

Original article

Effects of combined increase in temperature and CO₂ concentration on the weathering activity of phototrophic organisms inhabiting granitic rocks and its implications in terms of cultural heritage conservation



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ABSTRACT

The increase in CO₂ concentration and temperature observed in the current context of climate change may cause changes in the behavior of phototrophic organisms colonising stone cultural heritage, leading to undesirable changes in terms of biodeterioration. In this study, we examined the combined effect of both parameters (18°C or 24°C and ambient or high CO₂ concentration) on the physiological state of organisms and on granite weathering through experiments involving a green alga (*Bracteacoccus minor*) and a cyanobacterium (*Synechocystis* sp. PCC 6803) grown in medium containing granite. The results revealed an increase in the green algae population relative to the cyanobacteria at lower temperature and a reduction in biomass and efficiency during the early stages of culture development at the higher temperature. In addition, although rock weathering was favoured by modification of the solution equilibrium through cation uptake by the organisms no significant differences in response to environmental conditions were detected.

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1. Introduction

Greenhouse gas (GHG) emissions, particularly carbon dioxide (CO₂), have been increasing since the Industrial Revolution and are the main cause of climate change and global warming. Atmospheric CO₂ concentration has reached an average level of 419 ppm and up to 500 ppm in urban areas, which is linearly related to global warming, with a 0.6 to 4°C increase in global average temperature expected in the coming decades [1]. Although this information is widely known, many of the final implications have yet to be understood and studied in order to try to mitigate the effects.

Climate change has a direct impact on cultural heritage monuments and buildings [2], making their conservation a real challenge due to the difficulty of providing protection from the elements, which interact with the substrate and also with surface-colonising organisms. Thus, changes in climatic conditions are expected to directly affect the surfaces of rocky substrates through physico-chemical processes but also the way in which organisms colonise and degrade surfaces. In this respect, phototrophic organisms must

be considered as their metabolism is highly dependent on environmental conditions [3–5], as many aspects of life are mediated by temperature, water availability, light and/or the presence of CO₂.

Given the expected increases in CO₂ concentration and temperature, it is important to understand the combined effect of increasing both parameters on the biological deterioration processes occurring on the rock. The available literature mainly deals with how these changes affect organisms living on the substrate, but little is known about how the changes in the organisms translate into deterioration of the stone. Increased temperature has been related to a stimulatory effect on the growth and metabolism of the organisms [6–8]. Thus, while faster biofilm formation has been related to higher temperatures, the increase in biomass has been related to nutrient availability [9–12]. That is, if nutrients are limiting, an increase in water temperature may not increase the algal growth rate [13], making it difficult to separate the effect of temperature from the effects of other parameters. Moreover, optimal temperatures for photosynthesis vary among phototrophic species [14,15], and increasing temperatures could therefore lead to taxonomic change beyond changes in biomass [16,17]. In addition, the interactions between different groups of community-dwelling organisms may modulate the response of each group to warming and thus determine the outcome of the entire biofilm community [18,19]. That is,

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community changes may be generated that cannot be predicted by studying individual species. Considering the effects of temperature on patterns of colonisation on rocky cultural heritage, Cutler et al. [20] observed that for four sandstone heritage structures located in Belfast (Northern Ireland, UK), greening was more pronounced in colder areas than in warmer areas, with the pattern mainly being related to the evaporation of humidity. Ramirez et al. [21] corroborated that biofilm biodiversity on stone buildings was higher in colder areas.

In relation to the effect of changing CO₂ conditions, previous studies have demonstrated functional changes in biotic communities associated with increased atmospheric CO₂ concentrations such as increased productivity or changes in the quality of photosynthetic active tissue, which in turn affect community dynamics. Thus, Korner [22] and Schimel [23] hypothesised that exposure to higher environmental CO₂ levels may have a “fertilising effect” on photosynthesising organisms and thus enhance photosynthetic performance. Subsequent research on higher plants [24,25], *Chlorella* species [26] and phytoplankton communities [27] supported this hypothesis. However, this is not a generalised trend, as other studies involving marine microalgal cultures of *Nannochloropsis gaditana* and *Nannochloropsis maculate* [28], cyanobacteria *Anabaena* sp. PCC 7120 [29] and *Gloeotrichia natans* [30] and various lichen species [31] have shown that there are no differences in the growth of species at high and normal CO₂ concentrations. Few studies have quantified the effect of increased atmospheric CO₂ concentrations on biofilm development, and some authors consider that the communities colonising the heritage are more likely to be limited by humidity, water availability and/or nutrients than by CO₂ [5,32]. However, an increase in the productivity of microorganisms (in particular cyanobacteria and green algae) colonising cultural heritage monuments has been reported to be expected as atmospheric CO₂ increases [33].

2. Research aim

As far as we know, most of the existing knowledge on the response of microalgae and cyanobacteria to climate change comes from studies on phytoplankton dynamics. However, there are no studies related to the deterioration of stone heritage by phototrophic organisms exposed to different temperatures and CO₂ concentrations. Thus, it is expected that changes in both parameters can lead to changes in metabolic and physiological state affecting the resilience of both algae and cyanobacteria and its weathering activity. Therefore, to test this hypothesis, a mixed culture of the alga *Bracteacoccus minor* and the cyanobacterium *Synechocystis* sp. PCC6803 in contact with fragmented granite rock and exposed to an increase in temperature and CO₂ concentration was analysed in terms of organism development, culture composition, pigment production, physiological state and biodeterioration rate.

3. Materials and methods

In order to study the effect of the increase in CO₂ and temperature on the physiological and deteriogenic activity of the organisms that commonly inhabit granite monuments, an experiment was designed in which samples of fragmented granite rock suspended in a liquid medium with and without organisms (a green alga and a cyanobacterium) were exposed to combinations of two different temperatures (18 and 24°C) and carbon dioxide (CO₂) concentrations (non-polluted city CO₂ concentration, 416.87 ppm or high CO₂ concentration) for 90 days. In addition, since research on climate change requires long periods of time to observe the effects, the experiment was carried out in a liquid medium, which accelerates the deterioration processes, and the temperature increase chosen was 6°C, tripling what is expected for the climate change

scenario under consideration. The effects were monitored by cell counts, chlorophyll fluorescence, colour variation and quantification of chlorophyll degradation, dissolved oxygen, and solubilized elements.

3.1. Culture and substrate preparation

The culture used was a 1:1 mixture (biomass) of two monospecific cultures, one of *Synechocystis* sp. PCC6803 (Cyanobacteria) and one of *Bracteacoccus minor* (Chlorophyta): because of the smaller cell volume of cyanobacteria, this is equivalent to a culture composed of 92% cyanobacteria cells and 8% microalgae cells. The culture was maintained in BG11 liquid culture medium [34] with aeration. These species were selected for their ease of cultivation, the possibility to count cells (non-filamentous) and also for their presence in the granitic material [35,36].

A commercial granite, Silvestre (two mica adamellitic granite with equigranular-panalotriomorphic texture and medium grain), was selected for the study. It is mainly composed of quartz (34%) albite (21%), microcline (15%), muscovite (18%), biotite (11%) and kaolinite (1%). With the aim of increasing the surface of the rock exposed to the environment and microorganisms, the granite material was crushed to produce fragments of size between 0 and 5 (three fractions: 2 to 5 mm, 0.5 to 2 mm and <0.5 mm in diameter).

3.2. Experimental set-up

A total of 24 test tubes were each filled with 18 g of fragmented granite (6 g of each size fraction were obtained by physical sieving; 5–2 mm, 2–0.5 mm and <0.5 mm) and sterilised at 121°C for 15 min. After sterilizing, 5 mL of BG11 culture medium and 50 mL of distilled water were added to half of the tubes (non-inoculated), and 5 mL of mixed culture (*Synechocystis* sp. PCC6803 and *Bracteacoccus minor*, including BG11 medium) and 50 mL of distilled water were added to the other half (inoculated). Aeration was supplied via a circuit from outside the laboratory building, by means of an aeration pump. Carbon dioxide was provided directly in the tubes by connecting them to a gas bottle. The tubes were held in a climatic chamber for 90 days with 1900 lux illumination and a 12:12 h light/dark photoperiod.

Both temperatures (18 or 24°C) and CO₂ concentrations (environmental CO₂ concentration -416.87 ppm- or high CO₂ concentration) were applied to inoculated samples and also to non-inoculated samples, which served as controls. Thus, a total of 24 sample tubes were analysed, with 3 replicates for each condition, resulting in 3 inoculated and 3 non-inoculated replicates at 18°C and environmental CO₂ concentration; 3 inoculated and 3 non-inoculated replicates at 24°C and environmental CO₂ concentration; 3 inoculated and 3 non-inoculated replicates at 18°C and high CO₂ concentration; 3 inoculated and 3 non-inoculated replicates at 24°C and high CO₂ concentration.

For CO₂ supply, half of the tubes received a higher CO₂ concentration (environmental CO₂ concentration plus 1.37 L/day of extra CO₂), by connecting the aeration system to a CO₂ gas cylinder and supplying a daily pulse of gas once a day. The other half of the tubes were only connected to the outside of the building for exposure to environmental CO₂ levels.

3.3. Monitoring biofilm formation and deteriogenic activity

3.3.1. Quantification of *Synechocystis* sp. PCC 6803 and *Bracteacoccus minor* cell numbers

The numbers of green algae and cyanobacteria cells were quantified at the beginning, middle (day 45) and end (day 90) of the ex-

periment. Cell numbers were counted using a 0.1 mm deep counting chamber or haematocytometer with a Neubauer step scale [37].

3.3.2. Chlorophyll fluorescence

Chlorophyll fluorescence analysis indirectly assesses the photosynthetic efficiency of organisms by measuring their fluorescence emission. The signal was measured in the samples and determined at 470, 645 and 665 nm by pulse-amplitude modulated (PAM) fluorometry, in a Phyto-PAM system (Heinz Walz GmbH, Effeltrich, Germany) equipped with a Phyto-EDF fibre optics emitter-detector unit. A total of nine readings were taken by immersing the measurement sensor in each sample tube. Samples were kept in the dark for 20 min before recording data. Minimal fluorescence in the dark-adapted state (F_0), maximal fluorescence in the dark-adapted state (F_M) and maximum quantum efficiency of PSII photochemistry (Y_{MAX} ; $F_M - F_0 / F_M$) parameters were obtained [38].

In addition, in order to study the variation in the relative microbial abundance, the $F_0470\text{nm}/F_0645\text{nm}$ ratio was calculated as the signal at 470 nm, related to chlorophyll *b* (algae), and the signal at 645 nm, related to allophycocyanin (cyanobacteria).

3.3.3. Colour variation

Colour quantification has been shown to be a reliable, non-destructive method for quantifying the development of phototrophic organisms on solid substrates [39]. Aliquots (1.5 mL) of the culture were taken from each culture each 15 days and filtered under vacuum through 0.45 μm nitrocellulose filters leaving the organisms deposited on the filter. Five colour measurements were made on each filter using a portable spectrophotometer Konica Minolta CM-700D (Konica Minolta, Osaka, Japan) with CM-S100 w software (Spectra Magic TM NX) and under the following conditions: D65 illuminant, 2nd observer and SAV type objective area with a diameter of 3 mm.

The data obtained were represented in CIELAB color space [40], where each colour is defined by three Cartesian or linear coordinates $L^* a^* b^*$, where L^* is a measure of lightness, varying from 100 absolute white to 0 absolute black, a^* is a measure of chromatic variations on the red-green axis (positive red, negative green) and b^* is a measure of chromatic variations on the yellow-blue axis (positive yellow, negative blue).

3.3.4. Quantification of phaeophytinization quotient

The phaeophytinization quotient (PQ) reflects the degradation of chlorophyll to phaeopigments as a consequence of environmental stress. It was calculated by extracting phytopigments every 15 days with dimethyl sulfoxide (DMSO). Aliquots (1.5 mL) of the culture were removed from each tube and filtered under vacuum with 0.45 μm nitrocellulose filters, and the filters were then re-suspended in 1.5 mL DMSO for 1 hour at 65°C with agitation [41,42]. At the end of the DMSO digestion, the samples were centrifuged for 10 min at 7000 g (Centric 150 Tehtnica), and the supernatant obtained was measured at different wavelengths using a UV-visible spectrophotometer (Varian Cary 100). The phaeophytinization quotient (PQ) was calculated by the ratio of absorbances of the extracts at 435 and 415 nm [43].

3.3.5. Quantification of dissolved oxygen

Dissolved oxygen is an important indicator and limiting factor in photosynthetic efficiency and thus CO_2 fixation [44]. In order to determine the concentration of dissolved oxygen in the samples exposed to the different temperature and CO_2 combinations, 1.5 mL aliquots of the culture were taken from each sample tube and measurements were carried out with an oximeter EI2 microsensor 10 μm (Unisense, Aarhus, Denmark) every 15 days from the beginning of the experiment.

3.3.6. Quantification of dissolved elements by flame atomic absorption-emission spectroscopy

In order to determine the effects of the conditions tested on the ability of the organisms to solubilize and/or take up elements from the substrate, in this case granite, the dissolved Na^+ , K^+ and Ca^{2+} and Mg^{2+} in each tested condition were quantified at the end of the experiment by atomic emission spectroscopy or atomic absorption spectroscopy. Aliquots (20 mL), previously vacuum-filtered through 0.45 μm nitrocellulose filters, were removed from each test tube and analysed by a flame atomic absorption-emission spectrometer SpectrAA 220 FS (Mulgrave, Victoria, Australia) Varian model with fast sequential module (FS-FAAS) and equipped with a conventional pneumatic nebuliser and spray chamber system. The flame composition was acetylene (2.0 $\text{L}\cdot\text{min}^{-1}$) and air (13.5 $\text{L}\cdot\text{min}^{-1}$). The suction flow rate of the nebuliser was maintained between 5.5 and 6.0 $\text{ml}\cdot\text{min}^{-1}$.

3.4. Statistical analysis

Data were subjected to a two-way ANOVA and a post hoc Tukey HSD test, considering the measured parameters as dependent variables and temperature and CO_2 concentration as fixed factors. Statistical analyses were carried out using SPSS Statistics v23.0 software (IBM, New York, U.S.).

4. Results

4.1. Quantification of cell number and variation in proportion of *Synechocystis* sp PCC 6803 and *Bracteacoccus minor* cells

Fig. 1 shows (a) the total cell number and (b) percentages of cyanobacterium and green algae in the inoculated samples on days 0, 45 and 90 of the experiment. All temperature and carbon dioxide (CO_2) conditions resulted in an increase in total cell content over time, and the increase was accentuated towards day 45 of the experiment, followed by a decrease towards day 90, although the number of cells was higher at the end of the experiment than at the beginning of the experiment.

However, the proportion of green algae and cyanobacterial cells in the samples varied widely throughout the experiment. In general, the proportion of green algae increased, and the proportion of cyanobacteria decreased over time. On day 45, differences were observed in cultures held at the different temperatures: samples held at 18°C showed an increase in the percentage of algae relative to cyanobacteria whereas there was no such increase in samples held at 24°C. On the final day of the experiment, the differences in cultures exposed to different temperatures were maintained, but the percentage of green algae increased in all cases. Differences were also observed between CO_2 exposure conditions, with samples exposed for 90 days to high levels of CO_2 showing a lower proportion of algae cells than samples exposed to environmental CO_2 levels.

4.2. Chlorophyll fluorescence

The variation in minimum fluorescence (F_0 ; culture growth indicator, more organisms, more amount of minimum fluorescence re-emitted [45]) over time for the different combinations of temperature and CO_2 concentration is presented in Fig. 2a and Table 1 (Supplementary material). An increase in the F_0 over time was observed for the cultures subjected to all temperature and CO_2 combinations. However, there was also a large difference between F_0 values of cultures subjected to different temperatures. On day 45, the interaction between the factors CO_2 concentration and temperature was significant, i.e. the effect of the temperature factor on the dependent variable F_0 depends on the levels adopted by the CO_2 concentration factor (Table 2, Supplementary material).

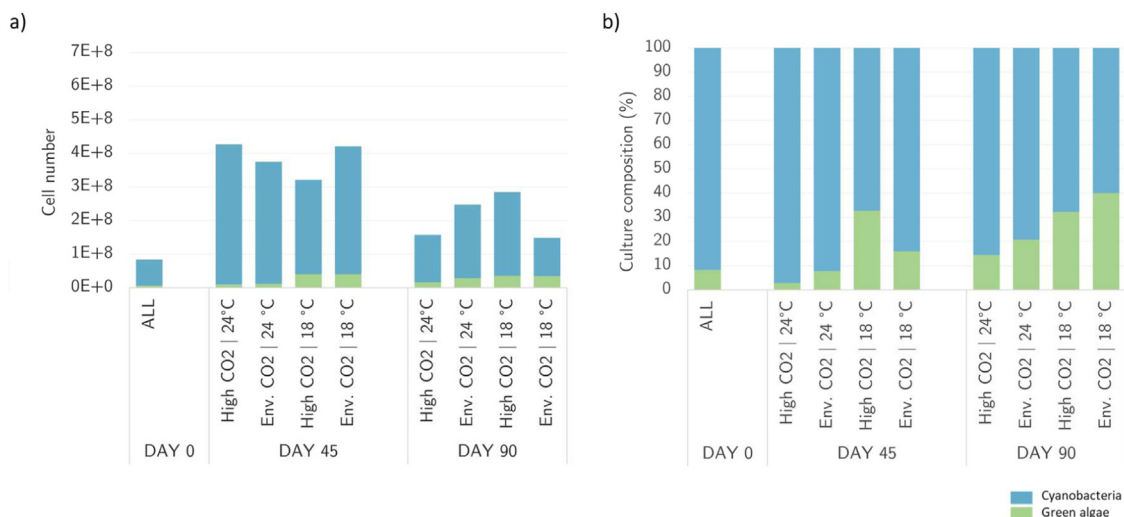


Fig. 1. a) Total cell number and b) percentages of cyanobacterium and green algae in the mixed culture at day 0, 45 and 90 of the experiment. Green algae: green; cyanobacteria: blue.

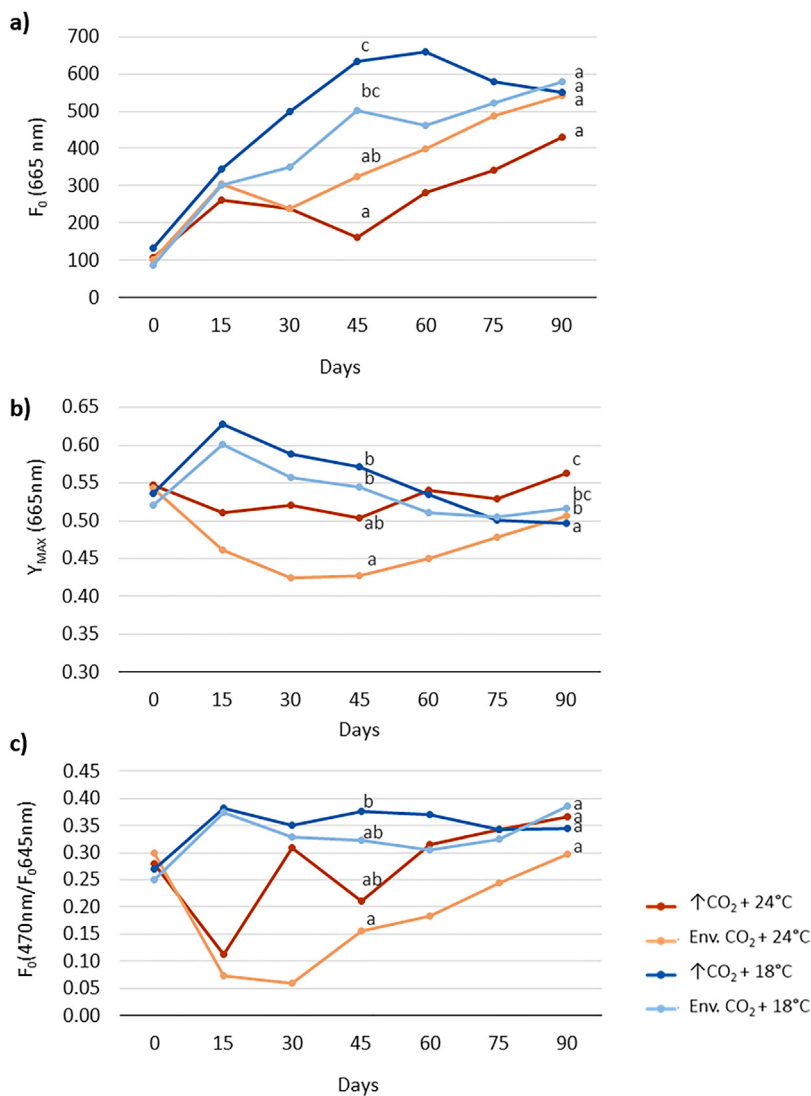


Fig. 2. Minimal fluorescence (F_0), maximum quantum efficiency of PSII photochemistry (Y_{MAX}) and F_0 470 nm / F_0 645 nm ratio of cultures subjected to different temperatures (18°C and 24°C; blue and orange, respectively) and CO₂ levels (environmental and high; light and dark, respectively) over the 90 days of the experiment. Different letters correspond to significant differences ($p < 0.05$) between the samples in relation to the different conditions.

In Samples held at 24°C, the minimum fluorescence values were lower in those exposed to higher CO₂ levels than those of the samples exposed to environmental CO₂ conditions. By contrast, at 18°C the cultures exposed to high levels of CO₂ exhibited higher F₀ values than those exposed to environmental levels. Under high CO₂ levels, differences in F₀ values between both assayed temperatures were significantly larger than under environmental CO₂ conditions. On the other hand, from day 45 onwards there was a change in the trends and F₀ values tended to become more similar. At the end of the experiment, day 90, the differences in the F₀ parameter for each combination of temperature and CO₂ concentration were not significant (Table 2, Supplementary material).

The maximum quantum yield (Y_{MAX}) indicates the efficiency of photosynthetic activity [46] (Fig. 2b, Table 1 in Supplementary material). In this experiment, differences were found in this parameter for the different temperatures and CO₂ levels. At day 45, significant differences were observed in Y_{MAX} between the two temperatures studied: photosynthetic efficiency of cultures maintained at 18°C was significantly higher than that of the samples maintained at 24°C. For both temperatures, the efficiency of the cultures exposed to high CO₂ levels was higher than that of the samples exposed to environmental CO₂ concentrations, however it was not statistically significant. At the end of the experiment, the interaction between the factors CO₂ concentration and temperature was significant, showing that those samples exposed to low CO₂ concentrations showed no difference in response to temperature, however, when the CO₂ concentration was high, those samples kept at 24°C had a significantly higher Y_{MAX} value than those kept at 18°C.

The F₀ 470 nm / F₀ 645 nm ratio (indicator of dominance of green algae (high values) or cyanobacteria (low values) [4]) is shown in Fig. 2c and Table 1 (Supplementary material). At day 45, cultures held at 18 °C showed a significantly higher ratio than those held at 24 °C (Table 2 in Supplementary material). From day 45 onwards, the ratio calculated for all cultures tended to converge, reaching higher values, in general, than those at the beginning of the experiment. At the end of the experiment, day 90, no significant differences were found for this variable in response to changes in CO₂ concentration and temperature (Table 2 in Supplementary material).

4.3. Quantification of colour change

Colour is an indicator of the level of development, senescence and physiological state of microbial cultures [47,48]. In general, throughout the experiment, all cultures became lighter, redder and yellower (Fig. 3; Table 1 in Supplementary material).

The L* parameter (Fig. 3a) varied significantly in the cultures with opposing trends depending on the temperature and the concentration of CO₂ applied: cultures held at 24°C and higher CO₂ concentration became lighter, while those held at 18°C and environmental CO₂ concentration, become darker. From day 45 onwards, the trends in the samples held at both temperatures changed and by day 90 the differences between the different treatments were reduced, maintaining significant differences depending on the applied temperature (Table 2 in Supplementary material). The parameter a* (Fig. 3b) showed a shift towards a redder colour in all cultures. Within this general pattern of redness, however, a significant effect of the interaction was observed between temperature and CO₂ concentration factors. In general terms, lower temperature produced greater redness, however, an increase in CO₂ concentration resulted in a reduction in redness at 18°C, but an increase at 24°C, regarding the values obtained under environmental CO₂ concentration. At the end of the experiment, samples held at 18°C were significantly redder than those held at 24°C (Table 2 in Supplementary material). The b* parameter showed a trend towards yellowing in all cultures. Once again, a change occurred on

day 45, with change in trend so that the increase in b* was reversed thereafter, with significant differences at day 45 between the values obtained at different temperatures (Table 2 in Supplementary material), with samples held at 18°C becoming yellower. A statistically significant colour variation associated with the effect of the interaction between the parameter temperature and CO₂ concentration was observed at the end of the experiment.

4.4. Quantification of phaeophytinization quotient

The phaeophytinization quotient (PQ) (Fig. 4; Table 1 in Supplementary material), which provides information on Chl *a* degradation (high values indicate low levels of chlorophyll *a* degradation), decreased in all samples over time, indicating an increase in chlorophyll *a* degradation. At day 45, significant differences were observed between the two temperatures studied: samples kept at 18°C showed higher PQ values than samples kept at 24°C, indicating greater degradation of the cultures held at 24°C. At the end of the experiment, although the differences between the different treatments were reduced, the statistical analysis showed differences resulting from the interaction of the two factors, temperature, and CO₂ concentration (Table 2 in Supplementary material).

4.5. Dissolved oxygen

The concentration of dissolved oxygen is an indicator of photosynthetic activity. Fig. 5 and Table 1 (Supplementary material) show the variation in the dissolved oxygen concentration relative to the beginning of the experiment (Δ dissolved O₂). The concentration of dissolved oxygen decreased over time in all samples regardless temperature and CO₂ exposure until day 75, increasing slightly towards the end of the experiment. However, in samples held at 18°C the amount of dissolved oxygen increased in the first days of the experiment. No significant differences were found in the concentration of dissolved oxygen in response to changes in temperature and CO₂ concentration (Table 2 in Supplementary material).

4.6. Dissolved element content

The dissolved Ca²⁺, Mg²⁺, Na⁺ and K⁺ contents are indicative of the weathering of the granite and at the same time, by comparison, of the incorporation of these elements in living organisms [49]. In Fig. 6, the bars represent the ratio between the final and initial concentrations of the Ca²⁺, Mg²⁺, Na⁺ and K⁺. For all tested conditions, the ratios were lower than 1 for the inoculated samples and higher than 1 for the non-inoculated samples. Ratios above 1 indicate higher concentrations of the dissolved element at the end than at the beginning of the experiment, while values below 1 indicate higher concentrations of the dissolved element at the beginning than at the end of the experiment. Thus, elements were released throughout the experiment but were used by organisms in the inoculated samples.

The concentration of elements was the same for the inoculated cultures subjected to the different conditions; however, there were some differences in the case of non-inoculated samples. For non-inoculated samples held at 24 °C there was an increase in the release of Mg²⁺ in the samples exposed to high CO₂ levels. For the other elements there were some variations, but on a smaller scale. However, the same comparison in samples held at 18 °C showed a different pattern, with an increase in the release of all of the elements, especially of Mg²⁺, under environmental CO₂ conditions. Thus, differences in the release of this element between temperatures were modulated by the CO₂ levels.

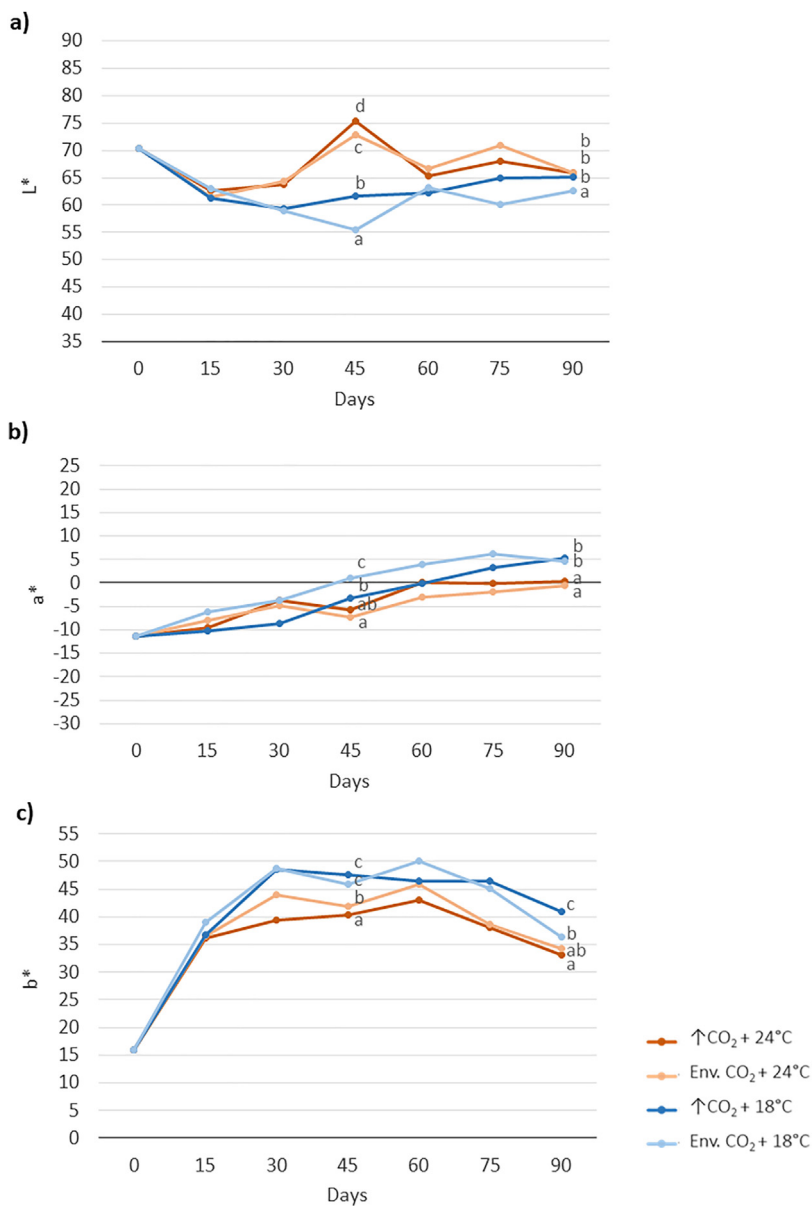


Fig. 3. L*, b* and a* parameters of cultures subjected to different temperatures (18°C and 24°C; blue and orange, respectively) and CO₂ levels (environmental and high; light and dark, respectively) over the 90 days of the experiment. Different letters correspond to significant differences (p < 0.05) between the samples in relation to the different conditions.

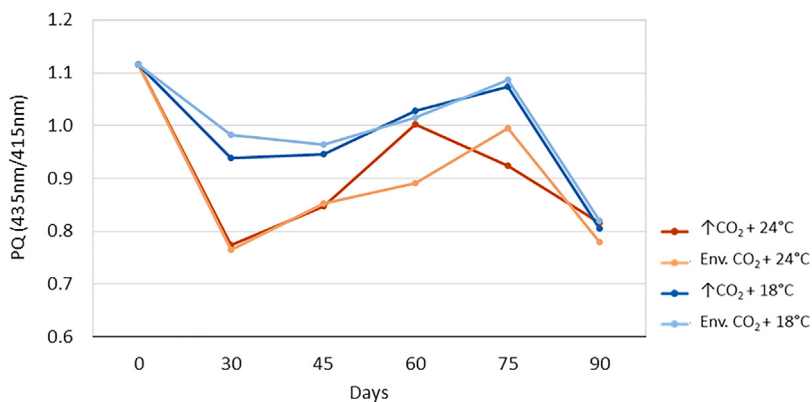


Fig. 4. PQ ratio of cultures subjected to different temperatures (18°C and 24°C; blue and orange, respectively) and CO₂ levels (environmental and high; light and dark, respectively) over the 90 days of the experiment. High values indicate low levels of chlorophyll a degradation.

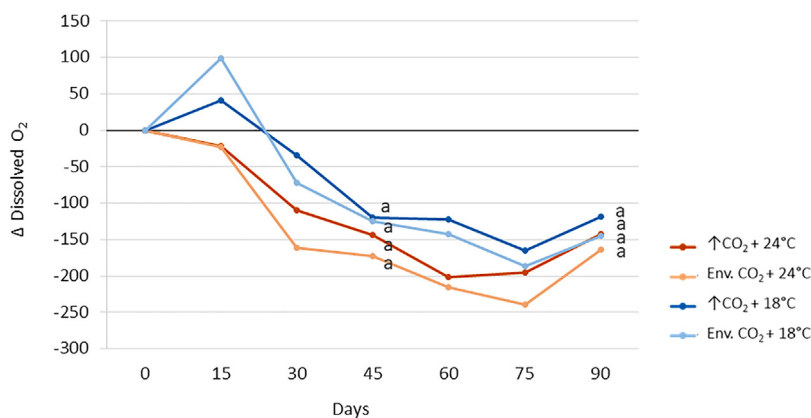


Fig. 5. Variation in the dissolved O₂ concentration of cultures subjected to different temperatures (18°C and 24°C; blue and orange, respectively) and CO₂ levels (environmental and high; light and dark, respectively) over the 90 days of the experiment. Different letters correspond to significant differences ($p < 0.05$) between the samples in relation to the different conditions.

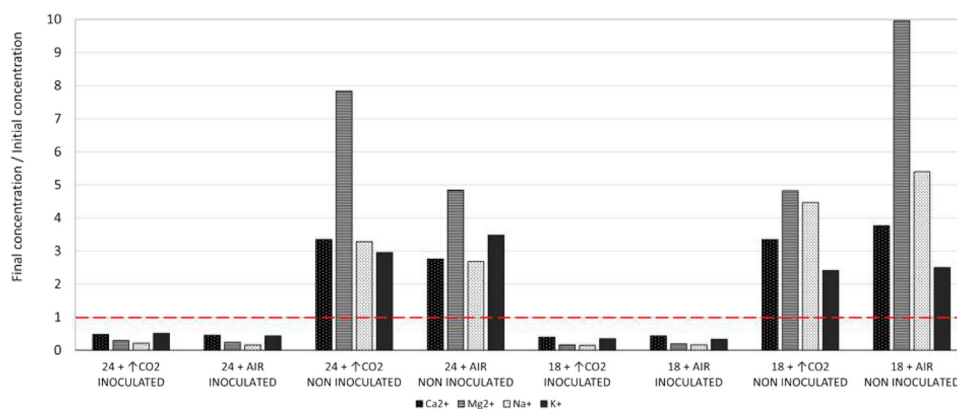


Fig. 6. Ratio between final and initial Ca²⁺, Mg²⁺, Na⁺ and K⁺ concentrations in cultures subjected to different temperatures (24°C and 18°C; left and right, respectively) and CO₂ levels (environmental -AIR- and high -CO₂-) at the end of the experiment. Since the initial concentration is the same, higher bars indicate higher final concentrations. Values below 1 (dotted line) indicate a higher initial concentration than the final concentrations.

5. Discussion

Results above show that variations in temperature and carbon dioxide (CO₂) concentration have an effect on the micro-organisms that commonly inhabit the cultural heritage, leading to a reduction in the proportion of green algae with increasing temperature, which caused a reduction in the total biomass of the culture throughout the experiment and a slight increase in its photosynthetic efficiency with increasing CO₂. Towards the end of the experiment, the differences tended to homogenise, so an increase in the duration of the experiment could provide information at successive stages. It was also found that the presence of organisms leads to a consumption or elimination of cations from the medium, i.e. it favours the deterioration of the granite as there is greater biological activity.

Analysis of the changes in cultures subjected to different temperatures (18 or 24°C) and CO₂ levels (environmental or elevated) showed that both parameters, both individually and together, affected the biofilm ecology, with an increase in the population of green algae relative to cyanobacteria at lower temperature (18°C) and high CO₂ concentration. However, it is important to differentiate which effects are caused by the environmental parameters applied and which effects are part of natural changes in the community. Thus, before considering the effect of environmental parameters, it is worth mentioning that cyanobacteria usually replicate faster than green algae, possibly due to their prokaryotic nature and smaller size [50,51]. In the mixed culture used in this

study, composed by a cyanobacterium, *Synechocystis* sp. PCC6803, and a green alga, *Bracteacoccus minor*, the former, which was initially more abundant, underwent a sudden initial growth burst (bloom), with cells reaching maximum numbers on day 45, followed by massive cell death. However, although the green alga did not undergo an initial bloom or massive death, the abundance increased gradually throughout the experiment. The total number of cells in cultures increased until day 45 but was greatly reduced at the end of the experiment, indicating death of the organisms. The reduction in total cell number, combined with the increase in the number of algal cells in the culture relative to that at the beginning of the experiment, shows that it was mainly the cyanobacteria that were negatively affected. The massive death of cyanobacteria and not of the algae in the mixture culture assayed may be due to programmed cell death, which occurs in cyanobacteria as a mechanism to regulate the population dynamic after bloom episodes [52–54]. This bloom dynamic was also corroborated by the depletion of O₂ (Fig. 5) observed in all cultures, and probably related with the process of decomposition [55]. In this study, the bloom dynamics and O₂ depletion were stronger at the higher temperature. However, this bloom occurs in a liquid medium, but could be different when the same community grows as a biofilm on a rock.

The reduction in total cell number contrasts with the increase in the F₀ parameter between the beginning and the end of the experiment. As F₀ is an indicator of biomass [56], a reduction in F₀ would be expected to occur as consequence of the reduction in cell number, but this was not observed. Thus, although the total num-

ber of cells decreased, the number of green algae increased and contributed more to the total biomass because of the larger size of the green algae (4 times larger diameter than cyanobacteria in this study) [45]. Also, some recent studies have shown that some environmental conditions and patterns could change this relationship, with an increase in biomass followed by a decrease of chlorophyll a content [57].

In relation to the response of the assayed organisms to the environmental conditions, two phases were identified: the period before and after 45 days. At the beginning of the experiment, the organisms were in the growth phase, and throughout the experiment they followed the usual phases of development and aging of a culture: (1) a lag phase with few initial cells, (2) an exponential growth phase in which cells produce more viable cells, (3) a phase of declining growth rate in which cessation of the exponential growth occurs (4) a stationary phase where cell death and growth are in balance, and finally, (5) a death phase in which the population declines [58,59]. Thus, the susceptibility of the organisms to environmental factors differed in the different phases.

Throughout the first 45 days, the cultures underwent explosive growth and the differences caused by variation in temperature and CO₂ levels were clearly observed. From then onwards, there was a change in the development and photosynthetic efficiency of organisms, mainly driven by temperature, and to a lesser extent by CO₂ concentration and the interaction of both temperature and CO₂. In this period, an increase in the percentage of green algae versus cyanobacteria was observed in cultures held at 18°C, with the opposite occurring in cultures held at 24°C (Fig. 1b and 3c). The shift in the algae-cyanobacteria balance in response to environmental factors has previously been reported. This is consistent with the findings of [60] regarding microorganisms colonising cultural heritage, where green algae predominated over cyanobacteria on colder areas of granite walls. Likewise, Gaylarde and Gaylarde [61] noted a greater presence of green algae in temperate and cold climates, and a wider distribution of cyanobacteria in tropical areas. In relation to the application of CO₂, although an increase in the competitiveness of cyanobacteria relative to green algae could be expected under high CO₂ concentrations [62], almost no changes were observed in relation to the effect of CO₂ on individual species.

Throughout the first 45 days, the highest temperature produced a decrease in the photosynthetic efficiency (Fig 2b). A decrease in the efficiency of the organisms in biofilms caused by the increase in temperature was previously reported by Villanueva et al. [12], but it could also be due to the previously mentioned higher percentage of cyanobacteria at 24°C, as at optimum state cyanobacteria are less efficient than green algae [63]. In terms of biomass, there was an increase in all cultures, with greater amounts reached in those cultures held at lower temperatures (Fig. 2a, Fig. 3a). In addition, PQ was higher in cultures maintained at 18°C than in those maintained at 24°C (Fig. 4), meaning that chlorophyll a degradation was lower at lower temperatures. The decrease in biomass with increasing temperature contrasts with the growth-enhancing effect reported by many other authors [64].

In terms of growth and photosynthetic efficiency, supply of CO₂ throughout the first 45 days of the experiment did not give rise to significant differences, although the performance appeared to improve slightly when additional CO₂ was provided. Thus, the results suggest a slight increase in CO₂-enhanced biomass at low temperature (Fig. 2a) and a slight increase in CO₂-enhanced photosynthetic efficiency in biofilms exposed to both temperatures (Fig. 2b). Some previous studies reported that increased CO₂ levels had no effect on the maximum quantum efficiency of PSII of phytoplankton communities [65–68]; however, other studies on marine and freshwater cyanobacteria related to an increase in biomass productivity, growth rates and carbon fixation with elevated CO₂ expo-

sure [69,70]. In the case of colonization of cultural heritage, Viles and Cutler [71] hypothesised that exposure to high CO₂ levels may enhance photosynthesis and favour the growth of photosynthesising organisms. This hypothesis was tested by Prieto et al. [5] and Vázquez-Nion et al. [72] in studies carried out on biofilms developed on granite rocks where high carbon concentrations favoured biofilm growth and increased photosynthetic pigments, although the effect seemed to be limited by water availability.

From day 45 onwards, when the culture was in the population decline phase, all parameters measured in cultures, regardless of the conditions applied, lead to the same point, and the differences caused by environmental factor in the previous phase (0–45 days) disappeared. Thus, although growth slowed towards the end of the experiment (Fig. 2a, Fig. 3a) and a massive cyanobacterial die-off occurred (Fig. 1a), the photosynthetic efficiency returned to initial values (Fig. 2b).

Furthermore, there was a constant change throughout the experiment toward a reddish colour in all samples (increase in parameter a*), with those held at 18°C being redder (Fig. 3b). This seems to contradict the increase in biomass, as an increase in the green component (decrease in a* parameter) would be expected as phototrophic biomass increased. However, stress conditions also affect a* so that this parameter could increase despite the increase in biomass. Thus, for the species studied here, colour changes due to different stress conditions such as nitrogen depletion in *Synechocystis* [73] or cell ageing in *Bracteacoccus minor* [74] have previously been observed.

Comparison of dissolved elements in the inoculated and non-inoculated tubes allowed us to examine the effects of the presence of organisms on the weathering of substrates. When granite comes into contact with water, the most mobile cations are expected to be released. Of these, K⁺, Na⁺, Ca²⁺ and Mg²⁺ are essential in biological processes and play an important role in saline-alkaline tolerance in phototrophic organisms [75]. Thus, in the present study, the concentrations of these cations in the inoculated test tubes should be related to the biological activity. At the end of the experiment, the concentration of dissolved elements was lower in all inoculated samples than the non-inoculated test tubes, i.e. for each of the studied conditions, cations were released from the rock, but they ceased to be in dissolution in the presence of the organisms. This may be because they are consumed by organisms as a source of nutrients; they may be attached to or sequestered by organisms; or organisms may produce substances that favour their chelation and precipitation. In either case, the elements are no longer in solution. This could be considered a type of biogeochemical assimilative degradation [76]. In addition, multivalent inorganic cations subtracted from the medium could interact with the EPS matrix, adhering to it and providing better mechanical properties [77,78].

Elements such as Mg²⁺ or Na⁺, which were released in greatest amounts in non-inoculated samples, were present at lower concentrations in inoculated samples, i.e. both release and uptake were greater. In this respect, in granite rocks, Mg²⁺ is mainly released from biotite, which is particularly susceptible to weathering [79]. The rapid release of Mg²⁺ from granite, together with its key participation as part of the chlorophyll molecule and photosynthetic process [80] make this one of the cations most commonly assimilated by phototrophic organisms.

The obtained results may point to a role of the organisms in the biogeochemical system equilibrium. As long as at the end of the experiment the organisms extracted cations from the solution, a further release of these labile cations from the minerals into the medium could be expected to maintain the equilibrium of the biogeochemical system. However, further experiments focused on that equilibrium should be designed. Therefore, changes observed in the quantity of dissolved elements seems to be consequence of the occurrence of organisms. Beyond that, environmental changes tested

did not produce significant changes in the removal of elements from the medium. However, considering that for the first 45 days of the experiment there were differences in biomass and photosynthetic efficiency, it would be interesting to extend the study in time to analyse the effect of these differences in the biogeochemical system equilibrium in a long-term period.

6. Conclusions

The development and physiological state of the phototrophic organisms under study were modulated by temperature to a greater extent than by CO₂. While increasing temperature was associated with lower biomass and photosynthetic efficiency of the organisms, increasing CO₂ concentration seemed to favour photosynthetic efficiency. The increase in temperature also led to a change in culture composition, favouring proliferation of cyanobacteria over green algae.

Thus, in terms of heritage conservation, the combination of both parameters (CO₂ concentration and temperature), which are expected to increase in the near future, will probably lead to an increase in colonization by cyanobacteria versus green algae, but a decrease in cover.

In addition, it was observed that in the presence of organisms there are fewer cations in solution, indicating an effect of the organisms on the biogeochemical system by modifying the chemical equilibrium, which could lead to an increased dissolution of elements from the substrate. To analyse the extent of this effect on the deterioration of the granite, new longer-term experiments need to be designed in which the dissolved elements will be monitored at different times.

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Data availability

All raw data can be provided by the corresponding authors upon request.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Beatriz Prieto reports financial support was provided by European Regional Development Fund, Agencia Estatal de Investigación and Xunta de Galicia. Elsa Fuentes reports financial support was provided by Xunta de Galicia. Diana Perez-Velón reports financial support was provided by Axencia Galega de Innovación (Xunta de Galicia).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.culher.2025.03.003](https://doi.org/10.1016/j.culher.2025.03.003).

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