Title:

The spatial organisation and microbial community structure of an epilithic biofilm

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Abstract

Microbial biofilms are common on lithic surfaces, including stone buildings. However, the ecology of these communities is poorly understood. Few studies have focussed on the spatial characteristics of lithobiontic biofilms, despite the fact that spatial structure has been demonstrated to influence ecosystem function (and hence biodegradation) and community diversity. Furthermore, relatively few studies have utilised molecular techniques to characterise these communities, even though molecular methods have revealed unexpected microbial diversity in other habitats. This study investigated 1) the spatial structure and 2) the taxonomic composition of an epilithic biofilm using molecular techniques, namely amplicon pyrosequencing and terminal restriction fragment length polymorphism (TRFLP). Dispersion indices and Mantel correlograms were used to test for the presence of spatial structure in the biofilm. Diversity metrics and rank-abundance distributions (RADs) were also generated. The study revealed spatial structure on a centimetre-scale in eukaryotic microbes (fungi and algae), but not the bacteria. Fungal and bacterial communities were highly diverse; algal communities much less so. The RADs were characterised by a distinctive 'hollow' (concave up) profile and long tails of rare taxa. These findings have implications for understanding the ecology of epilithic biofilms and the spatial heterogeneity of stone biodeterioration.

Keywords: amplicon pyrosequencing; TRFLP; lithobiontic microbes; green algae; bacteria; fungi; mantel correlograms; rank-abundance distributions

1 Introduction

Microbial biofilms are a common feature on both natural rock outcrops and stone buildings. Particular attention has been focused on the biofilms that form on stone buildings, as these communities can have a detrimental impact on both the appearance and physical structure of the stone (Warscheid & Braams, 2000, McNamara & Mitchell, 2005, Scheerer*, et al.*, 2009). However, whilst laboratory work has revealed some of the mechanisms by which biofilm-forming microbes contribute to the biodeterioration of stone, very little is known about the ecology of these organisms. In particular, the spatial structure of stone-dwelling (lithobiontic) microbial communities at sub-metre scales (i.e. the size of masonry building components) is poorly understood, as most previous studies have focussed on the micro- or field/landscape scale (Franklin & Mills, 2010). Furthermore, relatively few studies have used molecular (DNA) techniques to characterise the microbial communities of building stone. To address this knowledge gap, we conducted a high-resolution, molecular study of an epilithic biofilm in order to investigate both small- (centimetre) scale spatial structure and microbial community composition.

Studies of epilithic biofilms have largely neglected the spatial dimension of lithobiontic communities even though a) many biofilms on buildings are patchy on a scale visible to the naked eye and b) distribution of organisms in space (spatial structure) has been demonstrated to have a profound impact on ecosystem function (Franklin, 2005, Nunan*, et al.*, 2010) and hence the patterns and processes of biodeterioration. Microbial communities can obviously vary on very small spatial scales. Gleeson et al. (2005), for example, found that lithobiontic fungal communities can vary on the scale of individual mineral grains. Studies have investigated spatial variation on the scale of individual buildings (Rindi & Guiry, 2003, Rindi, 2007, Cutler*, et al.*, 2013) or on larger (regional) scales (Gaylarde & Gaylarde, 2005). However, there is almost no published work that looks at the intermediate scale of centimetres - metres, i.e. at a level that is of interest to building conservators (Franklin & Mills, 2010). Understanding the factors that structure microbial communities at this scale may shed light on the fundamental ecological processes that affect subaerial, lithobiontic biofilms and their influence on the deterioration of stone.

Previous studies have described the composition of lithobiontic microbial communities (see, e.g., Gleeson*, et al.*, 2005, Gorbushina, 2007, Gleeson*, et al.*, 2010). Most of this work has focussed on individual taxonomic groups (e.g. Cyanobacteria or fungi) and has been based on techniques such as microscopy and *in vitro* culturing of environmental samples. However, these techniques can only capture a tiny proportion of environmental microbial diversity. For

example, it is thought that only 1-10% of environmental microbes can be successfully cultured (Pace, 1997). Modern molecular techniques have been used in a variety of habitats including soil (Acosta-Martinez*, et al.*, 2008, Jones*, et al.*, 2009, Tedersoo*, et al.*, 2010) and marine habitats (Pommier*, et al.*, 2010, Gaidos*, et al.*, 2011), but, with a few exceptions, they have not been systematically applied to building stone (Cutler & Viles, 2010). Typically, molecular techniques reveal levels of microbial diversity much higher than those based on cultured environmental samples.

Our study was broad in that it encompassed the three main groups of microorganism (hereafter, microorganism types) living on building stone i.e. algae, fungi and bacteria. We applied two, widely used, molecular techniques (amplicon pyrosequencing and terminal restriction fragment length polymorphism (TRFLP)) to describe community structure, concentrating on the two-dimensional arrangement of microbes in a thin biofilm (i.e. predominantly epilithic microbes). In terms of spatial structure, we anticipated that samples close to each other would be more similar than widely-spaced samples in terms of community composition, due to centimetre scale patchiness in the biofilm. We also anticipated low taxonomic diversity, but high taxonomic richness, particularly in fungal and bacterial communities.

2 Materials and Methods

We used molecular methods to describe the spatial structure and composition of a biofilm on a sandstone slab. Broadly, sample-specific TRFLP was used to ascertain the spatial structure of each microbial group and amplicon pyrosequencing was used to establish microbial community composition.

2.1 Sampling

In order to assess potential variation in microbial communities at a centimetre-scale, we sampled a smooth, fine-grained sandstone slab at a high level of spatial resolution. The slab, which had been kept in an exposed location in a stonemason's yard in Oxford, UK, had obvious biological growth on its surfaces but was homogeneous in terms of surface texture and composition. Whilst sampling was carried out during the development of a non-destructive sampling technique for epilithic biofilms described in Cutler et al. (2012) the following research is based on new analyses of these samples. We focussed on the epilithic microbial community because surface-dwelling microorganisms are easiest to sample and are likely to have the greatest impact on the appearance of the stone. The sampling regime is described in detail in Cutler et al. (2012). Briefly, the surface of the slab was divided into a

6 x 4 grid of sample locations (24 samples in total), each measuring 30 x 30 mm and separated from neighbouring sample locations by a buffer zone of 25 mm. A section of sterile adhesive tape was applied to the surface of the stone to collect microbial cells from the biofilm. The biological material on the tape was then analyzed using molecular techniques.

2.2 Amplicon pyrosequencing and TRFLP

A modified CTAB extraction method was used to extract DNA from the tape samples: refer to Cutler et al. (2012) for a detailed protocol. Briefly, subsamples (20 µl) were taken from the DNA extracted from each of the 24 tape samples. These subsamples were pooled and cleaned with a Powerclean kit (MoBio Laboratories Inc, Carlsbad, CA) in accordance with the manufacturer's instructions. The pooled sample was then standardised to a DNA concentration of 20 ng/µl and analysed via tag-encoded FLX amplicon pyrosequencing, utilising a Roche 454 FLX instrument (454 Life Sciences, Branford, CT). The primer set euk516F (5'-GGAGGGCAAGTCTGGT-3'), euk1055R (5'-ARCGGCCATGCACCACC-3') was used for eukaryotes (primarily to characterise algae); the primers ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') were used for fungi and the primers 104F (5´-GGACGGGTGAGTAACACGTG-3´), 530R (5'-GTATTACCGCGGCTGCTG-3') for bacteria. The pyrosequencing was performed at the Research and Testing Laboratory (RTL, Lubbock, TX) based upon RTL protocols.

Mothur 1.32.1 (Schloss*, et al.*, 2009) was used to process raw sequence data generated by the amplicon pyrosequencing, following the pipelines described in Cutler et al. (2014). Briefly, bacterial 16S rRNA, eukaryotic 18S rRNA and fungal ITS flow files were trimmed and denoised with the mothur implementation of PyroNoise (Quince*, et al.*, 2009). Bacterial and eukaryotic rRNA sequences were aligned using the corresponding SILVA reference alignments (Quast*, et al.*, 2013) and only sequences spanning the targeted regions were kept. Data were denoised by clustering together sequences with 1 bp mismatch per 100 bp, and chimeras were removed using the mothur implementation of uchime (Edgar*, et al.*, 2011). Bacterial and eukaryotic rRNA sequences were classified against the SILVA reference databases using the Wang method (Wang*, et al.*, 2007), with a cutoff value of 60% for taxonomic assignment. Sequences were also clustered into operational taxonomic units (OTUs) at the 97% similarity level, which corresponds approximately to the species level. We screened the eukaryotic data and removed OTUs identified as Fungi, Embrophyta and Metazoa, as well as all unclassified Viridiplantae and Charophyta (i.e. only OTUs that could be confidently, if broadly, classified as algae remained).

For fungal ITS sequences, following denoising, the ITS1 region was extracted using the ITS Extractor tool on the PlutoF Workbench (Abarenkov*, et al.*, 2010, Nilsson*, et al.*, 2010) and sequences shorter than 100 bp were discarded. Chimeras were removed using the mothur implementations of uchime. OTU clustering was carried out from a distance matrix constructed in mothur using pairwise distance values. Sequences were classified against the UNITE+INSDC fungal ITS database (Abarenkov*, et al.*, 2010), modified as previously described (Cutler*, et al.*, 2014), with a cutoff value of 50% for taxonomic assignment.

All sequence data were uploaded with MIMARKS-compliant metadata to the NCBI Sequence Read Archive under Bioproject number PRJNA260418.

Alongside amplicon pyrosequencing, we also conducted a fresh analysis of TRFLP data associated with the same samples: refer to Cutler et al. (2012) for a full description of the original TRFLP protocols. In principle, amplicon pyrosequencing, or a similar, direct sequencing technique, could be used to analyse spatial structure in microbial communities. However, the large number of samples required for a fine-grained, statistically significant study meant that these techniques were considered too expensive when the analysis was initially performed.

2.3 Data Analysis

Because the TRFLP data were sample-specific, they could be used to investigate spatial variation on a scale of a few centimetres. Patchiness in the abundance of dominant taxa in each microorganism type was assessed by calculating dispersion indices. Morisita's Index, I_M, is a simple, global statistic that quantifies spatial patchiness. If the abundance of an OTU varies randomly in space, the observations follow a Poisson distribution; in this case I_M approximates unity. If abundance has a clumped (under-dispersed) pattern, $I_M > 1$. Conversely, in a regular (over-dispersed) arrangement, $I_M < 1$. The deviation from random expectation ($I_M = 1$) can be tested using critical values of the Chi-squared distribution with n-1 degrees of freedom. In this way, Morisita's index may be used to detect the presence of spatial structure.

The spatial structure of the epilithic community was also investigated with correlograms. Correlograms indicate the degree of spatial correlation between samples at different separation distances (Legendre & Legendre, 1998). In this case, the degree of correlation between samples at different locations was summarised by the Mantel statistic (Mantel, 1967). The Mantel statistic indicates the degree of correlation between two matrices, specifically, the correlation between a matrix of ecological dissimilarity and a matrix of

geographical distances (i.e. the physical separation between sample locations) derived from the same community. The significance of the Mantel statistic was estimated by means of permutation tests involving 999 randomisations of the ecological distance matrix. The Mantel correlograms were implemented using the *mantel.correlog* function in the *vegan* package (Oksanen*, et al.*, 2011).

Percent similarity (PS) was calculated from presence-absence data extracted from the TRFLP analysis and used to compare community composition across all the sampling locations for each microorganism type separately. Percent similarity is a metric based on similarities in community composition (Faith*, et al.*, 1987). In principle, PS figures provide an indicator of spatial heterogeneity in the microbial communities. High mean PS between samples implies a spatially homogeneous community (essentially the same taxa are observed in different locations). Conversely, low mean PS implies a relatively high degree of spatial variation in community composition, i.e. there are marked differences in community composition from location to location.

The overall taxonomic diversity for each microorganism type, expressed in terms of the Shannon index, H, and Simpson's index, J, was calculated from the pyrosequencing analysis (Hill*, et al.*, 2003). Community structure was investigated with rank-abundance plots which illustrated both taxonomic richness and evenness. Rank-abundance distributions (RADs) have been used to infer the mechanisms that underlie community diversity (Tokeshi, 1993, McGill*, et al.*, 2007). Microbial communities have been found to follow several different distributions. Curves describing distributions frequently reported in published literature (i.e. geometric, lognormal and power law models) were fitted to the pyrosequencing data using the *rad.fit* function in the *vegan* package (Oksanen*, et al.*, 2011).

3 Results

3.1 Spatial structure

Each microorganism type was dominated by just a handful of taxa: for example, the taxa illustrated in Fig. 1 accounted for 41% (algae), 68% (fungi) and 40% (bacteria) of total abundance in their respective categories. The abundance of the dominant OTUs varied across the surface of the slab (Fig. 1). Morisita indices were calculated for each of these OTUs (values given on Fig. 1). In each case, a highly significant degree of under-dispersion (clumping) was observed ($p < 0.001$), with the most marked clumping in the eukaryotic OTUs. At a community level, diversity varied across the surface of the slab in a similar

fashion (results not shown). Fungal and bacterial diversity were positively correlated: sites with high fungal diversity also tended to have high bacterial diversity (Spearman rank correlation: r = 0.4, p = 0.05). Algal diversity was uncorrelated with bacterial and fungal diversity (Spearman rank correlation: algal-fungal r = 0.3, p = 0.14; algal-bacterial r = -0.1, p = 0.69). The mean PS figure for the algal samples was very high (~90%). Bacterial communities exhibited intermediate levels of PS (64%). The figure for fungal communities was lower (50%).

Fig. 1

The Mantel correlograms based on the TRFLP analysis indicated significant spatial structure in algal and fungal samples ($p < 0.05$) (Fig. 2). Positive correlation was observed at the smallest spatial scale (< 8 cm) in these samples (Fig. 2a, b). No significant spatial structure was apparent in the bacterial communities (Fig. 2c).

Fig. 2

3.2 Community composition

A total of 74 algal OTUs, 244 fungal OTUs and 486 bacterial OTUs were identified by amplicon pyrosequencing (Table 1). The proportion of singletons was high in each case (34% of algal reads, 60% of fungal reads and 54% of bacterial reads).

In total, 2648 reads were classified as algae (Table 2). The sequences were divided between two phyla, the Chlorophyta and the Charophyta. The majority of sequences were associated with the Chlorophyta; sequences from the class Trebouxiophyceae were particularly abundant, accounting for 93.5% of the Chlorophyte reads. Most of these sequences belonged to the Prasiolales. In the Charophyta, the Klebsormidiophyceae were particularly abundant.

A total of 2626 fungal sequences were analysed. Two fungal phyla, the Ascomycota and Basidiomycota, accounted for 82.1% of these sequences, with each phylum having approximately equal representation (43.9% Ascomycetes, 38.2% Basidiomycetes). Overall, 17.9% of fungal reads were unclassified at a phylum level. The sequences from the Ascomycota were overwhelmingly from the subphylum Pezizomycota; the majority of these reads (811 out of a total of 1147) were unclassified below this level. Of the Ascomycete reads classified at a higher level of resolution, sequences associated with the family Capnodiales (class Dothideomycetes) were prominent (e.g. the diametiaceous fungi

Cladosporium sp. and *Batcheloromyces* sp.) In the Basidiomycota, most reads were associated with the Agaricomycotina (915 reads out of a total of 999 Basidiomycetes). The Agaricomycetes accounted for the bulk of these sequences. Lichenised fungi were not detected.

There were 2401 bacterial reads of which 98.5% were resolved to at least phylum level. Eleven bacterial phyla were represented, however, only three phyla, the Acidobacteria, Actinobacteria and Proteobacteria, accounted for >5% of the total (Table 2). Almost all the Actinobacterial reads were associated with the order Actinomycetales; the sub-orders Frankineae and Pseudonocardinae were notably abundant. Proteobacterial reads were dominated by the Alphaproteobacteria, with the Rhizobiales and Sphingomonadales prominent. However, levels of taxonomic resolution were generally low and most taxa were rare.

Table 1

Table 2

The RADs derived from the pyrosequencing analysis were characterised by high levels of dominance, a distinctive 'hollow' (concave up) profile and long tails of rare species (Fig. 3). Omitting singletons from the rank-abundance plots did not fundamentally change this shape, which closely approximated a zipf (power law) distribution.

Fig. 3

4 Discussion

4.1 Spatial variability

The Baas-Becking hypothesis, often summarised as "everything is everywhere - the environment selects" has been a remarkably persistent concept in microbial biogeography (Martiny*, et al.*, 2006). However, the hypothesis does not explicitly address issues of spatial scale and the factors driving the distribution of microorganisms at intermediate scales (from a few centimetres to a few metres) are largely unknown. If the 'everything is everywhere' model applied to our sample slab, then a spatially random distribution of microbes might be expected, given the apparent environmental homogeneity of this surface (in terms of surface

texture, meteorological conditions, etc.) However, this was not the case for eukaryotic microbes.

The mean PS figure for the algae was high, indicating a spatially homogeneous community (at least in terms of species composition): the same taxa frequently co-occurred. Despite this, spatial patchiness over short distances (<8 cm, or approximately equal to the spacing between adjacent sampling locations) was observed. In other words, at this scale each sampling location was positively correlated with adjacent sampling locations, on average. In general terms, spatial pattern in ecological communities can arise due to environmental patchiness (e.g. resource availability or disturbance), contagious ecological processes (e.g. dispersal, reproduction) or a combination of both (Fortin & Dale, 2005) In this case, the observed spatial patchiness may have been related to the contagious spread of an abundant species. In the pyrosequencing analysis, the most abundant OTU was associated with a *Klebsormidium* species, a filamentous alga which could structure the algal community by contagious spread. However, it is more likely that the spatial structure observed represents differences in OTU abundance (i.e. patch intensity), rather than the presence/absence of particular taxa, because community structure was similar across the sampling locations. In other words, what we observed was a compositionally homogeneous community that varied in relative abundance of different species. The contour plot of the dominant algal OTUs (Fig. 1), which features pronounced hotspots of abundance, and the I_M values for the same taxa supported this view. Rindi and Guiry (2003) and Cutler et al. (2013) noted that green algal communities can exhibit small-scale spatial heterogeneity. Our results are consistent with these observations. The presence of green algal patches on stone surfaces has been linked to geochemical and geophysical changes in stone (Cutler*, et al.*, 2013), so understanding characteristic scales of variation may assist in understanding patterns of biodeterioration/bioprotection.

The fungal community had a low mean PS figure. This indicated considerable differences in community composition between sampling locations. The distribution of the most abundant taxa was also highly patchy (Fig. 1). Spatial variation in fungal communities has been described previously (Cutler*, et al.*, 2013) and, as with algae, may be a function of environmental or ecological variation (or both). Like the algae, the fungi exhibited short-range spatial autocorrelation (Fig. 2b). When viewed in the context of low mean PS, this indicates sharp changes in community composition and species abundance across short spatial distances. Spatial pattern in the fungal samples could be due to the short-range development of patchy surface molds, although the low level of taxonomic resolution in many OTUs made it impossible to be definitive. As with algae, fungal activity has been closely linked with stone

biodeterioration, so it is likely that the patchy structure of abundant fungal OTUs is closely correlated with patchy discoloration and/or surface degradation.

The bacterial community had a moderate mean PS value as there was a high level of co-occurrence amongst the dominant OTUs. The I_M values calculated for these OTUs suggested spatial clumping. However, the associated contour plots indicated that the patches were larger and less intense than those observed for the eukaryotes (Fig. 1). The Mantel correlogram, which was based on the whole bacterial community, did not indicate significant spatial structure at the scale of measurement (Fig. 2c). It is possible that spatial structure was present at a scale smaller than the sampling unit. Whereas filamentary microorganisms (e.g. mycelium-forming fungi and certain algal species) may spread widely, the spatial range of assemblages of isolated bacterial cells may be limited. The absence of pattern in the bacterial community contrasted with the algal and fungal communities and suggested homogeneity in species composition and patch intensity. Interestingly, diversity in the heterotrophic fungal and bacterial communities was positively correlated, suggesting diversity 'hotspots' across the surface of the slab. Further study would be required to establish the factors that promote diversity in the heterotrophic community.

This study only concentrated on the two-dimensional arrangement of microbes in a thin biofilm. Whilst epilithic microbes have an extremely important impact on stone conservation (not least because they can cause surface discolouration), endolithic microbes also play a significant role in stone biodeterioration. Future studies could seek to establish the three-dimensional structure of lithobiontic communities, perhaps by analysing thin, stone sections from different depths at each sampling location.

4.2 Community composition and structure

In taxonomic terms, the microbial community was broadly similar to those previously reported for other stone substrates in temperate climates (e.g. John, 1988, Flores*, et al.*, 1997, Burford*, et al.*, 2003, Rindi & Guiry, 2004). The distinctive hollow shape of the RADs derived from the pyrosequencing data was also familiar from previous studies of microbial communities in different settings (e.g., Gans*, et al.*, 2005, Pommier*, et al.*, 2010, Inceoglu*, et al.*, 2011). Therefore, the high-resolution molecular study, whilst it provided additional detail on community composition and diversity, was largely consistent with previous models of microbial community structure.

4.2.1 Taxonomic composition

A review of literature on epilithic algal communities in Western Europe suggests a relatively small pool of widely dispersed, cosmopolitan species (Cutler*, et al.*, 2013). Our results are consistent with this scenario. In common with previous studies, green algae from the Chlorophyta (primarily of the order Trebouxiophyceae) were dominant. Microscopic Charophyta, notably from the order Klebsormidiophyceae, were also abundant. *Klebsormidium* spp. have been routinely reported on stone surfaces in humid habitats (e.g. Ortega-Calvo*, et al.*, 1993, Rindi & Guiry, 2003).

Several previous studies of lithobiontic fungal communities have indicated dominance by the Ascomycetes (e.g. Gleeson*, et al.*, 2010). This was not the case in our study: the representation of Ascomycetes and Basidiomycetes was only slightly in favour of the former. The relatively high proportion of Basidiomycetes and non-lithobiontic Ascomycetes may indicate a high level of allochthonous material (e.g. spores) across the surface of the slab (this material might be preferentially sampled when adhesive tape is used). Lichenized fungi were unexpectedly absent. Lichens are often found in lithic habitats and poorly developed/degraded (and unidentifiable) lichen thalli were present on the surface of the slab. However, these areas were not directly sampled and it may be that the tape sampling method we used is poorly suited to collecting material from firmly-adhered lichen thalli. In contrast, ubiquitous, diametiaceous hyphomycetes were relatively abundant. Taxa such as *Cladosporium* spp. are frequently observed in lithobiontic habitats (Burford*, et al.*, 2003); *Batcheloromyces* sp. is reported less commonly.

McNamara and Mitchell (2005) observed that bacterial communities on stone are typically dominated by taxa drawn from five phyla, i.e. the Proteobacteria, Actinobacteria, Bacteroidetes, Acidobacteria and low-GC Firmicutes group. All these phyla were present on the slab, although only Actinobacteria and Proteobacteria were abundant. Bacterial communities on stone are often closely related to soil communities and this was the case in our study. Several authors have commented that Actinobacteria are particularly abundant in temperate biofilms and this observation was consistent with our results (Scheerer*, et al.*, 2009). The Proteobacterial taxa found on the slab are cosmopolitan and have been reported in a wide range of habitats, so it is difficult to infer much from their presence.

4.2.2 Community structure

As expected, the bacteria were the most diverse group, followed by the fungi. Algal diversity was relatively low. In both the bacteria and the fungi, the diversity metrics generated from the pyrosequencing study were much higher than the equivalent figures derived from TRFLP data (by a factor of two: Cutler*, et al.*, 2012). This was unsurprising, as DNA fingerprinting

techniques such as TRFLP are known to underestimate microbial community richness (Lalande*, et al.*, 2013). Interestingly, Shannon and Simpson diversity for the algae were remarkably similar for both the pyrosquencing and TRFLP data. It appeared that in the case of algae, rare species (undetectable by TRFLP) did not contribute greatly to overall diversity. This suggests that algal community structure may be adequately captured by TRFLP in certain circumstances.

A number of previous studies have suggested generic models of microbial RADs, including geometric, lognormal and power law models. Jackson et al. (2001), for example, reported that a geometric relationship best described early successional bacterial communities in an aquatic biofilm. However, it is likely that simple geometric models only apply where the members of the community are all competing for the same niche (Dunbar*, et al.*, 2002) and this model was a poor fit for our data. Dunbar et al. (2002) proposed that the lognormal distribution is better suited to functionally and phylogenetically diverse assemblages and proposed the use of this distribution as a null model for microbial communities. Lognormal RADs can arise from the multiplicative effects of biotic and abiotic factors and this distribution does not necessarily depend on specific biological/ecological mechanisms. Lognormal distributions have been reported in several bacterial communities (Dunbar*, et al.*, 2002, Doroghazi & Buckley, 2008) but did not capture the essential characteristics of the lithobiontic RADs. Our results are most consistent with the power-law distributions that have been observed in a range of microbial communities from different settings (Gans*, et al.*, 2005, Pommier*, et al.*, 2010, Inceoglu*, et al.*, 2011).

5 Conclusions

Lithobiontic communities, especially those dominated by subaerial green algae, have been characterised as low-diversity assemblages (John, 1988). However, our study demonstrated that microbial communities on building stone can be heterogeneous, both in terms of spatial distribution and taxonomic composition. Different components of the microbial community exhibited different spatial patterns. If the results of our study apply more widely, lithobiontic eukaryotes should exhibit spatial structure over intermediate (centimetre) spatial scales, as well as the large- (metre-) scale patchiness often found to be associated with varying aspect and exposure. Spatial structure in lithobiontic bacterial communities, if it exists, is likely to be at a smaller scale than our sampling interval. DNA fingerprinting techniques, despite their inability to detect rare taxa, may be adequate for profiling green algae in these settings. These findings have implications for understanding spatial heterogeneity in the biodeterioration of stone as the observed patchiness of fungal and algal varieties is likely to

be correlated with centimetre-scale variation in stone degradation and soiling. Further study is required to elucidate the ecological relationships between the species that comprise these communities and the factors that generate spatial patchiness in eukaryotes, but not prokaryotic microbes.

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Figure captions

Fig. 1: Contour plots indicating the varying abundance (measured in fluorescence units) of dominant OTUs for each microorganism type. I_M = Morisita index; in each case the value is significantly > 1 ($p < 0.001$), indicating a patchy distribution. The white dots indicate sampling locations.

Fig. 2: Mantel correlograms for each microorganism type; filled circles indicate significant ($p < 0.05$) correlation in a given distance class.

Fig. 3: Rank-abundance plots for algal, fungal and bacterial OTUs (each OTU is represented by a point), based on amplicon pyrosequencing. Singletons have been omitted. The taxon ranked 1 is the most abundant in each case. The plots have been fitted with a zipf (power law) model, indicated by a red line.

Tables

	No. OTUs	Shannon's diversity, H	Simpson's diversity, J
Algae	74	2.3	0.81
Fungi	244	3.0	0.83
Bacteria	486	4.2	0.89

Table 1: Diversity metrics derived from amplicon pyrosequencing

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Algae		No. reads	Proportion of reads (%)
Chlorophyta			
Trebouxiophyceae		1454	54.9
Others		101	3.8
Charophyta			
Klebsormidiophyceae		1087	41.0
Others		6	0.2
	Totals	2648	100.0
Fungi			
Ascomycota			
Dothideomycetes[a]		314	12.0
Others[b]		840	32.0
Basidiomycota			
Agaricomycetes		965	36.7
Others		39	1.5
Unclassified at phylum level		468	17.8
	Totals	2626	100.0
Bacteria			
Acidobacteria			
Acidobacteria		127	5.3
Others		5	0.2
Actinobacteria			
Actinobacteridae		440	18.3
Others		55	2.3
Proteobacteria			
Alphaproteobacteria		1579	65.8
Others		88	3.7
Other bacterial phyla		107	4.5
	Totals	2401	100.0

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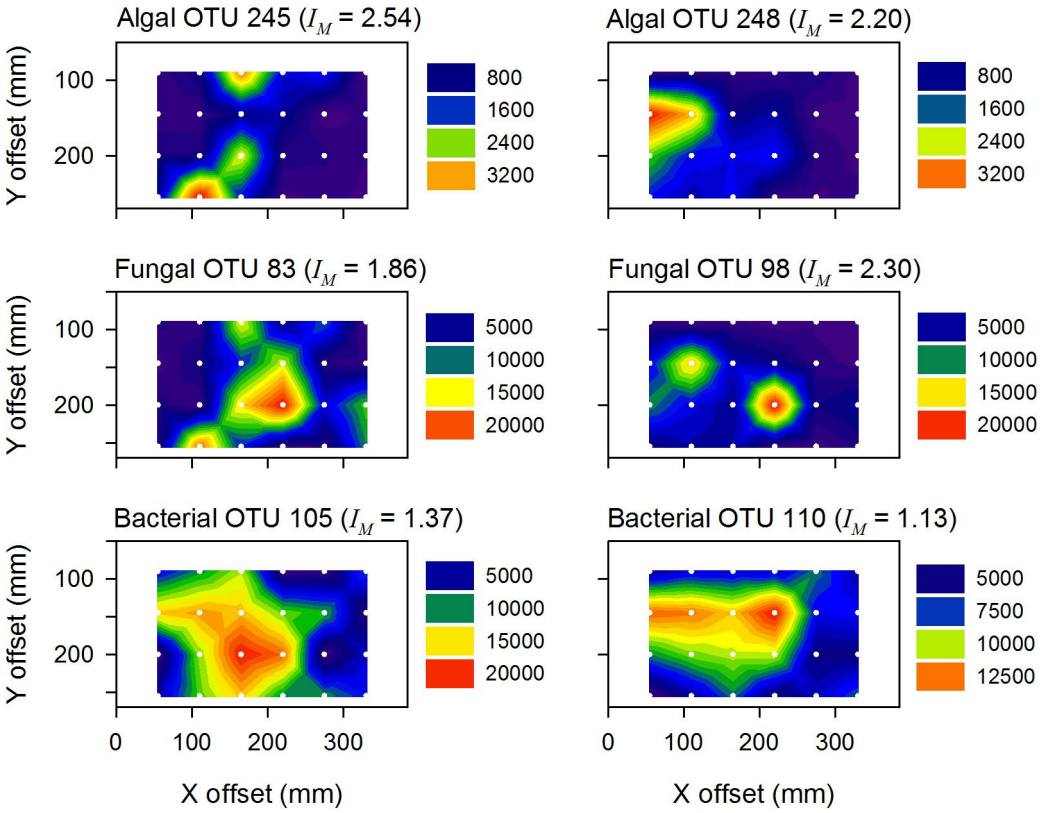
610 Table 2: Summary of pyrosequencing results; phyla are indicated with bold text

611

612 **Figures**

613



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616 Fig. 1

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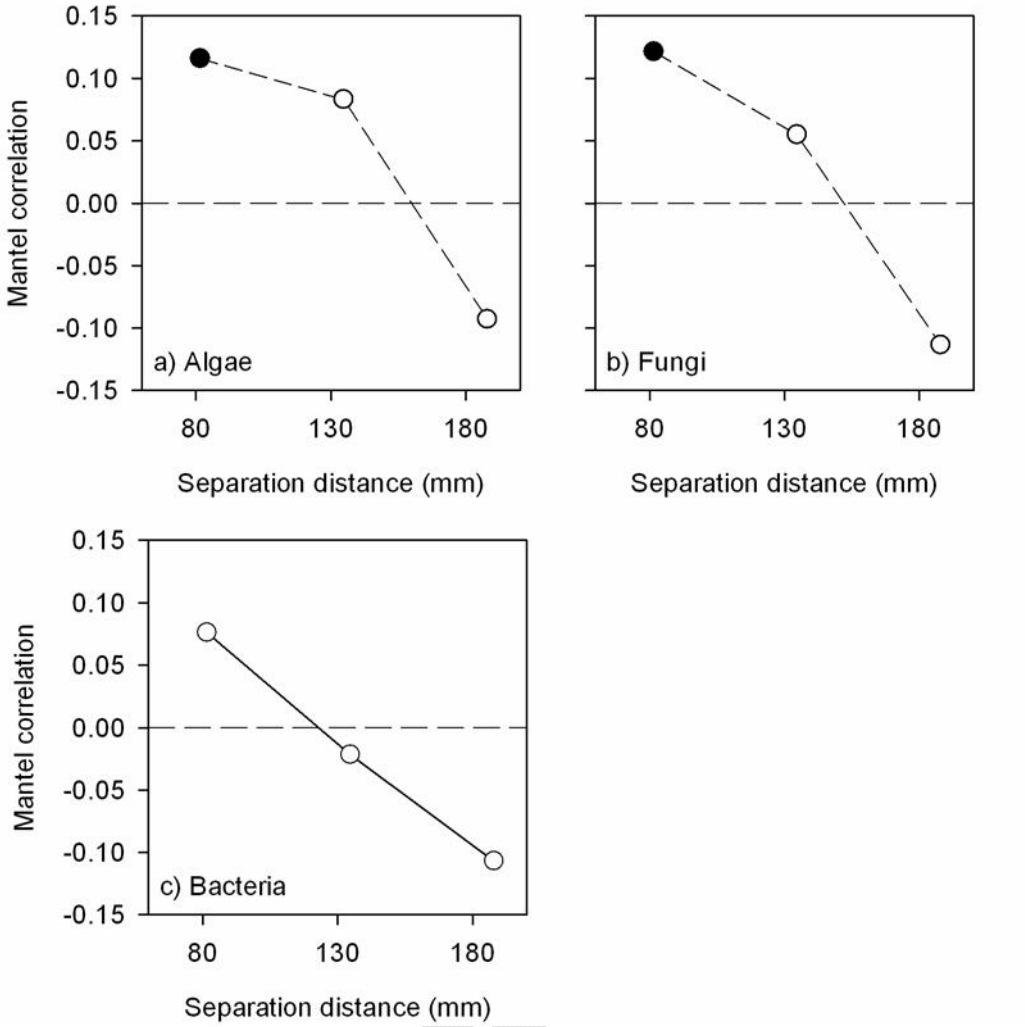

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620 Fig. 2
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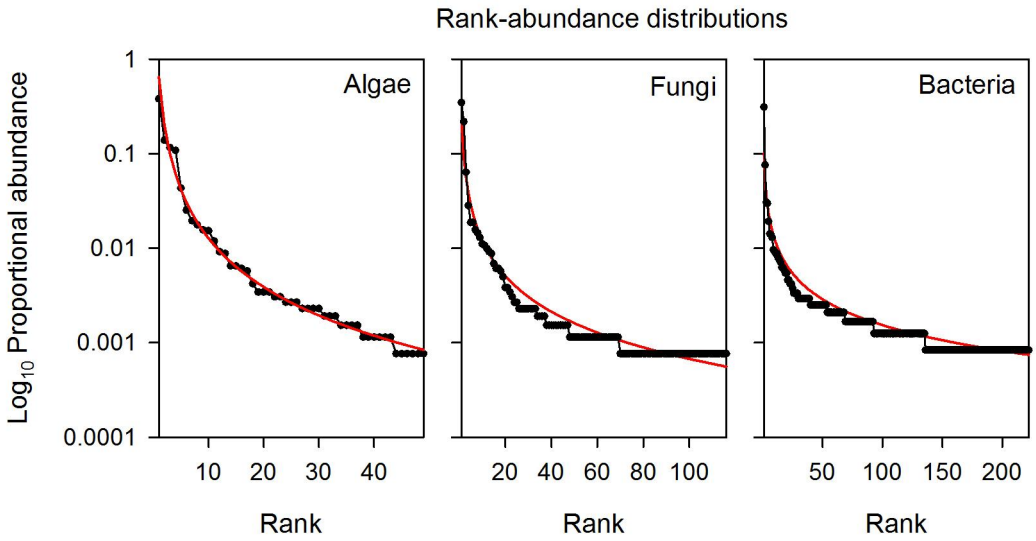

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624 Fig. 3