Subaerial biofilms on outdoor stone monuments: changing the perspective towards an ecological framework

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Abstract

Despite the appreciation of the role played by outdoor stone heritage in societal well-being and sustainable urban development, research efforts have not been completely successful in tackling the complex issues related to its conservation. One of the main problems is that we are continuously underestimating the role and behavior of microorganisms in form of biofilm (subaerial biofilms, SABs) to the management of stone artifacts. To this end, we discuss the necessity to approach the topic from an ecological perspective, through an overview of the characteristics of SABs that mediate different ecological interactions. Furthermore, we explore the application of functional traits ecology to unravel the mechanisms by which SABs might respond to a changing environment. Finally we guide and prioritize further research, in order to inform policy-makers, and to develop management strategies for protection prior to, or following after, active conservation treatment.

Introduction

Preserving the environment for future generations is one of the key concepts of sustainability, which is grounded in the need for intergenerational equity. The ongoing political and scientific debate on sustainability tends to focus on issues related to carbon emission, energy consumption, natural resource use and waste management, or the economic aspects of urban regeneration and growth (Tweed and Sutherland 2007). Increasingly, however, national governments and international institutions recognize cultural heritage as a non-renewable resource that is unique, non-replaceable or non-interchangeable, highlighting the intrinsic value of cultural heritage in contributing to the societal and economic well-being of communities (*inter alia* MEA 2005, EU Communication 2010, UNESCO 2013).

Thus, conservation and management of cultural heritage constitutes a strategic choice for the 21st century.

This fundamental principle has been recently recognized in the outcome document of the United Nations Conference on Sustainable Development - or Rio+20 - The Future We Want- (Brazil on 20-22 June 2012), by highlighting how '*many people, especially the poor, depend directly on ecosystems for their livelihoods, their economic, social and physical well-being, and their cultural heritage*' (emphasis added), or by calling for the '*conservation as appropriate of the natural and cultural heritage of human settlements, the revitalization of historic districts, and the rehabilitation of city centres*.'

The fundamental roles played by cultural heritage are threatened today by a number of factors, including climate change and microbial attack, leading to new challenges for heritage objects, especially those exposed to the outdoor environment. Preserving the fragile character of our cultural heritage, and managing it for the benefit of current and future generations is a major task for researchers and decision-makers worldwide.

Many of the world’s most precious artworks are made of stone (e.g. marble, limestone and sandstone) with a finite life, and they are slowly but irreversibly disappearing (Schreerer *et al.* 2009).

Despite the appreciation of the role played by stone heritage in many societies, research efforts have not been completely successful in tackling the complex issues related to its conservation, and the need to develop comprehensive approaches and methodologies for its management. One of the main gaps is that we are still understanding the contribution of microorganisms to the deterioration of stone, as for many decades chemical and physical deterioration was believed the main cause of material decay (Sterflinger and Piñar 2013). Stone monuments, apart from being ancient records that illuminate the cultural history of our planet, are dynamic repositories that support microbial life. The presence of green, yellow-brown or black patinas is all too familiar to anyone who has looked closely at a historic stone building or sculpture. These patinas are composed by densely packed microorganisms that operate within self-organized structures of micron to- millimeter scales (**Figure 1**). These microbial communities at the stone/air interface are called subaerial biofilms (SABs). SABs are made up of many microbial cells, generally of different types, which employ coordinated survival strategies to increase biocide resistance and microbial fitness, and to avoid loss of energy and nutrients (Stewart and Franklin 2008, Stone 2015). SABs can be viewed as multi-component open ecosystems sensitively tuned to the atmosphere and the stone substratum (Gorbushina 2007). As with any other ecosystem, understanding of the ecological and evolutionary mechanisms by which SABs organize themselves and respond to environmental changes will help to predict, and possibly ameliorate, system performance and their response to perturbations, improving the development of comprehensive approaches for the sustainable management of outdoor stone heritage in a changing environment.

Thus, in order to obtain a holistic view of the phenomena occurring at the stone surface, we should consider relationships between the biotope (stone), the biocenosis (SABs) and the surrounding environment (macro- and micro-climate).

The main goal of this paper is to argue for new lines of research in which SABs inhabiting stone monuments are viewed from an ecological perspective, and moving toward a system-level understanding of biofilm community organization and function. Conversely, SABs on stone monuments could act as interesting models for ecological study, offering exciting new opportunities for the development and testing of ecological principles, broadening understanding of microbial ecosystems and generating new insights in basic ecology.

This review is organized to provide the reader with: (1) an overview of what is known of ecology of SABs inhabiting stone monuments and the gaps in the literature, suggesting an objective framework for the factors that influence the structure and function of the microbial communities inhabiting stone surfaces; (2) the application of functional traits ecology to unravel the mechanisms by which SABs might respond to a changing environment; and (3) a summary of the salient points of the presented review and identification of the highest priority research areas for targeted research.

Ecology of SABs inhabiting outdoor stone materials

SABs and their inhabitants are shaped by the complex dipartite interactions between the atmosphere and the stone. The stone substratum acts as a putative source of minerals, while the air chemistry might offer inorganic and organic compounds (Villa *et al.* 2015). Furthermore, surface irregularities such as fissures, cracks and pores, provide microorganisms safe places against harsh environmental conditions. There, microorganisms take advantage of the accumulated moisture, as well as of the shelter from intense solar radiation, temperature fluctuations, wind and desiccation, and therefore they successfully colonize the lithic material (Gorbushina *et al.* 2002). Not surprising is the occurrence of endolithic communities, including photosynthetic communities, inside micro-cracks and pores of stone monuments (Crispim and Gaylarde 2005).

Microbial growth on stone surfaces follows the complex topography of the substrate and generates a patchy biofilm that spreads between the mineral grains filling depressions, fissures and intergranular spaces (Gorbushina 2007). However, SABs do not simply cover the lithic surface, but rather they interact with the stone in myriad ways, revealing a tight and clearly defined coupling between geochemical and biological processes that affect the lithic substrate in different ways **(Table 1)**. These properties translate into a characteristic set of ecological impacts, making SABs effective ecosystem engineers by their substantial effects on the physical and chemical properties of the habitat in which they live.

Taxonomic and phylogenetic studies of SABs have revealed lower diversity in SABs on stone surfaces compared to most natural systems (Gorbushina and Broughton 2009). The relatively low diversity is attributed to the extreme and fluctuating environmental conditions that microorganisms must endure. In fact, outdoor stone monuments are often stressful environments characterized by desiccation, low nutrient concentrations, large temperature variations and high exposure to wind, UV radiation and physical damage (Viles and Cutler 2012). Only microorganisms with a very broad range of tolerance to multiple and fluctuating stresses can establish themselves under these conditions (Zakharova *et al.* 2013).

However, despite the relatively low genetic diversity, SABs contain metabolically interactive, self-sustaining microbial communities, which promote cooperative interactions within the biofilm (Villa *et al.* 2015). An over-riding characteristic of SAB communities is that together, constituent microorganisms overcome environmental stresses better than any could individually.

This joint protection is rooted in the presence of the biofilm matrix, in the close contacts between different biofilm partners (e.g. mutually beneficial associations with cooperating microorganisms with different nutritional requirement) and in interactions with the mineral substrate and the atmosphere (Gorbushina 2007, **Figure 1**). Furthermore, the biofilm microenvironment provides the community as a whole with an enormous capability to become resistant to biocide exposure. Bacteria embedded in the biofilm matrix are remarkably more tolerant to biocides, up to 1,000-fold relative to planktonic cultures of the same bacterial strains, depending on the species-drug combination (Davies 2003). Conservation treatments with traditional doses of biocides are sometimes insufficient to destroy all members of the biofilm community, and this is a cause of concern for conservators (Cappitelli *et al.* 2011). Consequently, in the last few years, the efforts have been directed towards implementing and developing preventive strategies (Cappitelli *et al.* 2011).

The documented presence of specialized microorganisms (*Inter alia* Golubic *et al.* 1981, Friedmann and Ocampo-Friedmann 1984, Eppard *et al.* 1996, Laiz *et al.* 2009, Bastian *et al*. 2010, Cappitelli *et al.* 2012, Polo *et al.* 2012, Ettenauer *et al.* 2014), highlights the existence of multiple trophic levels (McNamara and Mitchell 2005) with a simultaneous bottom up (resource supply-driven) and top-down (food web structure-driven) control of ecosystem structure and function, emergent patterns of organization (Gorbushina 2007), ecological succession (Hoppert and Konig 2006) and ecosystem stability founded on diversity (Miller *et al.* 2009, **Figure 2**).

The above-mentioned characteristics are the hallmarks of a ‘complete’ and ‘complex’ environmental biological system.

Moreover, despite conditions perceived by us as ‘extreme’, the primary production rate of epilithic communities can be high, comparable on a gC m^{-2} y^{-1} basis to rates for many terrestrial and ocean ecosystems (Büdel 1999). Interestingly, this suggests that carbon fixation rates in less then 100 μm thick biofilms are broadly equivalent to those achieved across the ocean photic zone (Denef *et al.* 2010).

The relatively low species complexity, the defined ecological succession patterns and trophic level, the tight biological-geochemical coupling, and the high biological productivity are important features that make SABs inhabiting stone surfaces a good model system to generate simple and clearly defined hypotheses to be tested across a range of environments.

As these biological systems are involved in the processing of weathered rock material, they might be considered perfect model systems for studying biogeochemical processes and pedogenesis and promising indicators of climate changes, being coupling agents between the atmosphere and the lithosphere (Warscheid and Braams 2000, Gorbushina 2007, Villa *et al.* 2015). In addition, SABs demonstrate mutually neutral or even beneficial associations as, in such hostile environments, the metabolic costs of survival are so high that antibiosis is often an unaffordable luxury, making them potential system to study symbiosis (Gorbushina *et al.* 2005, Gorbushina and Broughton 2009).

The ability of SABs to impact the lithic substrate and to buffer and adapt to both natural and anthropogenic changes provides a number of significant ecosystem services essential to human communities and societies (**Table 2**).

Towards a traits-based approach to SAB ecology

We advocate that an improved appreciation of the ecology of SABs inhabiting outdoor stone materials will strengthen our ability to predict the impact of environmental change and to develop management strategies for protection prior to, or following after, an active conservation treatment.

Until now, the scientific community traditionally viewed SABs through a taxonomic lens, often resulting in the loss of ecological generality. Although genomes and metagenomes give a detailed cross section of the functional potential of a community, the functional traits (morphological, biochemical, physiological, structural, phenological, or behavioral characteristics) are those properties that interact directly with the environment, providing more relevant information in a community analysis with special emphasis on feedback responses to environmental change and biodecay phenomena of cultural heritage.

In a recent work, Villa and colleagues (2015) used a traits-based approach to reveal the metabolic capabilities of SABs inhabiting historic limestone tombstones in response to atmospheric sulfur pollution. They elucidated functional interaction networks and syntrophic interplays that enable cooperative growth in SAB communities (**Figure 3**). This study showed also the ability of SABs to perceive the external environment and to buffer environmental perturbations.

Thus, the long-standing question “what is there?” should switch to the questions “why is it there?”, “how does it interact with the external environment?”, and “how does it respond to a disturbance event?”. In addition, recent developments in community ecology have begun to recognize that microbial assemblages cannot be defined without reference to their environments (Konopka 2009). An appreciation for the tight interrelationship between microbes and their physical and chemical environments is particularly important for delineation of microbial communities and their ability to respond to a changing environment (O’Donnell *et al.* 2007).

Because functional traits mediate the interactions among microorganisms as well as between microorganisms and the environment, it has been argued that trait-based approaches provide more relevant information in a community analysis and ecosystem service than taxonomic or phylogenetic attributes (Violle *et al.* 2007, Boon *et al.* 2014, Krause *et al.* 2014). As Cohan & Perry (2007) state, ‘…*the recognized “species” of bacterial systematics frequently contain a diversity of populations that are distinct in their biochemistry, physiology, genome content and ecology; classifying an unknown organism to its species thus tells us only vaguely about the organism’s way* *of life*'. Functional differences, even in only few critical pathways, could reflect dramatically altered ecosystem properties and could impact the services or disservices that human societies derive from them (Luck *et al.* 2009). This functional approach is instrumental for unraveling the role of SABs in biodeterioration or bioprotection of stone monuments, as detecting microorganisms does not automatically imply an involvement in the biodecay process of the lithic substrate. The axiomatic correlation among microorganisms and stone decay is matter of controversy, as it is far from clear why some communities are deteriorative and others are protective or, indeed, why can be deteriorative under some environmental conditions and bioprotective under others (Viles and Cutler 2012, Bartoli *et al.* 2014, Pinna 2014). Only a traits-based approach may reveal the dual role of SABs and their inhabitants, and how this dual role affects conservation strategies.

Trait-based approach to predicting feedback responses of SABs to a changing environment

Frameworks that group microorganisms into functional groups along a few trait axes have helped to summarize biological variation and has led to the development of hypotheses to explain the origins of functional diversity, the distribution and abundance of species, and the consequences of functional traits for ecosystem functioning (Chagnon *et al.* 2013).

For example, a simple model sees the characterization of microorganisms according to their life-history strategy: r-strategists (termed copiotrophs in microbial ecology) have high growth rates and low resource use efficiency, and K-strategists (termed oligotrophs in microbial ecology) have low growth rates and high resource use efficiency (Fierer *et al.* 2007). This assumed fundamental trade-off between growth rate and resource use efficiency might underlie the capacity of microbial communities to respond to disturbance, as community structure will change if the taxa present differences in this trade-off (Wallenstein and Hall 2012). There is evidence from both plant and soil communities that K-strategists are more resistant (the ability of a community property or process to remain unchanged in the face of a specific disturbance), but less resilient (the ability of a community property or process to recover after a specific disturbance, often reported as a rate of return), to climate change-related disturbances than r-strategists (Bapiri *et al.* 2010, Lennon *et al.* 2012), and a trade-off between resistance and resilience is widely documented (De Vries *et al.* 2012). De Vries and Shade (2013) proposed that simple measures that characterize microbial communities along the r-K spectrum could inform their ability to resist and recover from climate change related disturbances.

Nevertheless, the r-K framework has been criticized for its oversimplification of life history strategies along a single axis that combines both disturbance and resource availability. Other models that integrate additional axes have thus been proposed to more completely characterize diversity while at the same time remain simple and tractable (Chagnon *et al.* 2013). The Competitor-Stress tolerant-Ruderal (CSR) framework developed for plants, overcomes some limitations of other models by classifying plant life history strategies according to the functional traits associated with responses to two major environmental filters, namely stress and disturbance (Grime 1977). Stress refers to persistent adverse environmental conditions (e.g. increasing temperature and UV levels, decreasing moisture levels), whereas disturbance refers to episodic events leading to significant loss of functional biomass (e.g. fire, drought, storms or erosion). The C-S-R framework identifies three main life history strategies: 1) ‘competitors’ are adapted for rapid resource utilization and long-term site occupation, 2) ‘stress tolerators’ are adapted to persist in low-resource environments owing to resource conservation strategies, and 3) ‘ruderals’ cope with frequent disturbance by relying on high colonization ability, rapid production of low cost biomass and short reproductive cycles (Prosser *et al.* 2007).

Recently, Viles and Cutler (2012) employed the C-S-R framework as an example to show how trait-based classification approach can predict the responses of heritage biota in terms of biodeterioration, bioprotection and biological soiling to environmental changes. According to Hoppert and König (2006), opportunistic and ruderal taxa within SABs colonizing stone monuments are more likely to be deteriorative, as they colonize rapidly after disturbance and use a range of strategies to derive nutrients from the substrate (e.g. rapid, destabilizing, endolithic growth). Such strategies may cause further disturbance to the surface through weathering, favoring ongoing ruderal colonization. By contrast, stress-tolerant species are likely to be less deteriorative as, according to Hoppert and König (2006), they do not cause disruption of the surface. Indeed, some of the strategies they use to cope with stress (e.g. pigmentation) may even have bioprotective role by protecting the artistic surface from weathering (Viles and Cutler 2012). Following this path, Viles and Cutler (2012) predicted that areas likely to experience increased frequency of climatic disturbances are likely to experience a shift from bioprotective to biodeteriorative conditions. Furthermore, areas that are likely to face increased stresses (e.g. decreased precipitation) will show a reduction in soiling rates, a switch to stress-tolerators and knock-on decline in biodeterioration. They envisioned situations where conditions change from stressed to disturbed (or *vice versa*), producing no net change in soiling rate, but a switch between biodeterioration and bioprotection.

We summarized current knowledge of functional traits of the main microbial groups of a mature SAB (**Table 3**), and incorporate them into the C-S-R framework to conceptualize SAB life strategies in order to better predict of their responses to environmental changes (**Figure 4**).

The trait-based approach proposed provides a simplified representation of SAB life strategies. Associations in nature will likely be much more complex because SAB communities will rarely be at any of the three extremes of the C-S-R triangle, but most of the time will rather have a mixed life history. Moreover, microorganisms can display competitive, ruderal or stress-tolerant morphotypes at different stages of SAB development and under different environmental conditions. However, we argue that integrating such a trait-based approach into an established life history classification scheme, such as the C-S-R framework, can provide more mechanistic insights about the relationship among SABs, stone and the environment. The idea would be to assign taxonomic and functional information of a specific biofilm community retrieved on the artistic surface within the three dimensions of C-S-R classification framework, providing the basis to predict and assess SAB distribution, prevalence and response to stresses and disturbances. The same approach was recently used by Ho *et al.* (2013) to classify the observational ecological characteristics of methane-oxidizing bacteria and exploiting their life strategies to optimize the performance of this community in respect to a desirable set of outputs.

Moving ahead: future research directions

Understanding the ecology of SABs is arguably one of the most compelling intellectual challenges facing contemporary ecology. Although worthy for its intellectual merits alone, developing such an understanding is essential to the management of outdoor cultural heritage for their benefits in culture-related economic activities, socio-political development, urban sustainability, education and environmental protection.

Predicting how under a changing environment SABs will influence the ecosystem processes they mediate requires an approach that links change in fitness of individuals to population dynamics, community composition and function. In particular, looking at the structure of functional traits on a community-wide scale could provide us insight about the processes carried out by SABs and, in turn, about what traits are associated with a particular environmental condition. A deeper understanding of ecosystem function might represent a way to manipulate the growth of SABs on surfaces.

We think that several topics of research should be prioritized in order to predict the feedback response of SABs to anthropogenic changes, and to develop microorganism-mediated approaches to protect artistic surfaces and mitigate the effect of stresses and disturbances.

First, we need to understand and quantify the functional traits of SABs that may impact their fitness in a given habitat and their responses to a changing environment. Second, we need to map taxonomic information into a functional space, in order to assign ecological niches to different microbial taxa and elucidate SAB/stone and SAB/atmosphere interactions. Third, we need to quantify the biodeterioration of artistic surfaces and the effects of environmental changes on stone geochemistry. Fourth, we need to improve our understanding of microbial responses to simultaneous environmental stressors (Staudt *et al.* 2013). Finally, we need to create a framework to incorporate biological (omics), environmental, chemical and geological data into mathematical models, in order to offer a system-level understanding of the phenomenon, reducing uncertainty and improving quantitative estimation and prediction.

Recent advances in 'omic' technologies, computational science and the ease with which data can be shared and forwarded will provide the opportunity to integrate knowledge across disciplines to generate an increasingly comprehensive understanding of the SABs responses to a changing environment, and how they will influence the ecosystem where they are growing in. Progress will require collaborative research among different disciplines. We envision that contributions by five different groups will be particularly useful:

1) Partnerships between conservators, heritage managers and ecosystem scientists to sample SABs on outdoor stone surfaces across a global-scale gradient of biomes. The comparison of the taxonomic and functional dimension of SABs over a wide range of both spatial- and

timescales, will lead to hypotheses about the relationships between environmental changes and potential microbiological damage. This effort should be coordinated with ongoing long-term research networks and utilize existing data sets to the fullest possible extent.

2) Collaborations with molecular biologists and bioinformaticians to apply next generation sequencing technologies to look for functional patterns in the samples collected from around the world. This information would allow testing hypotheses about the time and mode of SABs response to a changing environment.
3) Collaborations with biochemists to identify biomarkers in the form of metabolites, proteins or transcript pools that signify ecosystem state at the onset of a transition. This knowledge will inform hypotheses about the potential role for SABs in C, N, P and S dynamics in a changing environment.
4) Work together with mathematicians to incorporate space- and time-resolved omics and environmental data into new models to test hypotheses about the role of SABs for biogeochemical cycles, biodeterioration vs. bioprotection of stone, ecosystem productivity and climate.
5) Most importantly, research findings should be used to build relationship and open lines of communication between researchers and stakeholders, to facilitate the translation of research findings into actions. Researchers can profile their achievements and stakeholders can be informed of research outcomes and influence research challenges.

The complexity of the phenomenon under investigation requires interdisciplinary research if we are to attain the predictive capability that could inform policy makers. The potential for interdisciplinary research ultimately hinges on the extent to which individuals want to engage in it, and equally importantly if they have the opportunity to do so. Granting agencies are encouraging multidisciplinary approaches by increasingly providing support for crosscutting research efforts. There is no better time for seizing the opportunity to establish and fine-tune the collaboration with co-workers in other fields.

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Tables

Table 1: Mechanisms by which SABs can alter and engineer their habitat.

Mechanisms	**References**
Respiration of bacteria and fungi increases local CO_2 concentrations: • Formation of H_2CO_3, which decreases the pH of the stone surface and leaches out carbonates, phosphates and silicates.	Warscheid and Braams 2000, Dakal and Cameotra 2012, Sterflinger and Piñar 2014
Production of ligand-based agents (e.g. organic anions, siderophores): • Chelation of Ca, Mg, and Fe, which promote the dissolution of cationic constituents.	Warscheid and Braams 2000, Hoffland *et al.* 2004, Dakal and Cameotra 2012
Production of acids: • Promotion of the dissolution and/or chelation of cations. • Weakening of the mineral lattice by dissolution of metal cations. • Precipitation of calcium oxalate.	Warscheid and Braams 2000, McNamara and Mitchell 2005, Gorbushina 2007, Sterflinger and Piñar 2014
Production of extracellular polymeric substances (EPS): • Dessication/hydration cycles of the EPS cause separation of particles. • Regulation of the humidity, thermal transmission and water vapor diffusion, reducing thermo-hydric stresses to the stone. • Wrapping the grains with a biogenic matrix temporarily stabilizes the surface and reduces weathering.	Warscheid and Braams 2000, Crispim and Gaylarde 2004, Gorbushina 2007, Pinna 2014, Sterflinger and Piñar 2014
Uptake and accumulation of sulfur and calcium into the cells: • Weakening of the stone matrix. • Growth of cells forces separation of mineral grains.	Crispim and Gaylarde 2004, Scheerer *et al.* 2009, Dakal and Cameotra 2012
Endolithic growth: • Contribution to the breakdown of rock crystalline structures.	Golubic *et al.* 1981, Crispim and Gaylarde 2004, Scheerer *et al.* 2009
Hyphae and filamentous growth: • Contribution to the breakdown of rock crystalline structures.	Sterflinger and Krumbein 1997, Warscheid and Braams 2000, Hoffland *et al.* 2004
Create a multitude of varnish-like coatings: • Discoloration. • Discolored areas may absorb more sunlight, which increases physical stress by expansion and contraction caused by temperature changes.	Warscheid and Braams 2000, Gorbushina *et al.* 2002, Crispim and Gaylarde 2004, Noack-Schönmann *et al.* 2014

Table 2: Ecosystem services provided by SABs inhabiting stone surfaces

Service	**Mechanism**
Biogeochemical cycles	Nutrient cycling, specific elemental transformation (e.g. nitrification and sulfur oxidation).
Atmospheric change indicators	By intercepting compounds carried by the air, SABs and their activity are under the direct influence of the atmospheric input.
Climate regulators	Carbon sequestration, nutrient cycling, specific elemental transformation (e.g. nitrification, sulfur oxidation).
Culture and conservation of stone monuments with impacts on recreation, tourism and economy	Cultural heritage is often associated with the identity of an individual, a community or a society. Cultural heritage provides experiences shared across generations, as well as settings for communal interactions important to cultural ties. Conservation of stone monuments has indirect impacts on tourism and recreation activities. Tourism and recreation activities are estimated to contribute € 415 billion to the EU GDP and 3.4 million tourism enterprises account for 15.5 million jobs (EU Communication 2014). In addition, visitor's expenditure generates income for the local communities and infrastructure development.

Table 3: Ecological characteristics of the main microbial groups of SABs (Fungi, Bacteria and Algae) inhabiting stone surfaces. C: Competitor; R: Ruderal; S: Stress tolerator

FUNGI			
Group	**Ecological characteristics**	**References**	**Class**
Hyphomycetes (Hyp)	• Fast growing in comparison to MCF. • Different abilities to access limiting resources (e.g. production of siderophores). • Ability to scavenge nutrients from the air and rain. • Pigment production. • Hyphal growth and reproductive structures. • Production of asexual spores. • High dispersal rates in comparison to MCF. • Epilithic and endolithic growth.	Sterflinger and Krumbein 1997, Cutler and Viles 2010, Nai *et al.* 2013, Sterflinger and Piñar 2013	C C/R
Micro colonial fungi (MCF)	• Slow growing in comparison to Hyphomycetes. • Accumulation of storage compounds. • High resistance to desiccation, UV radiation and osmotic stress. • Swollen, isodiameteric cells with thick, melanin containing cell walls. • Compact microcolonies on and inside the stone. • No aerial mycelium. • Capacity to survive long period of suspended metabolism. • Create a multitude of varnish-like coatings. • Production of survival propagules.	Sterflinger and Krumbein 1997, Nai *et al.* 2013, Cutler and Viles 2010, Sterflinger and Piñar 2013	C C/S

BACTERIA

Group	Ecological characteristics	References	Class
Cyanobacteria (Cya)	• Simple nutritional requirement. • Slow growing. • Ability to store essential nutrients and metabolites. • Production of photosynthetic and/or protective pigments. • Production of exopolymers. • Harbor a number of repair and tolerance mechanisms to counter the effects of UV and oxidative stress. • Efficient response to moisture status. • Epilithic and endolithic growth. • Limited mobility.	Crispim and Gaylarde 2005, Scheerer *et al.* 2009, Sterflinger and Piñar 2013	S S/C
Actinobacteria (Act)	• High growth rate. • High cellular turnover rates and short life cycle. • Small cell size. • Metabolic plasticity and rapid response to different substrates. • Production of soluble pigments. • Hyphal growth. • Endolithic growth. • Early production of asexual spores. • More efficient dispersal mechanisms.	Eppard *et al.* 1996, Gorbushina 2007, Scheerer *et al.* 2009, Sterflinger and Piñar 2013	R R/C R/S
Lithotrophs (Lit)	• Simple nutritional requirement. • Slow growing. • Release of inorganic and organic acids. • Accumulation of storage compounds. • Small cell size.	Golubic et al. 1981, Warscheid and Braams 2000	S/C

ALGAE			
Group	**Ecological characteristics**	**References**	**Class**
Green algae (GA)	• Simple nutritional requirement. • Slow growing. • Accumulate organic osmolytes to face osmotic stress. • Protection against oxidative stress via non-photochemical quenching. • Cope with high light condition by producing protective carotenes and xanthophyles. • Able to use water vapor. • Mixotrophy. • Algal propagules can remain viable in the atmosphere for extended period.	Gorbushina 2007, Scheerer *et al.* 2009, Cutler and Viles 2010	S S/C

Figure captions

Figure 1: A SAB growing on the white marble of the Lincoln Memorial, Washington, DC. (A) Antefix on the roof of the Lincoln Memorial. (B-C) Close-up shots of a vertical SAB on the Jefferson Memorial. (D) Confocal laser scanning imaging of a biofilm taken from this location. Blue are microcolonies of photoautotrophic microbes, green are chemoheterotrophic microbes, and red are extracellular polymeric substances.

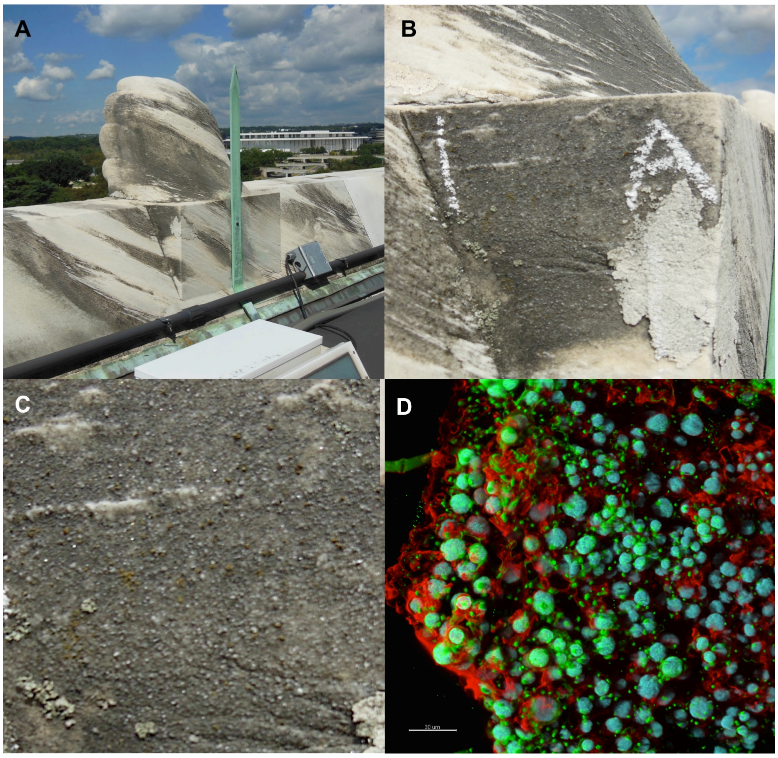


Figure 2: Multiple trophic levels in SABs inhabiting stone surfaces. The microbial food web in SABs is influenced by both bottom-up (resource-supply driven) and top-down (predation-driven) forces.

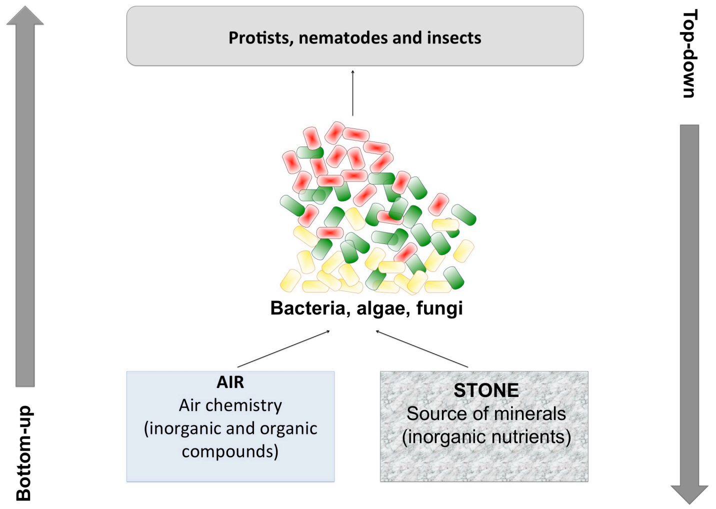


Figure 3: Interdependent cycling of nutrients that occurs among the main functional groups retrieved on a tombstone located in a polluted environment. The organic carbon produced by cyanobacteria during photosynthesis supports the growth of microorganisms that require organic matter as energy source such as sulfate reducing bacteria (SRB), and sulfur oxidizing bacteria (SOB). SOB consumes the oxygen produced by cyanobacteria, creating the anaerobic environment for SRB and anoxygenic phototrophic sulfur bacteria. The SOB quickly remove the metabolic products of SRB, S^{2-}, that could inhibit cyanobacteria and at higher concentrations also SRB.

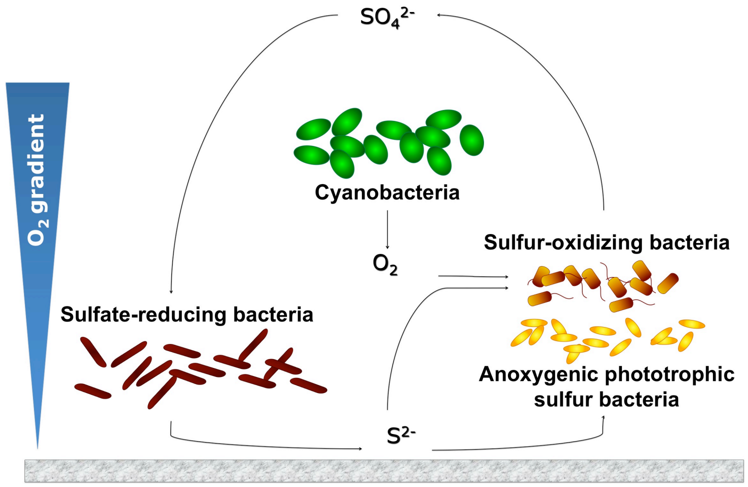
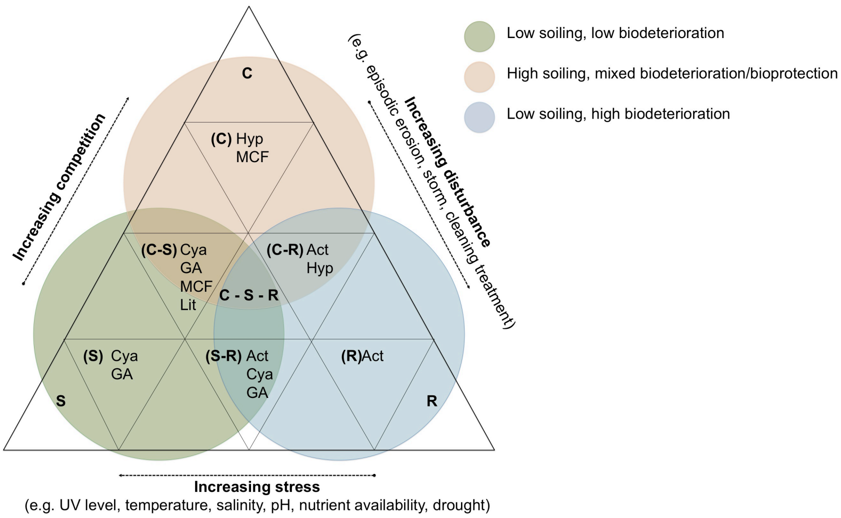


Figure 4: Reflection of SAB microbial traits on the Competitor (C)-Ruderal (R)-Stress tolerator (S) life strategy framework as was proposed for plants by Grime (1977). Hyp: Hyphomycetes; MCF: Micro Colonial Fungi; Cya: Cyanobacteria; Act: Actinobacteria; GA: Green Algae; Lit: Lithotrophs.


Low soiling, low biodeterioration
High soiling, mixed biodeterioration/bioprotection
Low soiling, high biodeterioration
C
(C) Hyp
MCF
(C-S) Cya
GA
MCF
Lit
(C-R) Act
Hyp
C - S - R
(S) Cya
GA
(S-R) Act
Cya
GA
(R)Act
S
R
Increasing competition
Increasing disturbance
(e.g. episodic erosion, storm, cleaning treatment)
Increasing stress
(e.g. UV level, temperature, salinity, pH, nutrient availability, drought)

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