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A laboratory approach on the combined effects of granite bioreceptivity and parameters modified by climate change on the development of subaerial biofilms on cultural heritage

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ABSTRACT

Conservation of cultural heritage buildings and monuments may be negatively affected by the impact of climate change on substrates and colonizing microorganisms. In this study, four types of commercial granite, in which the bioreceptivity ranged from very low to very high, were inoculated with a multispecies microbial culture and exposed to different temperatures (18 and 24 °C) and levels of water availability (1 day, 3 days and 7 days of water availability/week) to simulate different climatic conditions. The effects on biofilm formation of the interactions between the substrate bioreceptivity and the environmental parameters were investigated. Biofilm growth and photosynthetic efficiency were monitored over 42 days by pulse-amplitude modulated (PAM) fluorometry and spectrophotometric colour measurement. The primary bioreceptivity of the granite was a determining factor in the organisms' capacity to attach to the substrate and interacted through the experiment with the climatic conditions by modifying the development of the microorganisms. Water availability was a limiting factor for the colonizing microorganisms, in terms of both growth and photosynthetic efficiency. At the higher temperature, metabolic reactions were enhanced under water restriction (but not under maximum water availability) and the microbial ecology was altered, leading to a higher proportion of cyanobacteria relative to algae.

1. Introduction

Cultural heritage is a legacy left by our ancestors, with which we live at present and which we must pass on to future generations. Once lost, it is unrecoverable, and conservation and protection of cultural heritage buildings and monuments against harmful agents, such as rapid environmental changes, is therefore of particular importance (UNESCO World Heritage Centre, 2007). Since the Industrial Revolution in the 18th century, the levels of greenhouse gases of anthropogenic origin have been increasing continuously, leading to an increase in the average temperature of between 0.6 and 4 °C. Global warming produces changes in the climate, with fluctuations occurring much faster than at any other time in the Earth's history. One of the most commonly studied consequences is the change in precipitation patterns and the distribution of droughts.

In this context, there is growing concern about the extent to which these changes will affect cultural heritage, as the construction materials used are usually fragile or weathered as a consequence of their long existence. These materials are not designed to withstand the "new" climatic conditions, and despite having been exposed to environmental fluctuations for many years, they have never experienced the current rate of change (Haugen et al., 2018). In recent years, this matter has begun to be given special attention, with numerous studies providing new insights into the relationship between environmental changes and cultural heritage (Sabbioni et al., 2009; Brimblecombe et al., 2011; Fatorić and Seekamp, 2017; Sesana et al., 2020). Information about the effects can lead to an oriented preventive conservation, focusing resources on the heritage monuments most susceptible to deterioration.

Stone is one of the materials most commonly and widely used throughout history, and despite its strength and durability, it is affected by deterioration processes. Climate is one of the main causes of degradation, due to both the direct physical-chemical effects on the stone and to the impact it has on the activity of microorganisms that inhabit the stone surface. The complex ecology of stone-built monuments is strongly affected by environmental changes, as the ecological niche is exposed to environment stressors. Biofilms, which are communities of algae, fungi,

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cyanobacteria and bacteria embedded in a matrix of exopolysaccharides, are habitual inhabitants of stone walls in cultural heritage monuments. Subaerial biofilms occur at the interface between the atmosphere and the substrate, and any environmental changes will influence the microorganisms, in turn, altering the stone (Viles and Cutler, 2012; Favero-Longo and Viles, 2020). In this context, the concept of bioreceptivity, defined by Guillitte (1995) as "the capacity of a material to be colonized by living organisms", is of particular importance. The bioreceptivity of materials can be modulated by climatic factors, making the materials more susceptible to colonization; however, climatic factors can also affect the microorganisms and their survival. Although changes in climatic factors can increase the bioreceptivity of the substrate as it degrades, little is known about the importance of microorganisms in mediating these changes. The complex network of interactions makes it difficult to determine the impact of environmental changes as a result of the interaction between the initial state of the stone and the type of microorganisms colonizing the stone.

Among the parameters affected by climate change, temperature and water availability have the strongest impact on phototrophic organisms, while also affecting the integrity of the substrate (Ariño et al., 2010). Modifications in porosity and roughness can favour water retention, and water availability regulates the growth of microorganisms (Cutler et al., 2013a; Gladis-Schmacka et al., 2014) and their physiology (Häubner et al., 2006; Karsten and Holzinger, 2012; Prieto et al., 2020; Fuentes and Prieto, 2021). Temperature, on the other hand, can alter the microbial composition of ecosystems, causing displacement of species and modification of metabolic rates (Beardall and Raven, 2004; Ariño et al., 2010; Hodkinson et al., 2011). Although the individual effects of temperature and water availability on microorganisms have been well studied, little is known about how the interaction with each other affects the development of subaerial biofilms, and even less about the differences linked to the primary bioreceptivity of stones.

At present, climate conditions may be changing faster than the ability of the environment to adapt. In this context, the study of the effect of different factors associated with climate change in relation to substrate bioreceptivity is of particular interest. Study of the interactions between microbial growth and environmental changes, taking into account the bioreceptivity of the stone, is important as these interactions can influence the conservation of cultural heritage (Gorbushina, 2007; Joh and Lee, 2012; Viles and Cutler, 2012; Witt et al., 2012). In this paper, we report a first approach to investigating the combined effects of temperature, water availability and stone primary bioreceptivity on the growth of phototrophic organisms on granite. With this aim, we monitored the development of biofilms on granite samples differing in bioreceptivity and subjected to different environmental conditions, by using pulse-amplitude modulated (PAM) fluorometry and spectrophotometric color measurement.

2. Materials and methods

2.1. Preparation of granite samples, culture inoculation and experimental design

Four different types of commercially available granite, commonly used as construction and ornamental material, were used to evaluate the development of microbial colonization under different environmental conditions. The ornamental granites were selected taking into account their bioreceptivity index (protocol developed by Vázquez-Nion et al., 2018): two with low bioreceptivity (*Negro Sudafrica (NS) and Silvestre B (BLUE)*) and two with high bioreceptivity (*Silvestre AM (SAM)* and *Silvestre G (GOLDEN)*) (Fig. 1). The characteristics of these granites are listed in Table 1.

Eighteen 4 × 4 × 2 cm specimens of each granitic rock were prepared. Sawn surface finish was applied to all of them. After autoclaving, a volume of 2 ml of a multi-species culture characterized by Vázquez-Nion et al. (2016), in exponential growth phase, was inoculated onto the upper surface of each specimen using a micropipette. The culture is mainly composed of the green algae *Bracteacoccus minor* (Schmidle ex Chodat) Petrová, *Stichococcus bacillaris* Nägeli and *Chlorella* sp., and the cyanobacteria *Aphanocapsa* sp. and *Leptolyngbya cebennensis* (Gomont) I.Umezaki and M.Watanabe.

The inoculated samples were held in climatic chambers (SCLAB PGA-1228/2 HR and AIR-FRIO AIR-1330-HRTLV) with 12 h light/dark cycles (LED tubes, 6500 K, 1500 lm) and constant relative humidity (80%), at two different temperatures and 3 different levels of water availability: 18 °C and 24 °C, and 7 days (H7, non-days of drought), 3 days (H3, with 4 days of drought) and 1 day (H1, with 6 days of drought) of water availability per week (Fig. 2). For the water availability experiment, the samples were placed horizontally in trays with sterile water up to the sample surface. Water was regularly renewed to maintain stable level. The H3 and H1 drought periods were performed by removing samples from water. These water-dry cycles were repeated weekly for 42 days.

2.2. Monitoring biofilm formation

The chlorophyll fluorescence (proxy for growth (Eggert et al., 2006) and physiological state (Genty et al., 1989)) and the colour variation (proxy for biomass and pigment production (Prieto et al., 2002)) were monitored immediately after inoculation of the samples and 7, 15, 28 and 42 days later, to determine any effects on biofilm development.

Fluorescence signals in the samples were determined at 470, 645 and 665 nm by pulse-amplitude modulated (PAM) fluorometry, in a Phyto-PAM system (Heinz Walz GmbH) equipped with a Phyto-EDF fibre optics emitter-detector unit. The samples surfaces were hydrated with water using a hand-held atomiser and held in darkness for 20 min before the data were recorded, in order to allow the oxido-reduction state of the PSII centres. A total of nine random readings were made on the surface of each sample, to measure the following variables: minimal fluorescence in the dark-adapted state (F_0, proxy for biomass), i.e. with all the PSII centres open, and the maximal fluorescence in the dark adapted state (F_M), i.e. with all the PSII centres closed. The values of these

Fig. 1. Photograph of the four types of granites used in the experiment (from low to high bioreceptivity index).

Table 1
Description of the lithotype studied.

Commercial name	Abbreviation	Classification	B.I.[a]	Texture	Size (cm)	Open porosity (%)
Negro Sudáfrica	**NS**	Gabbro	1.7 - Low	Fine/medium grained	4 × 4 × 2	0.53
Silvestre B	**Blue**	Monzogranite	2.2 - Low	Fine grained	4 × 4 × 2	0.90
Silvestre G	**Golden**	Monzogranite	8.3 - High	Fine grained	4 × 4 × 2	2.53
Silvestre AM	**SAM**	Monzogranite	8.4 - High	Medium grained	4 × 4 × 2	3.66

[a] Bioreceptivity index (B.I.) was calculated following the protocol developed by Vázquez-Nion et al. (2018)

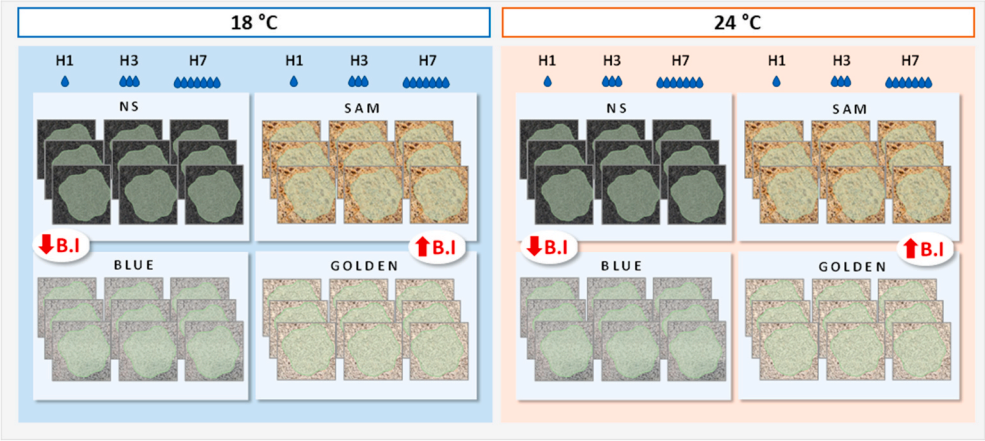

Fig. 2. Schematic diagram of the experimental design applied to each group of samples (N = 3) divided into the four different granites (NS, BLUE, GOLDEN and SAM), three levels of water availability (H1, H3 and H7) and two temperatures (18 and 24 °C). ↓B.I indicates granites characterized by low bioreceptivity and ↑ B.I indicates granites characterized by high bioreceptivity.

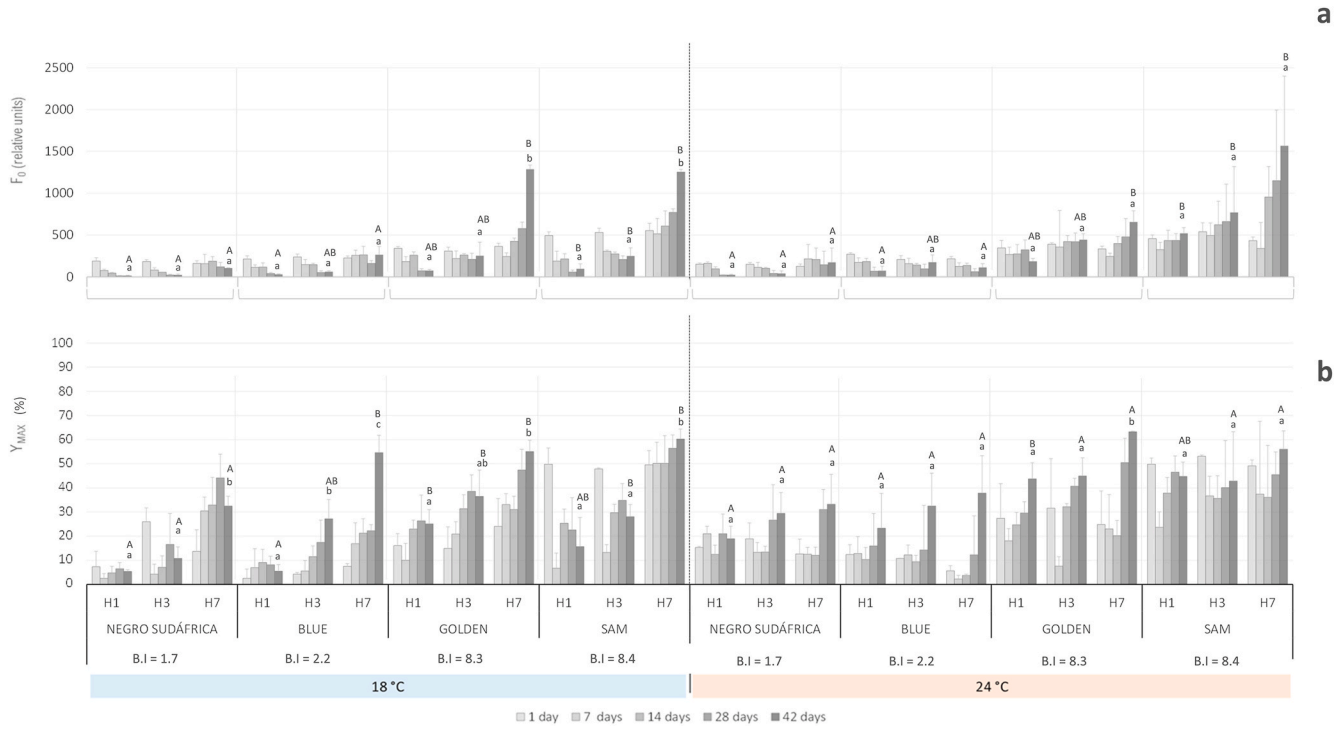

Fig. 3. F_0 (biomass proxy) (a) and Y_{MAX} (biofilm photosynthetic efficiency) (b) values for the different biofilms formed on the granite samples (NS, SAM, BLUE and GOLDEN) under different conditions of water availability (H1, H3 and H7) and temperature (18 and 24 °C) at 1, 7, 14, 28 and 42 days (from darker to lighter red, respectively). Different lower-case letters indicate significant differences at the end of the experiment between the three levels of water availability (for each group formed by granite type and temperature), while the different capital letters indicate significant differences between the four different types of granite (for each group formed by water availability and temperature) (Duncan post-hoc test at $\rho > 0.01$).

variables were used to calculate the maximum quantum efficiency of PSII photochemistry ($Y_{MAX,}$ proxy for physiological state), as $(F_M\text{-}F_0)/F_M$ (Logan, 2005). In addition, in order to study the variation in the relative microbial abundance, the $F_0$470nm/$F_0$645nm ratio was calculated as the signal at 470 nm, related to chlorophyll *b* (algae), and the signal at 645 nm, related to allophycocyanin (cyanobacteria). High values of the ratio indicate a dominance of green algae, while low values indicate dominance of cyanobacteria (Fuentes and Prieto, 2021).

Colour variations were determined by the spectrophotometric colour technique, using a portable spectrophotometer (Konica Minolta CM-700d) with an 8 mm diameter target area and equipped with CM-S100w software (Spectra Magic TM NX). The analytical conditions were as follows: D65 illuminant, 2° observer and SCI mode. Nine random measurements were made directly on the SABs surfaces. The data were analyzed using the CIELAB colour system (CIE, 1978), where a* denotes the red/green value (a* (+) = red; a* (−) = green) and b* indicates the yellow/blue value (b* (+) = yellow, b* (−) = blue). These parameters were represented as variations in the values relative to the original colour of the stone, thus yielding Δa* and Δb*. To analyse data, color variation of 2 CIELAB units was used as indicative of differences as this is the threshold of perceptible changes noticed by experienced observers (Mokrzycki and Tatol, 2011).

2.3. Statistical analysis

The chlorophyll fluorescence derived data (F_0, Y_{MAX} and $F_0$470nm/$F_0$645nm ratio) obtained just after inoculation and at the end of the experiment were subjected to a Three-Way ANOVA with a post-hoc Duncan's test (p-value≤0.01). For this purpose, SPSS statistical program (version 23.0) was used.

3. Results

The minimum fluorescence (F_0) of the biofilm (biomass proxy) over time is represented in Fig. 3a, for each group of samples of granite rocks subjected to different temperatures (18 and 24) and different humidity levels (H7, H3 and H1). For NS, the fluorescence was very low under all of conditions and decreased over time. This was observed in the samples maintained at 18 °C and at 24 °C. In the case of BLUE rock, F_0 was very low, reaching slightly higher values at the end of the experiment at 18 °C, but only for maximum water availability (H7). The values were low in the samples held at 24 °C, regardless of the level of water availability, and tended to decrease over time. The F_0 values for the GOLDEN samples increased over time in the samples subjected to maximum water availability (H7) at both 18 and 24 °C, but in the latter case lower maximum values of F_0 were obtained. When water availability was reduced, the fluorescence was much lower, decreasing over time, both at 18 and 24 °C. For the SAM granite, the highest F_0 values are found, especially at maximum water availability (H7). In this scenario (H7), the biofilm fluorescence increased over time, both at 18 and 24 °C. However, the F_0 values for the biofilms subjected to 3 and 1 days of water availability (H3 and H1) varied with the temperature. At 18 °C the values decreased over time, while at 24 °C this parameter tended to increase, not reaching values as high as with maximum water availability.

The maximum yield (Y_{MAX}), which indicates the photosynthetic efficiency, is represented in Fig. 3b. For the NS samples, the microorganism efficiency generally increased over time. The highest photosynthetic efficiency (45%) was reached on day 28 at 18 °C with maximum water availability (H7). At the end of the experiment, the efficiency was about 30% for samples exposed to maximum water availability, and below this value for the lower levels of water availability (H3 and H1), both at 18 and 24 °C. An increase in photosynthetic efficiency over time at both 18 and 24 °C was observed for the BLUE samples. At 18 °C the highest photosynthetic efficiency for those samples (55%) was observed at the end of the experiment for the biofilms with maximum water availability (H7) but when the availability was decreased to 1 day (H1), the maximum photosynthetic efficiency was greatly reduced (<10%). This did not occur when samples were held at 24 °C, as the maximum photosynthetic efficiency at the end of the experiment with maximum water availability was lower (37%) and when the water availability decreased, the photosynthetic efficiency did not vary greatly (>20%). For GOLDEN samples, the photosynthetic efficiency increased over time for maximum water availability (H7) and 18 °C, exceeding 50% by the end of the experiment. However, when the water availability was decreased, although the trend continued upwards, the values were below 40%. When biofilms were exposed to 24 °C, the microbial abundance increased over time (between 44 and 63%) for all levels of water availability, reaching 63% for maximum water availability (H7). Finally, SAM photosynthetic efficiency was high when biofilms were exposed to maximum water availability and 18 °C, reaching an efficiency of 60% by the end of the experiment. In the samples exposed to 24 °C, the photosynthetic efficiency reached 55%. However, for the microorganisms exposed to restricted water levels (both H3 and H1) at 18 °C, the efficiency decreased over time, reaching lower values than for maximum water availability (H7). However, at 24 °C the efficiency remained constant over time for H3 and H1 (exceeding 40% efficiency) and scarcely differing from the values obtained for H7.

Fig. 4 represents differences in the F_0 470 nm/F_0 645 nm ratio (relative to the initial value) at 7, 14, 28 and 42 days. This ratio provides information about the proportion of algae and cyanobacteria in the biofilms. As the signal at 470 nm is related to chl b (algae) and the signal at 645 nm, to allophycocyanin (cyanobacteria), high values indicate a predominance of green algae while low values indicate a predominance of cyanobacteria. When the samples were held at 18 °C, the ratio increased over time for all levels of water availability, relative to the value at the beginning of the experiment for NS, BLUE and GOLDEN granites. For the SAM samples, there was no great variation at the end of the experiment relative to the initial value in the case of samples in which water availability was restricted (H1 and H3) although an increase was noted in the samples subjected to maximum water availability (H7). In the samples held at 24 °C, this trend was somewhat modified. For the microorganisms growing on the granites with highest levels of bioreceptivity (SAM and GOLDEN) and in which water availability was maximum (H7) the index decreased. This was also observed for NS under maximum water availability until day 28 but then reversed towards the end of the experiment.

The colour variations of the biofilms (subtracting the values of the uninoculated substrate) at the end of the experiment with respect to the values obtained just after inoculation are shown in Fig. 5, with Δa* plotted against Δb* both for 18 and 24 °C (left and right respectively). Considering that the central point implies no difference to the just inoculated samples, for NS and BLUE granites both parameters scarcely varied over time (below the threshold of 2 CIELAB units) for any of the conditions studied. In GOLDEN samples a marked trend towards greening (Δa* decreases) and yellowing (Δb* increases) was observed for maximum water availably (H7) and 18 °C. However, the colour of the same samples under the same water conditions but kept at 24 °C, trend to greening but not yellowing. In the case of SAM granite, both Δa* and Δb* values of biofilms subjected to maximum water availably (H7) and both temperatures were over the 2 CIELAB units' threshold. In summary, more notable colour changes towards greening and yellowing were observed in SAM and GOLDEN granites, especially for maximum water availability, while the colour of the other biofilms did not vary during the experiment. Furthermore, for all granites, the differences in colour variation among the three different levels of water availability (H1, H3 and H7) are greater at 18 than at 24 °C.

4. Discussion

The parameters derived from chlorophyll fluorescence and colour

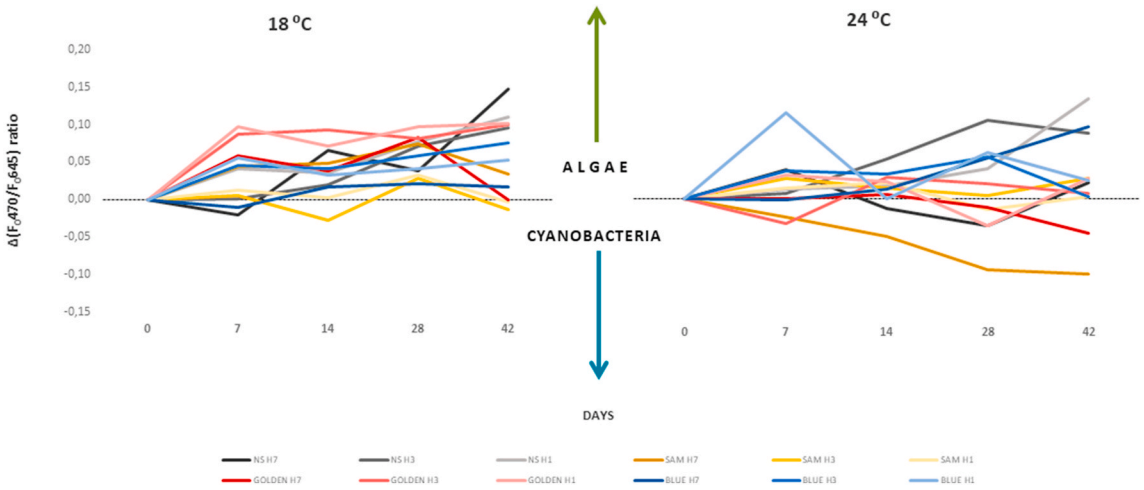

Fig. 4. Changes in the F_0470/F_0645 ratio over time (42 days) for the different biofilms formed on the granite samples (NS, SAM, BLUE and GOLDEN) at 18 and 24 °C.

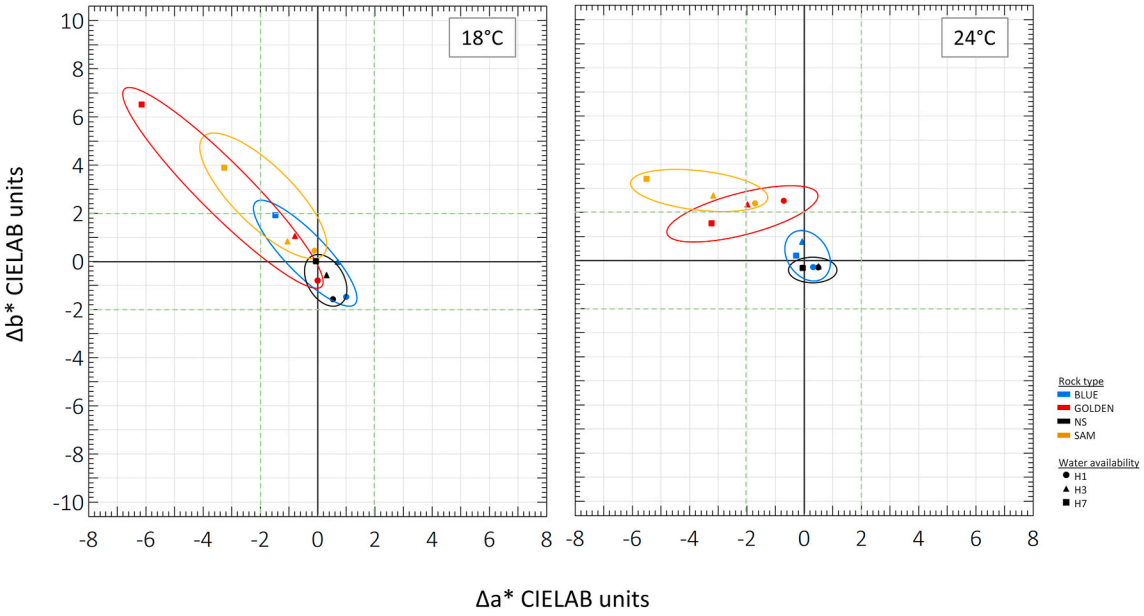

Fig. 5. Plot of Δb* against Δa* colour changes in the biofilms at the end of the experiment (day 42) with respect to just after inoculation, at two different temperatures (18 and 24 °C). Rock substrates are indicated in different colours (black, NS; yellow, SAM; blue, BLUE and red, GOLDEN) and water availability is represented by different shapes (circle, H1; triangle, H2 and square H3). The dashed green line marks the threshold of 2 CIELAB units, after which colour changes are perceived by an experienced observer.

quantification provide estimates of the growth and the physiological state of the biofilms (Eggert et al., 2006; Vázquez-Nion et al., 2016; Genova et al., 2020). Both techniques revealed differences in growth in relation to bioreceptivity and the environmental parameters, with the highest growth and photosynthetic efficiency in the biofilms on samples supplied with maximum water availability for 7 days (H7). Temperature did not appear to have a significant effect on growth, but significantly affected the photosynthetic efficiency (Table 2). Depending on the type of granite, the climatic factors, temperature and water availability, had an effect on the biological colonization as well as on the biofilm photosynthetic efficiency.

Biocolonization of materials depends on both the prevailing climatic conditions and the bioreceptivity of the material (Guillitte, 1995). Although many studies have investigated the bioreceptivity of stone (Prieto and Silva, 2005; Miller et al., 2012; Vázquez-Nion et al., 2018; Favero-Longo and Viles, 2020; Sanmartín et al., 2021) and the effect of environmental conditions on microbial colonization (Ariño et al., 2010; Cutler et al., 2013b; Gladis-Schmacka et al., 2014; Gaylarde and AuthorAnonymous, 2020) no studies have addressed both factors at the same time. The bioreceptivity of a material, specifically of granite, depends on the surface roughness, porosity and chemical composition (Guillitte, 1995; Prieto and Silva, 2005; Miller et al., 2012). In this study, the biomass remaining on the surface of the sample 24 h after inoculation was analyzed by determining the minimum fluorescence parameter. The fluorescence signals (F_0) and maximum yield (Y_{MAX}) differed significantly between the different types of granite (between groups) (Table 2), but not in the 18 samples of the same type of granite (within group). This indicates that the settlement varied on different substrates. This different response is consistent with the bioreceptivity index (BI) of each type of granite considered, as the mean F_0 values increased with those of the bioreceptivity index. The substantial difference in the abundance of organisms on the granite samples with different levels of bioreceptivity, only 24 h after inoculation, may indicate that the properties of the stone that determine bioreceptivity are important during

Table 2
Results of three-way ANOVA of Phyto-PAM measurements just after inoculation and at the end of the experiment, considering type of granite, water availability and temperature as factors. p-values < 0.01 are indicated in bold type.

		Just after inoculation		End of the experiment	
	Factors	F	p-value[a]	F	p-value[a]
F_0					
	TEMPERATURE	1.965	0.167	2.852	0.098
	WATER	0.818	0.448	42.792	**0.000**
	GRANITE B.I.	**186.867**	**0.000**[b]	39.543	**0.000**
	TEMPERATURE * WATER	2.463	0.096	3.457	0.040
	TEMPERATURE * GRANITE B.I.	2.254	0.094	5.156	**0.004**
	WATER * GRANITE B.I.	0.953	0.467	9.066	**0.000**
	TEMPERATURE * WATER * GRANITE B.I.	1.424	0.225	1.364	0.248
Y_{MAX}	TEMPERATURE	4.828	0.033	19.304	**0.000**
	WATER	1.239	0.299	23.669	**0.000**
	GRANITE B.I.	**103.370**	**0.000**[b]	51.018	**0.000**
	TEMPERATURE * WATER	1.723	0.189	1.339	**0.000**
	TEMPERATURE * GRANITE B.I.	1.363	0.265	9.507	0.273
	WATER * GRANITE B.I.	0.869	0.524	0.723	0.633
	TEMPERATURE * WATER * GRANITE B.I.	0.847	0.540	1.014	0.427
$\Delta F_{0(470\ nm)}/F_{0\ (645)}$	TEMPERATURE	-	-	4.585	0.037
	WATER	-	-	0.287	0.752
	GRANITE B.I.	-	-	22.126	**0.000**
	TEMPERATURE * WATER	-	-	3.507	0.038
	TEMPERATURE * GRANITE B.I.	-	-	5.569	**0.002**
	WATER * GRANITE B.I.	-	-	1.194	0.325
	TEMPERATURE * WATER * GRANITE B.I.	-	-	5.989	**0.000**

[a] p-value is significant when is < 0.01.
[b] For both, F_0 and Y_{MAX}, the Duncan post-hoc test ($\rho > 0.01$) revealed significant differences just after inoculation between all the types of granites following the next order: NS < BLUE < GOLDEN < SAM.

the initial steps of colonization. Although the effect of surface roughness was reduced in this study by placing the samples horizontally, other properties such as open porosity, bulk density and capillary water (Prieto and Silva, 2005) appear to be key factors in determining establishment of the microorganisms on the surface. This is consistent with the findings reported by D'Orazio et al. (2014), who investigated the effect of chemical composition, porosity and water retention on different types of bricks and observed that surface roughness and total porosity are the most important physical properties determining the adhesion of algal cells to the substrate. In the present study, the effect of granite bioreceptivity on biofilm attachment was observed after inoculation. Moreover, it was also found a significative interaction effect of bioreceptivity and climatic factors in the biofilm development at the end of the experiment. Thus, in those cases where the substrate favors the anchorage of the organisms, favorable environmental conditions such as high-water availability (H7) or increase in temperature led to a higher growth of the biofilm (Table 2). This is a very important finding that must be taken into account in laboratory experiments in which different types of stone are tested, in order to avoid attributing the observed effects to environmental parameters rather than to the substrate bioreceptivity. However, Manso et al. (2015), who studied microbial colonization on different cement mixtures, found that the main differences in biofouling were caused by changes in humidity, while roughness and porosity did not have substantial effects. Thus, these results lead to study the role of bioreceptivity on the initial stages of biocolonization independently of climatic conditions, as well as to the study of main parameters (roughness, porosity, capillarity, etc.) determining the initial adhesion of organisms and therefore the final colonization.

The environmental effects, in terms of growth and physiological state, began to be observed 14 days after the inoculation. At the end of the experiment, biofilm growth increased under maximum water availability, and the growth slowed down when the water availability was interrupted by periods of drought. Drought periods of 4–6 days reduced statistically the efficiency and growth of phototrophic biofilms (Table 2). The major effect of water in controlling growth of the biofilms (more important than temperature) is consistent with the findings of other studies, identifying water availability as one of the most important factors in the development of phototrophic organisms (Häubner et al., 2006; Gladis and Schumann, 2011; Fuentes and Prieto, 2021). Moreover, biofilm growth was not correlated with total precipitation, but rather with the frequency of rainy days, i.e. longer periods of exposure to high humidity favour growth of microorganisms (Viles and Cutler, 2012; Gladis-Schmacka et al., 2014). Thus, in the context of different climate change scenarios in which an increase in the duration of droughts is predicted (Hewitson et al., 2014), a reduction in the development of subaerial biofilms may be expected, especially during the summer period.

The effect of water availability on growth and physiological state of biofilms was also reflected by colour variation data especially for biofilms developed on SAM and GOLDEN granites, where colour differences greater than 2 CIELAB units, for both Δa* and Δb* (more yellow and green), indicate a biofilm development perceptible to the human eye. This is a very important subject to be taken into account when biocolonisation of cultural heritage is involved as perception of biofilm development do means aesthetical biodeterioration. To this respect, beyond the chemical and physical changes that organisms can cause in the substrate, colour modifications such as those reported in this work have been pointed as cause of aesthetic deterioration of monuments (Prieto et al., 2006; Smith et al., 2011; Sanmartín et al., 2012).

The growth of organisms also depends on temperature and nutrient availability. In general, increased temperature, up to an optimal level, tends to lead to acceleration of metabolic (Calvin cycle) reactions (Falkowski, 1980). The optimal temperature for growth of algae and cyanobacteria is species specific (Baumert and Petzodt, 2008; Franz et al., 2012). We observed that temperature statistically affects photosynthetic efficiency (Y_{MAX} parameter), which was higher at 24 °C than at 18 °C. Although in one previous study, photosynthetic efficiency did not vary, differences in growth were observed and despite the wide range of temperature ranges at which growth is viable, the best results were obtained between 21 and 24 °C (Häubner et al., 2006). In the present study, although no direct effect of temperature was observed in terms of biofilm growth, both the direct effect of temperature and the interaction with water clearly affected the photosynthetic efficiency (Table 2). At 18 °C, the photosynthetic efficiency of the biofilm was significantly higher at maximum water availability than with restricted water availability. However, at 24 °C, although the photosynthetic efficiency of the biofilms tended to be higher with maximum water availability, the difference was not statistically significant. The photosynthetic efficiency of the biofilms with restricted water availability (H3 and H1) was higher at 24 °C than at 18 °C, while at maximum water availability (H7) there were no differences in this parameter at the different temperatures. This different behavior of the biofilm's development under changing temperature conditions is also evident by colour data as colour differences among the 3 levels of water availability were greater in biofilms kept at 18° than on those at 24°. These results, together with those obtained from chlorophyll fluorescence could indicate a buffering effect of water (Garrett and Grisham, 2013), which would explain why the photosynthetic efficiency of the microorganisms only increased in response to an increase in temperature when the availability of water was restricted.

Taking into account the specific responses of different types of

microorganisms to the environmental conditions and the fact that biofilms are complex communities of organisms, competition for resources will lead to some species being selected over others. In response to changes in environmental conditions, cyanobacteria and algae will compete with each other, largely in response to an increase in temperature where the former is favored (Crispim et al., 2003; Gaylarde and Gaylarde, 2005). In the present study, the F_0470nm/F_0645 nm ratio provided information about the proportion of algae and cyanobacteria in the biofilms. In the biofilms in which the physiological state was optimal (SAM and GOLDEN samples at H7) the proportion of cyanobacteria increased when the samples were maintained at 24 °C (negative values of $\Delta F_0470nm/F_0645$ nm ratio), but not at 18 °C. That is, under ideal growth conditions, i.e., a highly bioreceptive substrate and high-water availability, temperature is the factor controlling diversity in biofilms as a rise in temperature favors the development of cyanobacteria versus algae (Table 2). Thus, the present findings indicate that in the context of the expected global warming, cyanobacteria will be predominant in areas where an increase in the frequency of rain is predicted.

5. Conclusions

The effect of the combination of primary bioreceptivity together with climatic parameters was for the first time evaluated. The results show an initial effect of bioreceptivity which is modulated by temperature and water availability along the experiment. Once organisms are stablished, a reduction in water availability reduces biofilm development, while temperature acts as a metabolic activator, improving the efficiency of the organisms even under unfavorable water conditions. In turn, when conditions are optimal, i.e., highly bioreceptive substrate and total water availability, an increase in temperature favors the development of cyanobacteria versus algae.

Declaration of competing interest

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.

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References

Ariño, X., Llop, E., Gómez-Bolea, A., Saiz-Jimenez, C., 2010. Effects of climatic change on microorganisms colonizing cultural heritage stone materials. Climate change and cultural heritage 193–198.

Baumert, H.Z., Petzodt, T., 2008. The role of temperature, cellular quota and nutrient concentrations for photosynthesis, growth and light-dark acclimation in phytoplankton. Limnologica. https://doi.org/10.1016/j.limno.2008.06.002.

Beardall, J., Raven, J.A., 2004. The potential effects of global climate change on microalgal photosynthesis, growth and ecology. Phycologia 43, 26–40. https://doi.org/10.2216/i0031-8884-43-1-26.1.

Brimblecombe, P., Grossi, C.M., Harris, I., 2011. Climate change critical to cultural heritage. In: Environmental Earth Sciences. https://doi.org/10.1007/978-3-540-95991-5-20.

CIE, 1978. Commission internationale d'Eclairage. Recommendations on uniform colour spaces, colour difference equations, psychometrice colour terms. J. Oral Rehabil.

Crispim, C.A., Gaylarde, P.M., Gaylarde, C.C., 2003. Algal and cyanobacterial biofilms on calcareous historic buildings. Curr. Microbiol. https://doi.org/10.1007/s00284-002-3815-5.

Cutler, N.A., Oliver, A.E., Viles, H.A., Ahmad, S., Whiteley, A.S., 2013a. The characterisation of eukaryotic microbial communities onsandstone buildings in Belfast, UK, using TRFLP and 454 pyrosequencing. Int. Biodeterior. Biodegrad. 82, 124–133. https://doi.org/10.1016/j.ibiod.2013.03.010.

Cutler, N.A., Viles, H.A., Ahmad, S., McCabe, S., Smith, B.J., 2013b. Algal "greening" and the conservation of stone heritage structures. Sci. Total Environ. 442, 152–164. https://doi.org/10.1016/j.scitotenv.2012.10.050.

D'Orazio, M., Cursio, G., Graziani, L., Aquilanti, L., Osimani, A., Clementi, F., Yéprémian, C., Lariccia, V., Amoroso, S., 2014. Effects of water absorption and surface roughness on the bioreceptivity of ETICS compared to clay bricks. Build. Environ. https://doi.org/10.1016/j.buildenv.2014.03.018.

Eggert, A., Häubner, N., Klausch, S., Karsten, U., Schumann, R., 2006. Quantification of algal biofilms colonising building materials: chlorophyll α measured by PAM-fluorometry as a biomass parameter. Biofouling. https://doi.org/10.1080/08927010600579090.

Falkowski, P.G., 1980. Light-shade adaptation in marine phytoplankton. In: Primary Productivity in the Sea. https://doi.org/10.1007/978-1-4684-3890-1_6.

Fatorić, S., Seekamp, E., 2017. Are cultural heritage and resources threatened by climate change? A systematic literature review. Climatic Change. https://doi.org/10.1007/s10584-017-1929-9.

Favero-Longo, S.E., Viles, H.A., 2020. A review of the nature, role and control of lithobionts on stone cultural heritage: weighing-up and managing biodeterioration and bioprotection. World J. Microbiol. Biotechnol. https://doi.org/10.1007/s11274-020-02878-3.

Franz, A., Lehr, F., Posten, C., Schaub, G., 2012. Modeling microalgae cultivation productivities in different geographic locations - estimation method for idealized photobioreactors. Biotechnol. J. https://doi.org/10.1002/biot.201000379.

Fuentes, E., Prieto, B., 2021. Recovery capacity of subaerial biofilms grown on granite buildings subjected to simulated drought in a climate change context. Microb. Ecol. 1–9 https://doi.org/10.1007/s00248-021-01692-0.

Garrett, R.H., Grisham, C.M., 2013. Biochemistry, third ed. Thomson books, Cole, Belmont, USA.

Gaylarde C, C., 2020. Influence of Environment on Microbial Colonization of Historic Stone Buildings with Emphasis on Cyanobacteria. https://doi.org/10.3390/heritage3040081. Heritage.

Gaylarde, C.C., Gaylarde, P.M., 2005. A comparative study of the major microbial biomass of biofilms on exteriors of buildings in Europe and Latin America. Int. Biodeterior. Biodegrad. https://doi.org/10.1016/j.ibiod.2004.10.001.

Genova, C., Fuentes, E., Sanmartín, P., Favero, G., Prieto, B., 2020. Phytochemical compounds as cleaning agents on granite colonized by phototrophic subaerial biofilms. Coatings 10, 295. https://doi.org/10.3390/coatings10030295.

Genty, B., Briantais, J.M., Baker, N.R., 1989. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochim. Biophys. Acta Gen. Subj. 990, 87–92. https://doi.org/10.1016/S0304-4165(89)80016-9.

Gladis, F., Schumann, R., 2011. Influence of material properties and photocatalysis on phototrophic growth in multi-year roof weathering. Int. Biodeterior. Biodegrad. 65, 36–44. https://doi.org/10.1016/j.ibiod.2010.05.014.

Gladis-Schmacka, F., Glatzel, S., Karsten, U., Böttcher, H., Schumann, R., 2014. Influence of local climate and climate change on aeroterrestrial phototrophic biofilms. Biofouling 30, 401–414. https://doi.org/10.1080/08927014.2013.878334.

Gorbushina, A.A., 2007. Life on the rocks. Environ. Microbiol. 9, 1613–1631. https://doi.org/10.1111/j.1462-2920.2007.01301.x.

Guillitte, O., 1995. Bioreceptivity: a new concept for building ecology studies. Sci. Total Environ. https://doi.org/10.1016/0048-9697(95)04582-L.

Häubner, N., Schumann, R., Karsten, U., 2006. Aeroterrestrial microalgae growing in biofilms on facades - response to temperature and water stress. Microb. Ecol. 51, 285–293. https://doi.org/10.1007/s00248-006-9016-1.

Haugen, A., Bertolin, C., Leijonhufvud, G., Olstad, T., Broström, T., 2018. A methodology for long-term monitoring of climate change impacts on historic buildings. Geosciences 8, 370. https://doi.org/10.3390/geosciences8100370.

Hewitson, B., Janetos, A.C., Carter, T.R., Giorgi, F., Jones, R.G., Kwon, W.-T., Mearns, L. O., Schipper, E.L.F., van Aalst, M., 2014. Regional context. In: Barros, V.R., Field, C. B., Dokken, D.J., Mastrandrea, M.D., Mach, K.J., Bilir, T.E., Chatterjee, M., Ebi, K.L., Estrada, Y.O., Genova, R.C., Girma, B., Kissel, E.S., Levy, A.N., MacCracken, S., Mastrandrea, P.R., White, L.L. (Eds.), Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part B: Regional Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, pp. 1133–1197.

Hodkinson, T.R., Jones, M.B., Waldren, S., Parnell, J.A.N., 2011. Climate Change, Ecology and Systematics, Climate Change, Ecology and Systematics. https://doi.org/10.1017/CBO9780511974540.

Joh, G., Lee, J., 2012. Cyanobacterial biofilms on sedimentation basins in a water treatment plant in South Korea. J. Appl. Phycol. https://doi.org/10.1007/s10811-011-9678-z.

Karsten, U., Holzinger, A., 2012. Light, temperature, and desiccation effects on photosynthetic activity, and drought-induced ultrastructural changes in the green alga Klebsormidium dissectum (streptophyta) from a high alpine soil crust. Microb. Ecol. 63, 51–63. https://doi.org/10.1007/s00248-011-9924-6.

Logan, B.A., 2005. Chlorophyll a fluorescence: a signature of photosynthesis. J. Torrey Bot. Soc. https://doi.org/10.3159/1095-5674(2005)132[650a:br]2.0.co;2.

Manso, S., Calvo-Torras, M.Á., De Belie, N., Segura, I., Aguado, A., 2015. Evaluation of natural colonisation of cementitious materials: effect of bioreceptivity and environmental conditions. Sci. Total Environ. https://doi.org/10.1016/j.scitotenv.2015.01.086.

Miller, A.Z., Sanmartín, P., Pereira-Pardo, L., Dionísio, A., Saiz-Jimenez, C., Macedo, M. F., Prieto, B., 2012. Bioreceptivity of building stones: a review. Sci. Total Environ. https://doi.org/10.1016/j.scitotenv.2012.03.026.

Mokrzycki, W.S., Tatol, M., 2011. Colour difference ΔE - a survey. Mach. Graph. Vis. 20 (4), 383–411.

Prieto, B., Silva, B., 2005. Estimation of the potential bioreceptivity of granitic rocks from their intrinsic properties. Int. Biodeterior. Biodegrad. https://doi.org/10.1016/j.ibiod.2005.08.001.

Prieto, B., Rivas, T., Silva, B., 2002. Rapid quantification of phototrophic microorganisms and their physiological state through their colour. Biofouling. https://doi.org/10.1080/08927010290014908.

Prieto, B., Silva, B., Aira, N., Álvarez, L., 2006. Toward a definition of a bioreceptivity index for granitic rocks: perception of the change in appearance of the rock. Int. Biodeterior. Biodegrad. 58, 150–154. https://doi.org/10.1016/j.ibiod.2006.06.015.

Prieto, B., Vázquez-Nion, D., Fuentes, E., Durán-Román, A.G., 2020. Response of subaerial biofilms growing on stone-built cultural heritage to changing water regime and CO2 conditions. Int. Biodeterior. Biodegrad. https://doi.org/10.1016/j.ibiod.2019.104882.

Sabbioni, C., Cassar, M., Brimblecombe, P., Lefevre, R.A., 2009. Vulnerability of cultural heritage to climate change. Pollut. Atmos. 157–169.

Sanmartín, P., Vázquez-Nion, D., Silva, B., Prieto, B., 2012. Spectrophotometric color measurement for early detection and monitoring of greening on granite buildings. Biofouling 28, 329–338. https://doi.org/10.1080/08927014.2012.673220.

Sanmartín, P., Miller, A.Z., Prieto, B., Viles, H.A., 2021. Revisiting and reanalysing the concept of bioreceptivity 25 years on. Sci. Total Environ. https://doi.org/10.1016/j.scitotenv.2021.145314.

Sesana, E., Gagnon, A.S., Bonazza, A., Hughes, J.J., 2020. An integrated approach for assessing the vulnerability of World Heritage Sites to climate change impacts. J. Cult. Herit. https://doi.org/10.1016/j.culher.2019.06.013.

Smith, B.J., McCabe, S., McAllister, D., Adamson, C., Viles, H.A., Curran, J.M., 2011. A commentary on climate change, stone decay dynamics and the 'greening' of natural stone buildings: new perspectives on 'deep wetting'. Environmental Earth Sciences 63, 1691–1700. https://doi.org/10.1007/s12665-010-0766-1.

UNESCO World Heritage Centre, 2007. Case Studies on Climate Change and World Heritage.

Vázquez-Nion, D., Rodríguez-Castro, J., López-Rodríguez, M.C., Fernández-Silva, I., Prieto, B., 2016. Subaerial biofilms on granitic historic buildings: microbial diversity and development of phototrophic multi-species cultures. Biofouling 32, 657–669. https://doi.org/10.1080/08927014.2016.1183121.

Vázquez-Nion, D., Silva, B., Prieto, B., 2018. Bioreceptivity index for granitic rocks used as construction material. Sci. Total Environ. https://doi.org/10.1016/j.scitotenv.2018.03.171.

Vázquez-Nion, Daniel, Silva, B., Prieto, B., 2018. Influence of the properties of granitic rocks on their bioreceptivity to subaerial phototrophic biofilms. Sci. Total Environ. https://doi.org/10.1016/j.scitotenv.2017.08.015.

Viles, H.A., Cutler, N.A., 2012. Global environmental change and the biology of heritage structures. Global Change Biol. 18, 2406–2418. https://doi.org/10.1111/j.1365-2486.2012.02713.x.

Witt, V., Wild, C., Uthicke, S., 2012. Interactive climate change and runoff effects alter O2 fluxes and bacterial community composition of coastal biofilms from the Great Barrier Reef. Aquat. Microb. Ecol. 66, 117–131. https://doi.org/10.3354/ame01562.