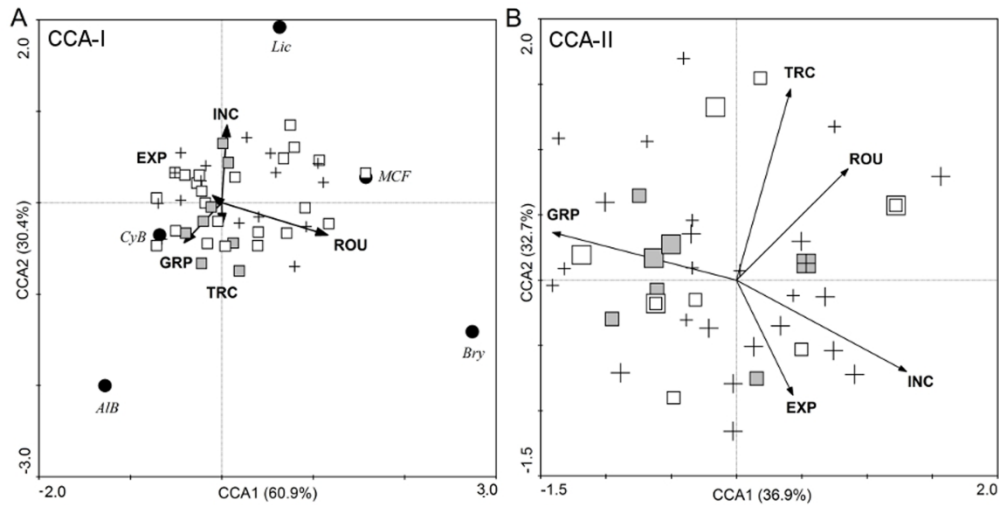


Fig. 4. Factorial map in the canonical correspondence analysis showing the position of plots having a different conservation history with the contributions of lithobiontic covers (A, CCA-I) and specific lichen frequencies (B, CCA-II), together with environmental factors (tree cover, TRC; surface micromorphology, ROU; inclination, INC; distance from bare or vegetated ground upstream, GRP; exposition, EXP). Symbols indicate different lithobionts (black circles: lichens, Lic; bryophytes, Bry; cyanobacterial biofilm, CyB; green algal biofilm, AIB; 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)

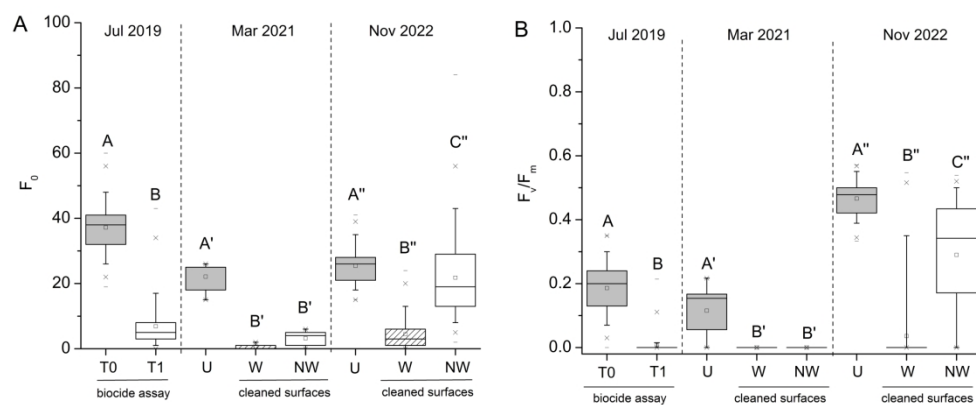


Fig. 5. Basal fluorescence (F_0 , A) and maximum quantum efficiency of Photosystem II photochemistry (B, F_v/F_m) quantified on Rock 70 during preliminary biocide assays (July 2019; T0, one day before biocide application, T1, one day after biocide application), and 20 (March 2021) and 40 (November 2022) 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, $p < 0.05$).

136x59mm (500 x 500 DPI)

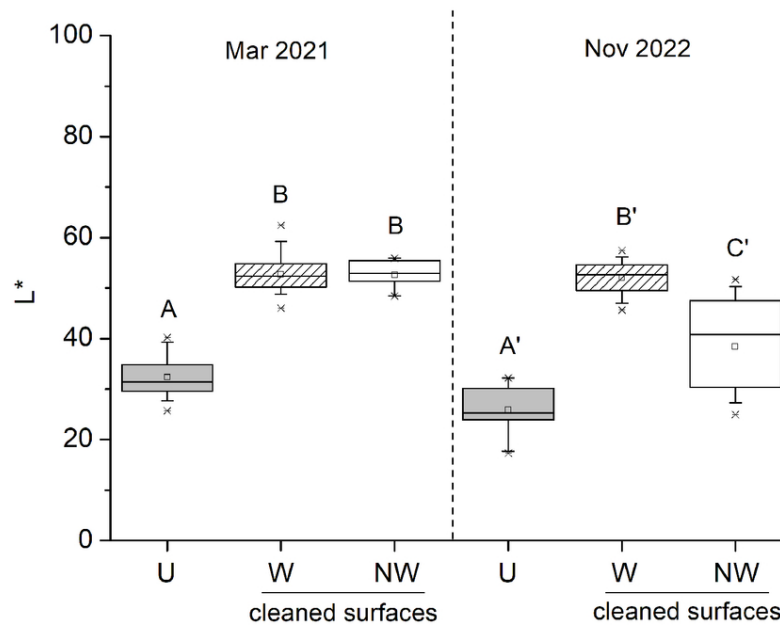


Fig. 6. Lightness of the surface (L^*) of Rock 70 quantified 20 (March 2021) and 40 (November 2022) months after the cleaning in areas of the outcrop protected (W) and non-protected (NW) by the wall, and on uncleaned areas as control (U). At each measuring time point, box-plots which do not share at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

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

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

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Repr.	Ecological indicator values				All the plots (n=27)			
	pH	IR	AR	EU	Occurrence (plot %)	Cover (%)		Freque
						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

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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Frequency(%)	3YC rocks (n= 19 plots)					Occurrence (plot %)
	Max.	Cover (%)		Frequency(%)		
		Occurrence (plot %)	Av.	Max.	Av.	
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	-	-	-	-	-	-

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

-	-	-	-	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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ncy(%)

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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