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THEMATIC ISSUE

Algal and fungal diversity on various dimension stone substrata in the Saale/Unstrut region

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Abstract

Physical, chemical and biogenic weathering considerably threatens all historic stone monuments. Microorganisms, though inconspicuous, are key players of stone surface colonization and penetration. This study highlights eukaryotic microbial communities on dimension stone surfaces from two representative monuments of the "cultural landscape corridor" in the Saale–Unstrut area. The historical buildings were erected from local Triassic limestone and sandstone and are prone to various deteriorative mechanisms. Generally, trebouxiophyceaen algae and ascomycete fungi dominate among the latter dematiaceous fungi and lichen fungi are abundant. Inside the stone substratum, ascomycetes, mosses and even large soil organisms (tardigrades) are present. This may be taken as a hint for the formation of pores with large radii, which are "risk indicators" for progressive weathering and degradation of the rock matrix.

Keywords Endoliths · Biogenic weathering · Dematiaceous fungi · Terrestrial algae

Introduction

The Saale–Unstrut area is located in the Burgenlandkreis (a district in South Saxony-Anhalt, Germany). The high density of edifices (churches, cathedrals, monasteries, historic industrial buildings and others) in the river valleys of the Unstrut and Saale creates the appearance of a closed "cultural landscape corridor" (cf. Hoppert et al. 2018). Most historic stone monuments were constructed by locally available Triassic sandstones ("Buntsandstein") and limestone ("Muschelkalk"). Both types are highly variable with respect to, e.g., grain size, cementation, porosity and hence general weathering susceptibility (Stück et al. 2013). Physical/chemical and biological weathering phenomena attack the stone surface simultaneously and depend on each other. Slightly weathered dimension stone, in particular, is susceptible to colonization by endolithic organisms like unicellular eukaryotic algae, bacteria, mosses and lichens (Gaylarde et al. 2003). Typical initial effects of microbiological colonization are discolorations of building stones, which mainly affect the appearance of architectural decoration (Gorbushina et al. 1993; Hallmann et al. 2011a, b). Progression of weathering leads to degradation of the building stone surface in various ways, which makes it difficult to attribute advanced decay phenomena to a single initial event such as, e.g., salt splitting or microbial growth (e.g., Stück et al. 2013; Hallmann et al. 2013a, b).

Generally, microbial endoliths penetrate stone just some µm up to few mm. However, depending on the pore size, stones may be colonized several centimeters below the surface in particular by (lichen) fungi and mosses (Hallmann et al. 2014a). Microorganisms and cryptogams benefit from the microhabitat inside the stone. Adverse environmental conditions, like high radiation, rapid desiccation, effect of extreme temperatures or

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grazing are reduced in this ecological niche (Griffin et al. 1991; Hoppert et al. 2004; Gorbushina 2007).

Algae, dematiaceous fungi (cf. Hallmann et al. 2011a, 2013b), and lichens (cf. González-Gómez et al. 2018) destain and deteriorate surfaces in advanced stages of colonization. Assessment of initial microbial colonization by molecular methods may be helpful to decide on further conservation measures.

The aim of this study is to give an inventory of stone associated (micro-)organisms on sandstone and limestone which may help to give, along with other physical methods of assessment of stone decay (Stück et al. 2013, 2018), a state-of-the-art report on monument degradation.

Materials and methods

Sampling site and preparation of samples

Samples from limestone lithologies (Muschelkalk, "Schaumkalk") originate from Saaleck Castle/Bad Kösen (Saaleck rampart: 51.109466 N, 11.701846 E, Saaleck wall base: 51.109526N, 11.701608E; cf. Fig. 1a, b). Samples of Buntsandstein ("Hardegsen-formation") were taken in early June 2012 from the location Blütengrund near Großjena/Naumburg (Saale) (51.183035N, 11.788119 E and 51.177971N, 11.793808E; Fig. 1c). Another sampling site close to this location (51.178110N, 11.793193E), the "Stone Album" ("Steinernes Bilderbuch"), consists of sandstone from the same formation (Fig. 1d).

Generally, sampling sites were allocated according to accessibility, compatibility of sampling spots with respect

Fig. 1 Sampling sites in the Saale–Unstrut area. **a** Rampart of Saaleck Castle. **b** Section of the wall base of the Saaleck Castle with outcropping rock (Schaumkalk). **c** Outcropping rock (Buntsandstein, Hardegsen-formation, Großjena). **d** Outcropping rock, "Stone Album" near Großjena (photograph provided by H. Stück); white dots in **b, c** mark selected sampling points

to monument preservation regulations and apparent signs of microbial colonization (discoloration, signs of biogenic weathering). In total, 60 sampling spots were selected. A sampling spot is defined as a small area of approximately 4 cm^2. In this area, samples were scratched from the surface with a sterile scalpel and were immediately transferred to sterile plastic containers. Among these 60 original samples, 23 could be further processed (19 environmental samples, 4 cultures, cf. Tables 1, 2, 3). Seven samples were taken from the location Blütengrund/Großjena (Buntsandstein, Hardegesen-Folge). The rock face is exactly W-exposed. The rock surface showed common signs of backweathering (cf. Fig. 1c; some sampling spots are marked by white dots), and spots were selected in a way that different microtopographies (resulting from backweathering) were included, but spots with apparent accumulation of soil, mosses and lichens (horizontal surfaces and clefts) were excluded. For surface samples, care was taken that no contamination from below the surface was collected (for sampling of depth profiles see below). Another two samples were taken from the site "Stone Album", from a vertical rock face in SSW exposition. Here, sampling was restricted due to monument protection regulations.

Saaleck was sampled on two exactly S-exposed and W-exposed vertical rock faces (cf. Fig. 1a, b). However, microtopography and hence exposition were again fluctuating according to irregularities of the natural rock face or the roughly trimmed dimension stone. In total, six sampling spots were selected from those sampling sites

Table 1 Algal phylotypes retrieved from all sampling spots on limestone and sandstone as well as from crude cultures

	Closest relative; Acc. No.	% sequence similarity	1	1a	2	3	4	4a	5	6	7	8	9	10	11	12	12a	13	13a
			limestone: Saaleck wall base				Saaleck rampart				Stone Album		Großjena						
Trebouxiophyceae	Phyllosiphon arisari PY9a1; JF304471	98-99	11	1	1	2			4	4			5	1					7
	Trebouxia aggregata SAG 219-1d; EU123942	99									3	12		4					
	Uncultured Trebouxiophyceae clone QE59 (Trebouxia); FJ790667	99	1		2		1		5	3			2						
	Edaphochlorella mirabilis Andreyeva 748-I; X74000	99		7															
	Pseudochloris wilhelmii C-1.1.9; X56102	99																	4
	Pseudostichococcus monallantoides SAG 380-1; JX185690	99																3	
	Elliptochloris sp. SAG 2117; FJ648515	99											1			1			
	Muriella terrestris ASIB V38; AB012845	99						2											
	Chlorella vulgaris NIES-227/SAG 211-11b; AB162910/FM205832	99																	1
	Chloroidium angustoellipsoideum SAG 2115; FM946019/ Chlorella reisiglii CCAP 11/8; FR865615	98					1												
	Desmococcus olivaceus SAG 1.92; EU434017	98												1					
	Diplosphaera (Stichococcus clone FGSsan_K35); JX391005	97-99									1								
	Neocystis brevis CCALA 393; JQ920362	99						1											
	Neocystis mucosa SAG 40.88; JQ920367	99						1											
	Stichococcus deasonii UTEX 1706; DQ275460	99											1						
	Xylochloris sp. SAG 2382; JQ988942	99						1											
Chlorophyceae	Uncultured Bracteacoccus clone; JX127180/Bracteacoccus sp. 668; U63103	99						5											1
	Chlamydomonas monadina ACKU 274-03; FR854377	99		4															
	Bracteacoccus sp. UT8-26; AF513376	99						2											
	Bracteacoccus sp. CNP1VF2; AF513378	95																	1
	Bracteacoccus terrestris CCAP 221/4; FR865690/ Pseudomuriella schumacherensis SAG 2137/ HQ292768	99																	1
	Scenedesmus sp. KGU-Y002; AB742453	99																	1
others	Klebsormidium sp. SAG 2155 (Charophyta/Klebsormidiaceae); EF372518	99-100											2	12					
	Trentepohlia aurea Handa-840 (Ulvophyceae); AB110783	98	1			2				1									
	Interfilum sp. SAG 2100 (Ulvophyceae); EU434033	99						1											

Samples from spots 1, 4, 12 and 13 were used for inoculation of cultures (arrows). Columns 1a, 4a, 12a and 13a show phylotypes retrieved from these cultures. Figures in blue and orange coloured columns indicate the numbers of sequences, assigned to a phylotype

Table 2 Phylotypes of protozoa, lichens and fungi, retrieved from all sampling spots on limestone and sandstone as well as from crude cultures

	Closest relative; Acc. No.	% sequence similarity	Saaleck wall base (limestone)			Saaleck rampart (limestone)				Stone Album (sandstone)		Großjena (sandstone)						
			1	1a	2	3	4	4a	5	6	7	8	9	10	11	12	12a	13	13a
Fungi	Knufia perforans CBS 885.95; JN040506/ Knufia endospora UAMH 10396; JN040509	99									2	5			19				
	Rhinocladiella sp. MA 4765; AJ972862	98-99							2		1	5	4	5	5	6			
	Penicillium solitum 20-01; JN642222	99					23												
	Arachnomyces kanei UAMH 5908; AF525308	99														9			
	Capnodiales sp. CCFEE 5205; GU250327	99			1						1		2	2	2				
	Glyphium elatum (now: Knufia sp.) CBS 268.34; AF346419/Coniosporium sp. CBS 665.80; Y11712	97-100	1				4		1	2									
	Pseudocoleophoma polygonicola KT 731; AB797256	98													5				
	Cladosporium bruhnei USN 11; JN397376	99			1		1				1		1						
	Uncultured Agaricomycotina clone; EU647051	99									1	2			1				
	Cordyceps brongniartii 546; AY282746	98-99				1				1									
	Cryomyces sp. CCFEE 5476; GU250352	97									2								
	Phaeosphaeria nodorum sn37-1; EU189213	99							2										
	Zygomycete sp. AM-2008a; EU428773	99																2	
	Passalora sp. CPC 12319; GU214668	99											1						
	Cheiromoniliophora elegans CBS 688.93; DQ018084	97									1								
	Cryptococcus carnescens CBS 973; AB085798	99			1														
	Dioszegia zsoltii like AS 2.2091; AF385444	96										1							
	Dothideales sp. like LM482; EF060783	91									1								
	Fibulobasidium murrhardtense CBS9109; GU327540	99					1												
	Lecophagus sp. ATCC 56071; AY635836	97																	1
	Paraphaeosphaeria sp. B19; GQ253350	98									1								
	Phoma sp. CPCC 480669; FJ515319	99									1								
	Pleospora herbarum ATCC 11681; U43458	99					1												
	Rhytidhysteron rufulum EB 0384; GU397368	97				1													
	Uncultured ascomycete clone; EU409872	99									1								
Lichens	Xanthoria elegans; AF088254	97-99	8		19	5	7		10	18	7								
	Caloplaca demissa; AF515609	97-99									6	21	2						
	Texosporium sancti-jacobi; U86696	97-98	1			2	2												
	Caloplaca holocarpa; AJ535281/Caloplaca verruculifera; AJ535284	97-98									2	2							
	Xanthomendoza hasseana 906 (110.8); AM494985	94									1								
Protozoa	Peritrichia sp. TS-2009a; GQ872428	97															24		
	Desmarella moniliformis; AF084231	96		6															
	Hartmannella vermiformis; AY680840	99		1													3		
	Adriamonas peritocrescens; AF243501	94		2															
	Uncultured apicomplexan clone; FJ410610	98		1															
	Thaumatomastigidae environm. clone; EF023480	95		1															
	Uncultured eukaryote clone (Amoeba sp.); AB505484	83															1		
Moss	Pottia truncata (Bryopsida); X95935	97-99	2															3	8
Metazoa	Oribatula tibialis/Phauloppia lucorum (Arthropoda); EU433990	97											10						

Samples from spots 1, 4, 12 and 13 were used for inoculation of cultures (arrows). Columns 1a, 4a, 12a and 13a show phylotypes retrieved from these cultures. Figures in blue and orange coloured columns indicate the numbers of sequences, assigned to a phylotype

(three spots from S-exposed wall base, three spots from W-exposed rampart).

For analysis of depth profiles, one sampling spot from Großjena and from Saaleck was selected, respectively. Both spots exhibited microtopographies with W- and S-exposed surfaces. Within a distance of 10 cm, both W- and S-exposed surfaces were sampled separately as described above. Then, the whole rocks were removed from the site and cracked with a chisel under sterile laboratory conditions within several hours after sampling. Samples were then taken, as

Table 3 Algal, fungal and other phylotypes retrieved from surface samples and from depths of 0.5 cm beneath the surface as indicated (W = west-exposed; S = south-exposed)

			Saaleck wall base limestone			Großjena sandstone		
			surface		0.5 cm	surface		0.5 cm
	Closest relative; Acc. No.	% sequence similarity	W 14	S 15	16	W 17	S 18	19
Algae	Trebouxia aggregata SAG 219-1d; EU123942	99	2	9			1	
	Diplosphaera (Stichococcus clone FGSsan_K35); JX391005	97-99	2	6				1
	Bracteacoccus bullatus CCALA 694; JQ259933	99				2		
	Chlorella reisiglii CCAP 11/8; FR865615/ Chloroidium angustoellipsoideum SAG 2115; FM946019	98-99	1				1	
	Chloroidium ellipsoideum SAG 3.95; FM946012	99-100	3	1				
	Chloroidium saccharophilum SAG 211-9a; FM946000	99		1			1	
	Dictyochloropsis splendida like UTEX 2612; GU017660	94	2					
	Elliptochloris sp. SAG 2117; FJ648515	99-100					2	
	Graesiella emersonii CCAP 211/55; FR865674	98	1				1	
	Pseudomuriella schumacherensis SAG 2137; HQ292768	99				2		
	Stichococcus deasonii UTEX 1706; DQ275460	99				1	1	
	Xylochloris sp. SAG 2382; JQ988942	99					2	
	Bracteacoccus terrestris CCAP 221/4; FR865690	99				1		
	Dictyochloropsis splendida UTEX 2612; GU017660	99	1					
	Klebsormidium sp. BCP-CNP2-VF35; JN795137	97				1		
	Leptochlorella corticola I2e; HE984579	98				1		
	Leptosira erumpens UTEX 979; Z68696	99	1					
	Myrmecia astigmatica IB T76; Z47208	99				1		
	Neocystis brevis CCALA 393; JQ920362	96						1
Mosses	Bryum capillare (now: Rosulabryum); AF205945	98-99				4	2	1
	Pottia truncata (Bryopsida); X95935	98-99		1		4	1	1
Meta-zoa	Halobiotus crispae Hc-Vellerup-1 (Tardigrada); EF620402	99						7
	Macrobiotus sapiens (Tardigrada); DQ839601	97			3			
Fungi	Uncultured Ascomycota clone; JN020194	98-100	1	7	1		6	1
	Rhinocladiella sp. MA 4765; AJ972862	97-99					6	1
	Caloplaca holocarpa; AJ535281/Caloplaca verruculifera; AJ535284	97-99	4	1				1
	Passalora sp. CPC 12319; GU214668	98			3			
	Aureobasidium pullulans like ZH1; JX303663	91		1				
	Capnodiales sp. CCFEE 5502; GU250357	100						1
	Chaetothyriales sp. CR07/2-1; FJ538966	99					1	
	Cladosporium bruhnei USN 11; JN397376	99		1				
	Clavariopsis aquatica WD(A)-00-1; FJ804122	99			1			
	Coniosporium apollinis CBS 352.98; JN040508	99	1					
	Cryptococcus carnescens CBS 973; AB085798	99	1					
	Elsinoe veneta AFTOL-ID 1360; DQ678007	98			1			
	Guignardia sp. IFB-GLP-4; GU380271	93			1			
	Herpotrichiellaceae sp. LM124; EF060490	83					1	
	Leptosphaeria maculans FI-IIII-FF2; JX967532	97					1	
	Leptosphaeria maculans like Unity; U04238	96	1					
	Paraphaeosphaeria sp. E5-3C; AB665311	99	1					
	Paraphaeosphaeria sp. B19; GQ253350	99			1			
	Penicillium solitum 20-01; JN642222	99			1			
	Phoma sp. DS1wsM30b; HM216186	98	1					
	Rachicladosporium paucitum CCFEE 5458; GU250347	97	1					
	Septoria dysentericae CPC 12328; GU214699	99		1				
	Uncultured ascomycete clone; AB074660	99	1					
	Uncultured Chytridiomycota clone; GQ995306	89	1					

Figures in blue and orange coloured columns indicate the number of sequences assigned to a phylotype

described above, from a spot below the surface as described in Hallmann et al. (2014a).

For preparation of crude cultures, aliquots of four selected samples (two from each sampling site, either Saaleck or Großjena) were suspended in 20 ml 3N BBM+V medium and Z Medium in 100-ml Erlenmeyer flasks (Starr and Zeikus 1993; Watanabe and Nozaki 1994), respectively. After inoculation, cultures were incubated at constant temperature of 18 °C. White fluorescent illumination with an intensity of 25 µmol photons m^{-2} s^{-1} was applied for four weeks, while the light:dark cycle was set to 14:10 h.

DNA extraction

Extraction of genomic DNA from stone samples was performed using the DNeasy PowerSoil DNA isolation kit (Qiagen, Hilden, Germany) according to the manufacturer's instruction. Successful DNA extraction (see below) required up to 250 mg of crushed samples. Samples were further processed as described in Hallmann et al. (2014a).

For preparation of crude cultures, an aliquot of 0.5 ml (0.25 ml of cultures from a sampling spot in BBM + V medium, and Z medium, respectively) was transferred to 2 ml beat-beating tubes and mixed with equivalent amounts of acid-washed glass beads (120–200 µm and 425–600 µm in diameter; Sigma-Aldrich, ST. Louis, MO, USA). These tubes were treated for 30 s at 5000 rpm in a Minibeadbeater (Biospec, Barlesville, OK, USA). DNA was extracted using the Invisorb® Spin Plant Mini Kit (Stratec Molecular, Berlin, Germany), following the manufacturer's instructions. Sampling on a 1% (w/v) agarose gel confirmed successful DNA extraction. Isolated DNA was stored at −20 °C until further processing.

Polymerase chain reaction (PCR) amplification

PCR amplification was performed for isolated biofilm DNA using eukaryote-specific primer combinations for 18S rRNA gene, 20F (5′ GTAGTCATATGCTTGTCTC 3′; Thüs et al. 2011) and 18L (5′ CACCTACGGAAACCTTGTTACGAC TT 3′; Hamby et al. 1988). Templates comprised approximately 10–100 ng of DNA. Amplification reaction mixture (25 µl) contained each dNTP at a concentration of 0.1 mM, 5 µl of 10× reaction buffer, 2 mM $MgCl_2$, each primer at a concentration of 0.2 µM, 2 U of Taq DNA polymerase (Bioline, Luckenwalde, Germany) and 4% (v/v) dimethyl sulfoxide (DMSO)-solution. PCR was performed in a thermocycler TProfessional Basic (Biometra, Göttingen, Germany) using the following program for the primer set 20F/18L: initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 50 °C for 1 min, extension at 72 °C for 3 min and final extension at 72 °C for 10 min. The PCR products were purified using the InvisorbR Spin PCRapid Kit (Stratec molecular). Aliquots of 2 µl of purified amplicons were analyzed by electrophoresis on a 1% (w/v) agarose gel to check for amplification.

18S rRNA gene cloning and sequencing

Cloning was carried out with the TOPO TA cloning kit (Life technologies, Carlsbad, CA, USA) using TOP 10 chemically competent One Shot—*Escherichia coli* cells (Life technologies), as supplied by the manufacturer. All eukaryotic clones were sequenced with the 18S rRNA gene standard sequencing primer 895R (5′AAATCCAAGAATTTCACCTC 3′) resulting in partial sequences including the hypervariable regions V2-V4 (Hodač et al. 2012). Sequencing reactions were performed by Macrogen Inc. (Seoul, South Korea).

Sequence analysis and phylogeny

Resulting sequences were manually corrected using the sequence analysis program SeqAssem (Hepperle 2004). These sequences were analyzed by BLASTn with NCBI database (Altschul et al. 1990, http://www.ncbi.nlm.nih.gov/). Analyzed sequences and reference sequences were imported into the ARB program (Ludwig et al. 2004, http://www.arb-home.de). To determine phylogenetic affiliations, relevant sequences were aligned with the homologous eukaryotic 18S rRNA gene sequences using the automatic alignment tool of the ARB program package. Potential chimeras were checked with Bellerophon (Huber et al. 2004). In addition, the first and the last 300 bp of putative chimeras were compared with similar rRNA gene sequences in NCBI and excluded from the dataset. After chimera check, 568 clones could be retrieved. These clones were assigned to 94 phylotypes (several eukaryote taxa), grouped at a similarity of 97% or higher to the closest related sequence retrieved by BLASTn search (Tables 1, 2, 3).

A phylogenetic tree (supplement Fig. 1) was constructed with representative full length sequences of algal phylotypes using the RAxML search algorithm for maximum likelihood (ML; Stamatakis et al. 2008), using the GTR + Γ + I model. The confidence of the tree topologies was tested by bootstrap analysis implemented in RAxML (100 replicates) and by Bayesian posterior probabilities (MB) using MrBayes 3.2 (Huelsenbeck and Ronquist 2001). Two parallel Markov chain Monte Carlo (MCMC) runs for one million generations each with one cold and three heated chains were conducted using the GTR + Γ + I model, with trees sampled every 100 generations. Sequence alignment was performed using *MAFFT* (Katoh and Toh 2008). The alignment consisted of 117 sequences with 1802 positions (738/534 variable/parsimony informative) in total. Sequences derived from 15 environmental clones, assigned to algal phylotypes (this study) and 97 isolates from culture collections as well

as environmental clones from other studies (cf. Hallmann et al. 2013a, b).

Representative sequences were deposited in GenBank under the following accession numbers: MH807077–MH807091.

Results

Exemplary for many dimension stones in the Saale–Unstrut region, the Schaumkalk at the base of Saaleck castle (cf. Fig. 1a, b) and the Buntsandstein (Hardegsen-formation, Fig. 1c, d) were selected for assessment of microbial diversity on stone surfaces. Tables 1 and 2 show data from each sampling spot and from crude cultures. In Fig. 2, summarized data from most abundant phylotypes (more than one per spot) at a sampling site are shown (crude cultures are excluded here). In total, 94 phylotypes were identified, mainly belonging to algae and filamentous fungi (including lichen fungi). Other retrieved phylotypes could be assigned to protozoa, arthropods, mosses and ferns. Clearly, phylotypes and numbers of retrieved sequences per phylotype varied; many phylotypes were just detected once in a sample. In cultures inoculated with samples from sandstone and limestone surfaces, phylotypes were identified that were not retrieved from clone libraries of the environmental samples. In particular, freshwater algae and protozoa of diverse phylogenetic groups were retrieved.

Among algae directly retrieved from stone surfaces (Table 1; Fig. 2), differences between limestone and sandstone are mainly due to the Trebouxiophyceae clone QE59, which is quite abundant in limestone samples, but present in just one sandstone sampling spot. We retrieved a *Phyllosiphon arisari* (putatively the species was wrongly assigned to a sequence; cf. Hallmann et al. 2013a; Procházková and Neustupa 2016)-related phylotype from both sets of samples. Other algae were present in small numbers (*Chlorella-, Stichococcus-, Pseudostichococcus-, Desmococcus*-related).

With respect to (lichen) fungal phylotypes, (Table 2; Fig. 2), differences between sandstone and limestone are more obvious. Although only spots without visible lichen thalli were processed, samples from Saaleck (limestone) were dominated by phylotypes of lichen fungi, in particular *Xanthoria elegans*, and to a lesser extent, *Texosporium sancti-jacobi*. Both Saaleck sampling sites appear to be similar. *Penicillium solitum*-related phylotypes are high abundant at only one spot from Saaleck

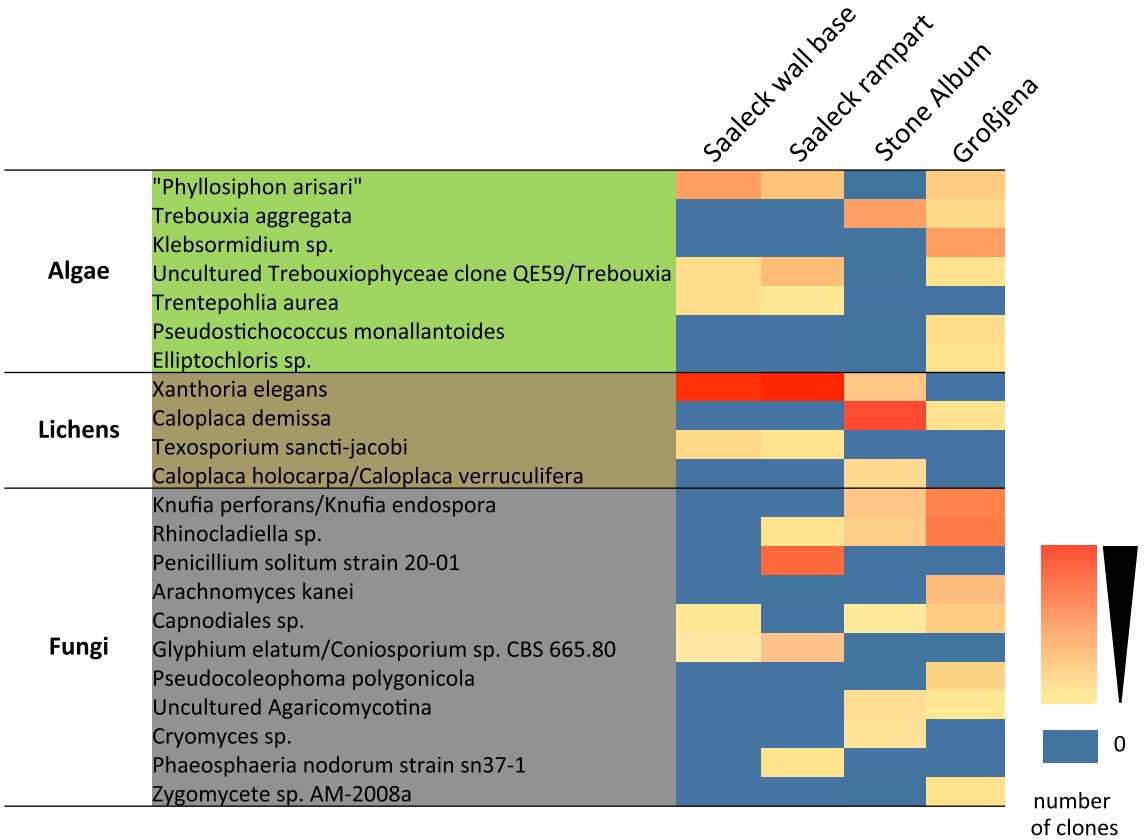

Fig. 2 Simplified heatmap displaying the number of retrieved clones, assigned to abundant phylotypes (sum over all sampling points; cf. Tables 1, 2) from two limestone (Saaleck) and two Buntsandstein (Großjena and Stone Album) sites

rampart wall (cf. Table 2). *Penicillium solitum* is a plant pathogenic fungus (Pitt et al. 1991). Others are related to insect- or other plant-associated fungi, like *Cordyceps brongniartii* (Shimazu et al. 1988) or *Rhytidhysteron rufulum, Cryptococcus carnescens, Cladosporium bruhnei, Glyphium elatum, Pleospora herbarum* and *Phaeosphaeria nodorum* (Takashima et al. 2003; Schubert et al. 2007; Hane et al. 2007; Woudenberg et al. 2017; Boehm et al. 2015; Chokpaiboon et al. 2016).

The fungal community on Buntsandstein is considerably different (Table 2). The *Xanthoria elegans* lichen fungus is missing, but on three spots, the *Caloplaca demissa* lichen fungus is abundant. *Rhinocladiella* and *Knufia perforans* are present in most of the sandstone sampling spots. *Rhinocladiella* is a typical rock-associated fungus (Sert et al. 2007; Hallmann et al. 2013b), but was also isolated from lichens (though it is not necessarily a lichen fungus, Harutyunyan et al. 2008). *Knufia* (*Coniosporium*) *perforans* has also been described as a rock-inhabiting fungus (Sterflinger et al. 1997). Also Capnodiales comprise plant and rock-associated phylotypes (Crous et al. 2009; Hallmann et al. 2013b). *Arachnomyces canei* has been described as a human pathogen (Gibas et al. 2002), but other members of the genus are rock-inhabiting fungi (Gueidan et al. 2008). Occasionally, we retrieved small animals (*Oribatula tibialis*) from the clone libraries, possibly due to the presence of eggs.

In summary, the calcicolous *Xanthoria* lichen fungus deserves attention due to its presence on most of the limestone surfaces, and *Rhinocladiella*, in particular, on Buntsandstein.

A small set of samples was taken to elucidate the inventory of endolithic organisms. Due to their microtopography (e.g., cliff formation, formation of edges due to backweathering and material loss), rock slope and surface exposition is variable. For comparison of organisms attached to a rock surface with a community at a depth of 0.5 cm below the surface, two surface samples (exposed roughly south and westwards in the field) were taken. The rock piece was removed, cracked and another sample from inside the rock was taken. A summary of the results is shown in Table 3.

From sampling site Großjena (sandstone), and Saaleck (limestone) phylotypes of algae (mainly Trebouxiophyceae), of mosses, and of fungi (mainly ascomycetes) were retrieved from surfaces, irrespective of their exposition. In samples taken at a depth of 0.5 cm, algae are missing almost completely. All other microorganisms belonged to plant-associated/plant-pathogenic fungi. From both sampling sites, tardigrade phylotypes were retrieved (*Halobiotus*/*Macrobiotus*).

Discussion

Specific microbial communities colonize all surfaces of rocks and dimension stone. Among eukaryotes, algae are the most important primary producers, but ascomycete fungi are also highly abundant (Gorbushina et al. 1993; Gorbushina 2007; Hallmann et al. 2011a, b, 2013a, b, 2014a, b, 2016). Among them, lichen fungi are one important group. Though no lichen thalli were visible on any of the sampled surfaces, phylotypes of lichen fungi and the lichen alga *Trebouxia* were abundant according to the analyzed clone libraries. Many sampling spots were dominated by either *Xanthoria elegans* or *Caloplaca (Lecanora) demiss*a lichen fungi. The preferred substrata of the lichen fungi correspond to those of the well-developed lichen, i.e., either calcareous rocks for *X. elegans* or siliceous rocks for *C. demissa*, though both lichen species have broad ecological amplitudes (cf. Wirth 1995).

Generally, lichen thalli were abundant on limestone and sandstone surfaces in the area. At the sampling spots, however, no thallus structures (cf. Wirth 1995) were observed. In spite of this, phylotypes of lichen fungi (i.e., fungal species, known to be part of a lichen symbiosis) and the lichen alga *Trebouxia* were detected. This may account for the presence of lichen prothalli, invisible to the naked eye, which may develop to a visible lichen thallus during the following years (cf. Sanders 2014). The presence of the lichen fungus of *Texosporium sancti-jacobi* may account for a rather flexible life style of some symbiotic partners in lichens. *Texosporium sancti-jacobi* thalli were found rarely on Western North American semi desert soils (McCune and Rosentreter 1992; Riefner and Rosentreter 2004), but not in other areas. Hence it must be assumed that the lichen fungus (or a closely related genus) is much more abundant than the well-developed lichen. This assumption is reasonable, because ascomycete fungi develop a sexual reproductive stage (teleomorph) rather rarely (frequently it is unknown), though the inconspicuous mycelia of the asexually reproductive stage are abundant (Cannon and Kirk 2000). In visible lichen thalli, in contrast, the lichen fungus frequently reaches its teleomorph state—which is also the case for *Texosporium* (Tibell and v. Hofsten 1968). Hence, the presence of lichen fungal mycelia, extending on the surface of or inside a substratum should be considered (cf. Hawksworth 1988). As a consequence, the building stone may be affected by lichen fungi, even when visible thalli are absent.

Many clones affiliated to non-lichen fungi were also detected. The largest subset of the retrieved fungi (among them *Glyphium elatum, Rhinocladiella* sp. and *Knufia* sp. are most abundant) exhibits melanized cell walls. These dematiaceous fungi are phylogenetically diverse and typical for extreme, dry, sun-exposed habitats. Many of them were found to be involved in colonization and degradation of natural and artificial building material (Gorbushina et al. 1993; Gorbushina 2007). Generally, the fungi discolor surfaces, but also penetrate existing fissures and enhance chemical and physical weathering processes.

Among algal phylotypes, we retrieved mainly Trebuxiophyceae (cf. Table 1; Fig. 2). Many algae of this group are

adapted to terrestrial habitats. *Trebouxia*, in particular, is the most important lichen alga and is putatively an obligate symbiont—in contrast to the fungal counterparts (cf. Amadijan 1988; Bates and Garcia-Pichel 2009). Another alga, "*Phyllosiphon arisari*" is also abundant and appears to be a common colonizer of dimension stone surfaces (cf. Hallmann et al. 2013a, b) and is mainly, but not exclusively, present on limestone surfaces. The diversity of trebouxiophyceaen vs. chlorophyceaen alga in our samples is supported by the phylogenetic tree (Fig. S1). Generally, Trebuxiophyceae may be considered as long-term sub-aeric rock colonizers, whereas the upcoming of other algal groups may be taken as an indicator for increasing moisture, or the presence of liquid water. Consequently, in liquid cultures, besides Trebouxiophyceae, also chlorophyceaen algae and protozoans were enriched. This feature has been repeatedly described and suggests the presence of a diaspore bank of algae and resting stages of protozoans that could become relevant when environmental conditions change, e.g., when the stone surface is moistened by rainfall (Hallmann et al. 2013a, b, 2014a, 2016).

Algal endoliths were first discovered in a zone of few millimeters beneath a surface of a rock in extreme dry and cold Antarctic valleys (Friedmann 1982). Colonization of rock substrata down to depths of several centimeters (Hallmann et al. 2014a; Cockell et al. 2017) or even hundreds of meters (e.g., Breuker et al. 2011) is also possible, though algae should be not expected. A 0.5-cm thick piece of the microcrystalline or amorphous rock absorbs all light (other than rocks consisting of rather large crystals, e.g., calcite); consequently photoautotrophic organisms were not retrieved from most of the samples in our study. Residual green algae in few sampling spots may be due to contamination from the stone surface, or transport by animal vectors. However, it is reasonable to assume that fungi penetrate the stone matrix, which is in particular true for porous rocks. As endoliths, fungal phylotypes do not necessarily belong to dematiaceous fungi, putatively because of the absence of stress by ultraviolet light. The presence of moss clones may be explained by the subterrestrial moss protonemata (Hallmann et al. 2014a).

Large animals such as tardigrades, which were exclusively endolithic in this study, indicated the presence of pores of several hundreds of µm in size. Tardigrades are omnivorous and feed on nematodes, moss, fungi or bacteria (Sánchez-Moreno et al. 2008; Schill et al. 2011). Their presence inside rocks may be taken as a strong signal for progressive degradation of a stone matrix.

Not all weathering phenomena cause damage in the sense of an actual loss of value. In many cases, discoloration and superficial colonization of dimension stone surfaces are of little relevance with respect to conservation of a monument. However, is certainly important to slow down the rapid weathering of the sandstone reliefs of the "Stone Album", because of its uniqueness as a rock monument in Central Europe (cf. Stück et al. 2018; Hoppert et al. 2018). Though colonization by algae and fungi is often perceived just as an esthetic limitation, endolithic colonization by fungi and by animals indicates penetrable (open) pores, which inevitably leads to surface deterioration and material loss. In this case, extended conservation and protection measures are necessary to prevent disappearance of relief elements.

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