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THE EFFECTS OF CYANOBACTERIAL BIOFILMS ON WATER TRANSPORT AND RETENTION OF NATURAL BUILDING STONES

ABSTRACT

Water affects the susceptibility of stone to alteration by facilitating physical, chemical and biological weathering. Stone properties determine water transport and retention, but it is also expected that biofilms and extracellular polymeric substances (EPS) could alter the water-stone relationship. A lot of research on this subject has been carried out on soils, but the effect on stones is understudied. For this reason, three sedimentary building stones, Ernzen, Euville and Savonnières, each with a different pore size distribution, were biofouled with cyanobacteria. Their relationship with the stone material was investigated by optical and electron microscopy, and the effect of cyanobacterial biofilms on water transport and retention was studied. The results showed that the cyanobacteria primarily colonize the building stones on the outer surface and have a limited effect on the water transport properties. They slightly reduced the capillary water uptake and drying rate of the stones but enhanced the water content in the stone and increased water vapor sorption. They induced (near) hydrophobic conditions, but this had no measurable effect on the gas permeability and water vapor diffusion. Moreover, swelling and shrinkage of the biofilms were observed, which could potentially induce physical weathering. It is expected that these changes could influence other forms of weathering, such as freeze-thaw weathering and salt weathering.

Keywords: Biofouling, Heritage conservation, Extracellular polymeric substances (EPS), Weathering, Biodeterioration

1. INTRODUCTION

When bacteria colonize rocks, like natural building stones, they form biofilms or communities embedded in extracellular polymeric substances (EPS) (Flemming et al.,

2016). The presence of biofilms and EPS might influence the stone properties. It is expected that biofilms and EPS alter the water transport and retention (Coombes and Naylor, 2012; McCabe et al., 2015; Favero-Longo and Viles, 2020; Schröer et al., 2021). Water is an important facilitator of stone alteration and deterioration. It enters stone by rainfall, infiltration (leakage or flooding) or capillary rise (Camuffo, 1995). It facilitates material dissolution (Cardell-Fernández et al., 2002) and transports and accumulates minerals such as damaging salts (Sawdy et al., 2008). Furthermore, water-ice phase transitions (Deprez et al., 2020) or the interaction of water with clay particles (Fontaine et al., 2015) can induce mechanical stress and physical weathering. Water is also vital for biological growth, and its availability will determine the amount of biological colonization (Ortega-Morales et al., 2004; Ramírez et al., 2010).

Biofilms could alter the surficial capillary water transport, vapor diffusion and even change the wettability (Warscheid et al., 1991; Warscheid, 1996; Warscheid and Braams, 2000; Polson et al., 2002; Karimi et al., 2012). On soils, considerable progress has been made in understanding the effect of biofilms on the water-substrate relationship. There, an effect was detected of cyanobacterial biofilms on the hydraulic conductivity, clogging, water infiltration and water uptake (Eldridge, 2001; Malam Issa et al., 2009; Rossi et al., 2012; Colica et al., 2014). Their effect on building stones is not well studied, but there is an agreement that biofilms would affect the water inside the stones, and a conceptual model was designed by McCabe et al. (2015) in which biofilms would make the stone increasingly non-breathable. Smith et al. (2011) suggested that algal biofilms tend to seal the surface and decrease the surface porosity and permeability of building stones. They proposed that algae biofilms aid moisture retention, facilitating higher water saturation degree to depth and even increase the dissolved salt penetration depth. Moreover, Coombes and Naylor (2012) detected reduced water absorption and evaporation rates on concrete covered with bio-chemical crusts, exposed to intertidal conditions. However, extensive experiments are missing on natural building stones, and it is unknown how biofilms affect moisture behavior inside the stone. Increased knowledge on this topic is necessary to understand the

influence of biofilms as climate change and decreasing pollution could favor biological growth. For example, in Northern Ireland, building stones have already responded to environmental change, leading to the algal greening of the material (Smith et al., 2011; Viles and Cutler, 2012).

This research aims to experimentally identify the influence of EPS and biofilms on water transport and retention within natural stones. Therefore, the effect of biofilms will be assessed on capillary water absorption, drying rate, gas permeability, water vapor diffusion, water contact angle and water vapor sorption on three sedimentary stones: Ernzen, Euville and Savonnières. Half of the samples were biofouled with the cyanobacterium *Phormidium autumnale.* Their relationship with the substrate was studied using spectrophotometry, (environmental) scanning electron and optical microscopy.

2. MATERIALS AND METHODS

2.1. Materials

Ernzen or Luxemburg sandstone is characterized by well to very well sorted fine to medium quartz grains cemented with sparitic or microsparitic iron-rich calcite or dolomite (Figure 1A) (Molenaar, 1998; Dusar et al., 2009). It is permeable and has an average porosity of 24.5%, although zones with a much lower porosity (<10%) exist (Molenaar, 1998). Ernzen contains a high amount of oversized secondary or moldic porosity, and is affected by the occurrence of some laminae of detrital clays (Molenaar, 1998; Dusar et al., 2009).

Euville limestone is a French grainstone, which consists of 98% calcite and is built up by crinoid fragments overgrown by syntaxial calcite (Figure 1B). Euville has an average porosity between 10 and 20%. The pores are heterogeneously distributed and consist of macropores between the sparitic cement. Micropores occur within the particles, in between the sparite crystals, within micrite around the shell fragments and occasionally within micritized crinoid fragments and intraclasts (Fronteau, 2000; Dusar et al., 2009; Dewanckele et al., 2014).

Savonnières limestone is a French light beige grainstone (Dunham, 1962) or a rounded oosparite (Folk, 1962) (Figure 1C). It consists for 98.8% out of calcite with 70% sparite and 30% micrite with dolomite as accessory mineral (Fronteau, 2000; Blows et al., 2003; Fronteau et al., 2010). It is a partially cemented limestone, dominated by mostly poorly preserved spherical to elliptical ooids (Figure 1C) (Dewanckele et al., 2014; Lebedev et al., 2014). Bivalve fragments are present, which are often locally concentrated, inducing a distinct layering and heterogeneity (Roels et al., 2000; Dewanckele et al., 2014; Lebedev et al., 2014). The total open porosity of Savonnières ranges between 22 and 41% (Fronteau, 2000). It has a complex pore system, with four types of distinguishable pores: (1) intragranular microporosity of the ooid walls, (2) intergranular microporosity between the sparitic cement, (3) triangular intergranular macroporosity in between the sparitic cement and (4) intragranular macroporosity or moldic porosity, induced by dissolution during diagenesis (Figure 1C). Larger macropores are only connected via micropores and can be considered as isolated regions. Besides the pores, microcracks and a secondary fracture porosity can be present (Derluyn et al., 2014).

These stones were chosen for their relevance as sedimentary building stones and their different porosities and pore structures. They are prominently used for buildings and monuments in northwestern Europe. Within the Grand Duchy of Luxemburg, the Ernzen sandstone is the most widespread and abundantly used building stone since the 12th Century. It was also an important building stone in Belgium during the end of the 19th and beginning of the 20th Century (Dusar et al., 2009). At the same time, Euville and Savonnières limestone were popular replacement and building stones. Within Belgium, they were by far the most popular French stone (Dusar et al., 2009; Dreesen et al., 2012; De Kock et al., 2013). Euville limestone was one of the most prestigious French stones (Fronteau, 2000; Dusar et al., 2009), and Savonnières limestone was also used in buildings of among others France, Germany, The Netherlands, Austria and Czechia (Lorenz and Lehrberger, 2013).

The model organisms were freshwater cyanobacteria. *Phormidium autumnale* ULC086 (also classified as CCALA 697), was acquired from the Belgian Co-ordinated Collections of Micro-Organisms (BCCM). They are filamentous and belong to the Oscillatoriales (Anagnostidis and Komárek, 1988). Freshwater types consist of simple, cylindrical, unbranched filaments (or trichomes) and form irregular colonies of more or less parallel filaments. Together with EPS, they adhere to the substrate and form mat-like structures (Strunecký et al., 2012). Cyanobacteria were chosen as they are one of the main EPS producers (Rossi and De Philippis, 2015) and among the most important colonizers of building stones (Gaylarde et al., 2012; Golubić et al., 2015). *Phormidium autumnale* occurs abundantly on natural building stones (Ortega-Calvo et al., 1991; Tomaselli et al., 2000; Rindi and Guiry, 2004; Macedo et al., 2009). The strain ULC086 was selected by its ability to form microbial mats, fast growth and durability. This strain was isolated from Ellesmere Island, Canada, out of the periphyton in a glacial stream (Strunecký et al., 2010).

2.2. Methods

2.2.1. Biofilm cultivation and biomass estimation

Phormidium autumnale ULC086 was cultured in BG11+ medium. It grew in Erlenmeyers deposited in an incubator at 23 °C, shaken at 120 rpm, and on the bench of the lab, with temperatures between 13 and 17 °C. All cultures were constantly illuminated by a LED strip, providing approximately 15 - 30 µmol $m^{-2}s^{-1}$ light. The same light intensity was maintained for all cultivation steps.

Biofilms were cultured on one side of Ernzen, Euville and Savonnières. Table 1 gives an overview of the biofouled samples used in the upcoming experiments, including their dimensions and how the biofilm was cultured. For each biofouled sample used to determine the water-transport properties, a non-biofouled, untreated sample was included to allow comparison. The cyanobacteria were partly disentangled by vortexing, and the stones were inoculated with a pipette. They grew on the bench to let them attach to the surface and were wetted by capillary uptake of water. Furthermore, every two or three days, BG11+

136 medium was added to the surface. It lasted mostly one week until, at most locations, green
137 discoloration was visible.

138 A dense biofilm was desired to study their effect on capillarity, drying, permeability, water
139 vapor diffusion, sorption, and to make thin sections (one sample per building stone). After
140 growth on the bench, these samples were placed on a water run-off setup in which the
141 cyanobacteria and broth were constantly flown over the samples. This setup was based on
142 De Muynck et al. (2009) (Figure 2). Above the setup, a LED strip was mounted. Growth
143 lasted until the complete stone surface was covered in dark green biofilms. This took
144 approximately one to two weeks. The time of biofouling was not fixed as the final biofilm
145 coverage was the most important. Extra inoculation was sometimes necessary to
146 accelerate growth. After biofilm development, the samples were submerged in tap water
147 for 24 hours to remove salts deposited on the stones.

148 It is expected that the amount of biofilm will have an impact on the water transport
149 properties. The amount of biofilm was estimated by measuring the color difference (ΔE)
150 before and after biofouling by spectrophotometry (Berger-Schunn, 1994). It is a simple,
151 fast and non-destructive technique that allows robust biomass quantification on building
152 stones (Prieto et al., 2004). Every sample used to study the water transport properties
153 was photographed, and the superficial colors were determined before and after biofouling.
154 The white balance of these pictures was corrected using a Mini ColorChecker Classic
155 (MacBeth). A Konica Minolta CM-600d spectrophotometer with an 8 mm aperture
156 determined the visible color spectrum between 400 and 700 nm and provided quantitative
157 color data in the CIE L*a*b* color space. The illuminant type D65, representing average
158 daylight, was used. For the cylindrical samples of 5 cm (for capillarity, drying and
159 permeability determination) and 8 cm diameter (for water vapor diffusion), respectively,
160 five and nine measurements were taken, and the mean was determined. For the smaller
161 biofouled samples, one measurement was performed in the center.

162 **2.2.2. Microscopy**

163 Biofouling was studied by Scanning Electron Microscopy (SEM), Environmental Scanning
164 Microscopy (ESEM) and optical microscopy on thin sections.

165 SEM images were acquired on two biofouled Ernzen, Euville and Savonnières samples of 2
166 x 2 x 1.5 cm. Images were acquired on the MIRA3 TESCAN SEM equipped with a Field
167 Emission Gun (FEG) from the Department of Geology (Ghent University, Belgium). All
168 samples were carbon-coated, and both secondary (SE) and backscattered electron (BSE)
169 images were using a voltage of 5 kV. Before visualization, the cells were fixated in 2.5%
170 glutaraldehyde in 0.1 N HEPES at a pH of 7.2 and dehydrated using an ethanol dehydration
171 series of 50%, 75%, 90% and three times 100%.

172 ESEM was performed on the biofouled sample of 0.6 x 0.6 x 0.5 cm of each stone without
173 any additional sample preparation. The biofilms were visualized in their hydrated state at
174 the Royal Institute for Cultural Heritage (KIK-IRPA, Brussels, Belgium) using a Carl Zeiss
175 EVO 15 LS SEM with LaB_6 filament. It was used as an ESEM at the extended pressure mode
176 and after installing a cooling stage to regulate the temperature. Images were taken at 3
177 °C, 20 kV using the backscatter detector. During the visualization, the relative humidity
178 (RH) ranged between 10 and 100%, in which the specimen chamber had respectively a
179 pressure between 76 Pa and 760 Pa.

180 Thin sections were prepared to investigate endolithic growth. All biofouled stones were
181 dried in the oven at 40 °C, embedded with epoxy resin and cut perpendicular to the
182 biofouled surface. The samples were polished with silicon carbide grinding paper (Stuers,
183 Ballerup, Denmark), after which ± 30 µm thin sections were prepared and analyzed using
184 a petrographic microscope (Axio Scope A1, Carl Zeiss Microscopy GmbH, Jena, Germany),
185 both under plane- and cross-polarized light. Pictures were taken using the AxioCam MRc 5
186 camera and processed with the AxioVision software (Zeiss, version 4.8.2) and ZEN 2.6
187 Lite.

188 **2.2.3. Water transport properties**

189 After biofouling, the samples were dried in the oven at 40 °C for 24 hours before starting
190 the water transport experiments to comply with the European standards. Preliminary tests
191 showed that *Phormidium autumnale* survived these conditions. Dried cells were rehydrated
192 by adding water and showed motion under the optical microscope. Reinoculating dried
193 biomass in BG11+ broth resulted in new growth.

194 The water transport properties were determined of the biofouled samples and compared
195 to untreated samples. This approach allowed to run each experiment simultaneously.
196 However, it was necessary to include enough replicates to compare both groups of
197 samples. Therefore, the number of replicates was chosen based on the European
198 Standards. Furthermore, the significance of the observed differences between untreated
199 and biofouled samples was determined by paired t-tests, in which p-values < 0.05 were
200 regarded as significant.

201 The capillary water absorption was measured, based on the European Standard EN 1925
202 (1999), on twelve 5 x 5 x 5 cm cylindrical samples of Ernzen, Euville and Savonnières,
203 which included for each stone six untreated and six biofouled samples. Ten minutes before
204 the start, water (0.1 – 0.3 mL) was sprayed on the surface to allow rehydration and
205 reactivation of the biofilms. The samples were weighed after rehydration and placed on a
206 support within a box filled with 3 ± 1 mm water with the biofilms facing the bottom. The
207 lowest cm of the sides was taped with parafilm to inhibit absorption from the sides. The
208 samples were weighed after 1, 3, 5, 7, 10, 15, 20, 30, 45, 60, 240 and 1440 minutes. For
209 Euville, additional measurements were performed after 75, 90 and 120 minutes, due to
210 the slower capillary water absorption. With these results, the capillary coefficient was
211 determined, which represents the initial or unsaturated capillary absorption rate.

212 After absorbing water by capillarity for 24 hours, the samples were immersed in water for
213 four days, and the drying rate was determined. Hereafter, they were packed with parafilm
214 and tape, leaving one side exposed to the environment. For the biofouled samples, this
215 was the side covered with biofilms. They were dried in a climatic chamber at 23 °C and
216 50% RH as described by the European Standard EN 16322 (2013). Their weight was

measured: every 20 minutes for the first hour, every hour during the next seven hours and twice a day, with at least six hours in between for the rest of the experiment on working days. During the weekend, one measurement was performed. The results were presented as the water content, expressed in m% of the water compared to the dry material over time in hours. The constant drying rate was determined during the first drying phase, by linear regression, just like the start of the second phase and the critical moisture content.

When a stable weight was reached, the permeability was determined with a minipermeameter "Tiny Perm II" (New England Research Inc., Hartford, USA). The Tiny Perm was mounted in a static upright position to provide reproducible testing, and three measurements were performed for each sample. The resulting Tiny Perm value (TP) was linked to the gas permeability K (mD) (Filomena et al., 2014).

The water vapor diffusion was determined according to the wet cup method (EN ISO 12572, 2016) on 30 cylindrical samples of 8 x 8 x 2 cm, ten of each stone, of which half were biofouled. Within the cup, a RH of 94% was established by a saturated KNO_3 solution (400 g/L). A climatic chamber established the surrounding conditions of 23 °C and 50% RH The rate of vapor transmission at a steady-state was determined by periodic weightings, and the water vapor resistance factor was determined according to EN ISO 12572 (2016), using the value $1.9*10^{-10}$ kg/(m.s.Pa) for the water vapor permeability.

The wettability was determined of six rectangular samples of 3 x 3 x 1 cm of Ernzen, Euville and Savonnières, of which half were slightly biofouled to maintain a straight surface to not hinder the analysis. Static water contact angle (WCA) measurements were performed in triplicates per sample. They were determined by a Krüss Drop Shape Analyzer (DSA25S, Krüss Gmbh, Germany) optical system. After a 2 µl droplet of distilled water was placed on the sample surface, a video (with a rate of 25 fps) was recorded and stored using a monochrome interline CCD video camera, PC-based acquisition, and data processing. Subsequently, the WCA was automatically measured on each frame containing the water droplet on the surface using the computer software provided with the goniometer applying

245 the Laplace-Young curve fitting method. All measurements were carried out under ambient
246 air and temperature conditions. The stones quickly absorbed the water droplet and, for this
247 reason, it was not possible to determine the static contact angle according to European
248 standard EN 15802 (2010). During absorption, the contact angle of a water droplet
249 decreased until zero, when it disappeared (Lee et al., 2016). The WCA was determined on
250 the first frame after the droplet touched the surface, assuming g that at that moment,
251 absorption did not start. Furthermore, based on the amounts of frames, the time until
252 complete absorption was determined.

253 Sorption was determined by the desiccator method (EN ISO 12571, 2013) on 36
254 rectangular samples of 3 x 3 x 1 cm, 12 of each stone, of which the half was biofouled.
255 The samples were placed in a series of desiccators with increased RH at ambient
256 temperature. It included the following theoretical RH's established above saturated solution
257 in equilibrium: 0% (silica gel), 33% ($MgCl_2$), 53% ($(MgNO_3)_2 \bullet 6H_2O$), 85% (KCl) and 94%
258 (KNO_3). Temperature and RH were monitored during the experiment. The samples were
259 weighed once a week until an equilibrium for each RH was reached. After determining the
260 moisture content at each RH, the sorption curve was drawn.

261 **3. RESULTS**

262 **3.1. Biofilm cultivation and measurement**

263 *Phormidium autumnale* developed dark green biofilms, resulting in a color difference (ΔE)
264 (Table 2). Figure 3 visualizes the biofouling of representative samples by presenting white-
265 balanced images and the ΔE of that specific sample. The highest color difference was
266 observed on the stones with biofilm cultured by the water run-off setup. For the different
267 stones, the mean ΔE ranged between 45.71 and 52.80 for the samples for water vapor
268 diffusion and sorption. A lower amount of biofilm developed on the samples later used for
269 capillary absorption, drying and permeability measurements, in which the mean value
270 ranged between 27.30 and 46.01. The mean color difference of the surface for the
271 wettability samples ranged between 6.95 and 18.64 (Table 2).

272 The coverage was not homogenous, especially when less biofilm was present (ΔE < 40)
273 (Figure 3A, D). Moreover, intermittent drying of extensively biofouled stones caused the
274 partial detachment and exfoliation of the biofilm from the surface (Figure 3B, C). These
275 partially detached biofilms were not removed but could fall off during the experiments,
276 which was mainly an issue for Euville and Savonnières samples. For this reason, less biofilm
277 was cultured on the stones to measure capillary absorption experiments.

278 **3.2. Microscopy**

279 Individual cells of *Phormidium autumnale* were recognized by electron microscopy. They
280 were attached to the surface, in which their filaments could follow surface irregularities
281 (Figure 4A) or were attached by discrete portions of EPS (Figure 4B). In case of high
282 concentrations, they formed biofilms, in which the cells were, more or less, aligned on
283 sheets of EPS (Figure 4C). They mainly covered the surface, sealed the pores (Figure 4D),
284 or locally entered pores and grew on the pore wall. Cracking of the biofilms could cut
285 through the individual cells (Figure 4C, D). No fungi or other algae were present, but at
286 higher magnifications (1000x and more as in Figure 4A, B), other rod-shaped or coccoid
287 bacteria were found on the biofilm. No signs of deterioration, such as pitting or dissolution
288 features were detected. There were no indications that these cyanobacteria grew
289 endolithic. Although, along with the cells and the EPS, some granular precipitates were
290 present, appearing brighter on the BSE images, suggesting an inorganic composition.
291 Similar observations were made by imaging the biofilms with optical microscopy.

292 The ESEM images revealed some cracked biofilms and filaments. Compared to the SEM
293 images, more EPS was visible. Furthermore, ESEM showed that when the RH decreased,
294 the biofilm immediately shrunk, while an RH increase caused the biofilm to swell. The effect
295 of changing RH is presented in Figure 5 on Euville. The effect was also recorded in a movie
296 and add to the Supplementary material. In here, the RH changed from 10% towards 100%
297 in steps of 10% over a total period of approximately 10 minutes. It shows that the biofilm
298 swelled in steps, almost synchronizing the increase in RH. Swelling-shirking of a biofilm
299 occurred over the whole range between 100 (Figure 5A) and 10% RH (Figure 5B). At a RH

300 of 10%, the biofilm almost disappeared out of the field of view. Increasing the RH caused
301 the biofilm to expand until it reached a similar state as Figure 5A. During this swelling-
302 shrinkage cycle, it was observed that the biofilm moved inorganic material, something like
303 a grain, transporting it over a distance of approximately 150 µm.

304 The petrographic examination of the thin sections visualized the biofilms covering the stone
305 surfaces of each sample. On most occasions, the biofilms were partly attached to the
306 surface while they covered the grain but spanned over the pores (Figure 6A). However,
307 sometimes they also colonized the pores and attached them to the inside (Figure 6B).
308 Similar observations were made by SEM (Figure 4) and ESEM (Figure 5). The penetration
309 was still very limited, and these pores were still a part of the outer edge of the stones. No
310 extensive endolithic colonization was detected, except within the two Euville samples on
311 which the biofilms solely grew on the bench. The thin sections of those samples revealed
312 biofilms inside the stones sample until a depth of at least 1 mm (Figure 6C, D).

313 **3.3. Water transport properties**

314 Biofouled samples have a reduced capillary water absorption compared to untreated
315 samples (Figure 7). The biofouled samples of Ernzen, Euville and Savonnières experienced
316 an mean decrease of the capillary coefficient of respectively 15.63, 15.89 and 25.02%
317 compared to the untreated samples (Table 3). However, only for Ernzen, this difference
318 was significant with a p-value of 0.0353. For Euville and Savonnières, these were
319 respectively 0.2744 and 0.0583. Variability was most prominent for the untreated samples
320 as biofouled samples have lower standard deviations of the capillary coefficients.

321 Biofouled samples obtained their capillary moisture content later in the absorption process,
322 compared to untreated samples. It illustrates the increased duration of capillary absorption
323 for all stones (Figure 7). The paired t-test showed that the effect of biofouling was
324 significant for all stones. The p-values for Ernzen, Euville and Savonnières were
325 respectively 0.0113, 0.0228 and 0.0043. The capillary moisture content of the biofouled

samples was slightly higher compared to the untreated ones (Table 4), but the difference was not significant.

After reaching the capillary moisture content, the biofouled samples of Ernzen and Savonnières absorbed respectively an additional 4.54 and 3.59 times the amount of water compared to their untreated counterparts. It was significant as the corresponding p-values were < 0.001. No significant difference was detected within Euville (Table 4). After 24 hours, the biofouled samples absorbed more water, adding overall, almost 1 m% water for Savonnières, 0.48 m% for Ernzen, but only 0.11 m% for Euville (Table 4). During the measurements, some biofilms detached from the surface after removing the non-absorbed water before every weighing. Biofilm removal was most pronounced for Savonnières samples and could reduce the observed effect of the biofilm.

Before drying, after 24 hours of capillary water uptake and four days full immersion, the water content of the biofouled samples was higher. The mean water content of the untreated and biofouled Ernzen was respectively 5.14 ± 0.39 and 5.81 ± 0.34 m%, 3.12 ± 0.26 and 3.41 ± 0.31 m% for Euville and 10.24 ± 0.92 and 11.70 ± 0.75 m% for Savonnières. These results differed, according to the paired t-test, significantly for Ernzen with a p-value of 0.0003. For Euville and Savonnières, the difference was almost significant with a p-value of respectively 0.0692 and 0.068.

Two drying periods can be distinguished in Figure 7, of which the results are presented in Table 5. The mean constant drying rate was lower for all biofouled building stones. The largest difference was measured for Ernzen with -2.65 and -2.00 $mg.g^{-1}h^{-1}$ for respectively the untreated and biofouled stones. The difference was only significant for Ernzen with p = 0.0066.

The constant drying phase of biofouled stones lasted longer but remained very similar for Euville and Savonnières (Table 5). The differences in critical moisture content for the untreated and biofouled stones were not consistent (Table 5). Moreover, they were not significant. An exception was Savonnières, where the biofouled samples had an increased

moisture content of importance, with a p-value of 0.0011. The difference for Euville was still relatively significant, as the p-value was 0.0535, which is only negligibly greater than 0.05.

During the second phase, the drying rate declined. After respectively 21, 31 and 23 days, Ernzen, Euville and Savonnières reached a stable weight leaving < 0.05 m% of residual water, with a negligible difference between the untreated and biofouled stones.

The values of the gas permeability were not consistent, highly variable, and no significant difference could be determined (Table 6). Moreover, the water vapor resistance factor was similar for the untreated and biofouled samples of Ernzen and Savonnières (Table 6). There was a slight difference for Euville as the mean water vapor factor for the untreated and biofouled samples were respectively 41.53 and 36.74. However, none of the results were significantly different, with p-values for Ernzen, Euville and Savonnières of respectively 0.5898, 0.13 and 0.6648.

Moreover, biofouled stones had a higher water contact angle (WCA) immediately after contact with the surface (Table 7, Figure 8). The difference was the largest on Savonnières, followed by Euville and Ernzen. The p-values of Ernzen, Euville, and Savonnières were respectively 0.0003, < 0.0001, and < 0.0001. These values show an important influence in the surface behavior of biofouled samples.

Even though biofouling increased the WCA, the droplets were still quickly absorbed (Table 7) inside the stone, and the biofilms swelled sometimes after adding the water. Biofouled Ernzen and Euville absorbed the water at a slower rate, while biofouled Savonnières absorbed water slightly faster, compared to the untreated samples. The differences were only significant for Ernzen, in which the p-value was 0.0196, compared to 0.0855 for Euville and 0.6065 for Savonnières. The rate of the droplet absorption differed between the measurements. It could reach a factor of 10 for the same stone type at the same condition (e.g. Euville 10.28 vs. 0.88 seconds) and resulted in very high standard deviations.

379 Furthermore, biofouled samples acquired more hygroscopic water by vapor sorption
380 compared to their untreated counterparts (Figure 9 and Table 8).

381 At a RH of 37.1%, the absorbed moisture was similar for Ernzen and Euville, while a larger
382 difference was measured for Savonnières (Table 8). At a RH of 98.2%, the measurements
383 obtained after one week were included. This was before equilibrium, but afterwards, the
384 samples became contaminated with mold. Paired t-tests showed that the difference
385 between the untreated and biofouled samples was often significant. Biofouled Ernzen
386 sorbed significantly more water at a RH of 91.5% (p-value = 0.0498). At other RH's, this
387 difference was less meaningful yet more important at 98.2% with a p-value of 0.0547.
388 Biofouled Euville limestone contained significantly more water at 60.6, 91.5 and 98.2%
389 RH, with p-values of respectively 0.0143, 0.0141 and 0.0187. At a RH of 37.1%, the
390 difference was almost notable with a p-value of 0.08. For Savonnières, a significant
391 difference was only present at a RH of 34.1 and 60.6% with a p-value of respectively
392 0.0143 and 0.0079. At higher RH's of 91.5 and 98.2%, the p-value rose again to
393 respectively 0.1232 and 0.0785.

394 **4. DISCUSSION**

395 Biofilms and EPS of *Phormidium autumnale* covering Ernzen, Euville and Savonnières
396 impacted water transport and retention. A summary of the results is presented in Table 9.
397 The changes were limited and not always significant within the given experimental
398 procedure. However, the effect on the biofilm was often similar on the different rock types
399 and thus consistent. Biofouling reduced the capillary water absorption at the surface of the
400 stones. It was most prominent on Savonnières limestone, which could be explained by the
401 high amount of biofouling compared to the other stones (Table 2). The increased water
402 content is related to the additional amount of water absorbed by the biofilm itself. The
403 decrease in capillary absorption does not agree with the previous findings of Warscheid et
404 al. (1991). They suggested that biofilms increased the rate of capillary water uptake after
405 comparing the capillarity of a biofouled outer wall with a biofilm-free inner wall.

406 Moreover, a relatively low amount of biofilms induced (near) hydrophobic conditions. This
407 was caused by EPS, which mainly consists of polysaccharides, nucleic acids, lipids and
408 proteins. These biomolecules have different hydrophobic or hydrophilic properties and thus
409 can change the wettability of a surface. Moreover, biofilms affected the contact angle of a
410 surface by changing the surface chemistry, roughness and reentrant topography (Epstein
411 et al., 2011; Tanaka et al., 2019) and was also examined by carbonatogenic bacteria (Elert
412 et al., 2021) and *Bacillus subtilis* (Epstein et al., 2011). Polson et al. (2010, 2002) detected
413 a change in wettability on quartz by a bacterial-fungal consortium that made the surface
414 hydrophobic, while an algal consortium left the surface hydrophilic. Its effect could be
415 limited as the water droplets were still quickly absorbed. Moreover, the duration of
416 absorption was highly variable. This could be related to the small droplet volume, the
417 different wettability of locally present biomolecules, local inhomogeneities of the building
418 stones, or incomplete colonization on the surface, which was overall limited to the outer
419 surface.

420 The increase in water retention was illustrated by a reduced drying rate and increased
421 sorption of hygroscopic water. The results agree with Warscheid et al. (1991) and Coombes
422 and Naylor (2012). It was expected from EPS as it protects bacteria from desiccation (Rossi
423 and De Philippis, 2015). The enhanced sorption of the biofouled samples is a result of the
424 hygroscopic behavior of the biofilm as observed under ESEM (Figure 5), where the size of
425 the biofilm was directly related to the surrounding RH No measurable effect could be
426 determined on gas permeability and water vapor diffusion. It shows together with Figure
427 7 that the second drying phase was not heavily hindered by the biofilms.

428 In their review, Warscheid and Braams (2000) suggested a potential increase in capillary
429 water uptake as biofilms narrow rock pores. However, the biofilms seemed to obstruct
430 water absorption and evaporation. As the pores of Ernzen, Euville and Savonnières are in
431 the typical capillary range (radius 0.1-1000 µm), the observed decrease in absorption rate
432 and increased water retention were expected by the narrowing surficial pores (Siegesmund
433 and Snethlage, 2014). Pore clogging and sealing of the superficial pore space were

434 observed by microscopy (Figure 4), which agrees with Smith et al. (2004, 2011).
435 Furthermore, the decreased absorption could also be influenced by the induced
436 hydrophobicity.

437 These experiments were not hindered by contamination besides the sorption experiment
438 at a RH of 98.2%. Contamination with heterotrophic bacteria might occur as visualized in
439 Figure 4, but compared to the cyanobacteria, their size was negligible. Cultivating biofilms
440 on the stones and estimating their amount by spectrophotometry were successful.
441 However, a lot of variation in the water transport properties and degree of biofouling
442 occurred, hindering quantitative analyses. Overall, *Phormidium autumnale* grew the
443 easiest on Savonnières, where it resulted in the fast development of dark green biofilms
444 and with mostly higher ΔE (Table 2). It was most likely related to the bioreceptivity. Growth
445 was limited to the surface (except limited endolithic growth visible on some thin sections
446 of Euville). Some of the biofilms were detached before and during the experiments, which
447 might have reduced their impact during the experiments. This was most likely caused by
448 the limited attachment of the biofilms on the surface and the cracks, both macroscopically
449 (Figure 3) and microscopically (Figure 4 and 5), which was most likely related to shrinkage
450 during drying. However, no intense drying occurred before acquiring ESEM imaging,
451 suggesting that it could rupture easily.

452 Generally, the data represented here confirmed the conceptual model of McCabe et al.
453 (2015). They proposed that biofilms alter the stone surface, leading to more effective
454 trapping of moisture. The moisture ingress would be slowed down due to a lower capillary
455 absorption rate, while moisture loss would be more difficult. They also suggested a
456 decrease in permeability, although this could not be observed.

457 Moreover, the effect of biofilms on building stones is comparable with previous findings of
458 soil research in arid to semi-arid regions. However, the effect of biofilms, described as
459 "biocrusts", on water infiltration and hydrology remains controversial (Xiao et al., 2019).
460 The results agreed with the previous findings, where the EPS matrix absorbed and delayed
461 water movement by expanding and becoming less fibrous. There, the EPS acted as a buffer

462 for the cells and also decreased or at least delayed the evaporation of water (Potts, 2001;
463 Mager and Thomas, 2011). This reduced the evaporation in soils (Xiao et al., 2010) and
464 could increase the water holding capacity (Rosenzweig et al., 2012; Adessi et al., 2018),
465 which was also detected by (Malam Issa et al., 2009) and related to the hygroscopic
466 properties of EPS. Some authors as Colica et al. (2014), Malam Issa et al. (2009), Xiao et
467 al. (2019) and Kidron et al. (1999) detected a decreased water infiltration and increased
468 run-off due to cyanobacterial EPS. This would be related to the hydrophobic nature of the
469 cyanobacterial compounds, preventing quick wetting of microbial crusts and reducing
470 porosity. Hydrophobic components could, also in our case, explain the slower capillarity.
471 However, others, including (Rossi et al., 2012), detected the opposite: an increased water
472 infiltration and hydraulic conductivity as EPS would facilitate water movement by creating
473 micropores and forming new waterways.

474 The induced changes in water transport and retention might be limited but could impact
475 stone alteration. According to McCabe et al. (2015), these changes would lead to water
476 accumulation after each wetting event, increasing the depth of the waterfront. This affects
477 deterioration and could trap salts at depth, which might prevent or drive future material
478 loss. Prolonged wetting increases the risk of freeze-thaw weathering (Siegesmund and
479 Snethlage, 2014) and enhances further colonization (Ortega-Morales et al., 2004; Ramírez
480 et al., 2010). Although biofouling might only affect the water redistribution on a microscale,
481 similar to some biological soil crusts (Keck et al., 2016), as water was still absorbed
482 relatively quickly. However, the reduced capillary absorption rate, together with the
483 induced hydrophobicity, suggested that biofilms obstruct absorption. Biofilms might lead
484 to increased run-off that could become important on e.g., an inclined wall or during intense
485 rain showers where water has little time to be absorbed inside the pores. Thus, biofilms
486 could act as natural waterproofing and play a protective role by reducing mineral
487 dissolution, salt weathering and freeze-thaw scattering (Polson et al., 2002). This could be
488 valuable, as the biofilms seemed to have little impact on the water transport properties.

489 Biofilms can also directly affect building stones. The observation of ESEM of swelling and
490 shrinking combined with the movement of a potential grain (Figure 5 and Supplementary
491 information) suggested that biofilms can potentially mechanically deteriorate natural
492 building stones, just like clays (Wangler and Scherer, 2008; Fontaine et al., 2015). This
493 was already suggested by Dornieden et al. (2000) and Warscheid (1996), but ESEM was
494 able to visualize this process in real-time. The movie included in the supplementary
495 information showed that also smaller RH changes affect the dimensions of the biofilm.
496 During the day, humidity is constantly changing in relation to varying temperatures (due
497 to day and night) or sudden rain events (Huby et al., 2020). These changes can be large
498 as e.g., micro-climatic monitoring by Huby et al. (2020) on the Saint-Remi Basilica in Reims
499 (France) detected a shift of 77% in RH during one day. Day-night cycles suggest that
500 biofilms will at least perform once a day swell- and shrinkage cycle. While the effect of one
501 cycle might be small, numerous cycles could have a relevant impact.

502 The biofilms might also affect the solubility of salts as SEM and ESEM showed along with
503 the cells and the EPS, some granular precipitates, appearing brighter on the BSE images,
504 suggesting an inorganic composition. These precipitates (Figures 4A, B) were most likely
505 salts of the growth medium as these were detected inside EPS during the examination of
506 the cultures under optical microscopy. EPS might prevent salt dissolution as the samples
507 were submerged in water to dissolve residual salts before microscopy. Another option could
508 be the precipitation of $CaCO_3$ linked to photosynthesis (Danin and Caneva, 1990; Albertano
509 et al., 2000; Ortega-Morales et al., 2000; Dittrich and Sibler, 2010).

510 **5. CONCLUSIONS AND FUTURE RESEARCH**

511 Within this research, the impact of biofilms on water transport and retention in building
512 stones was investigated. The effect was small but mostly in agreement with past work from
513 stones and soils. Biofouling reduced the capillarity, drying rate, but increased the moisture
514 content, water vapor sorption and induced near hydrophobic conditions. No effect could be
515 measured on the gas permeability and water vapor sorption. These changes will most likely
516 have a small effect but could enhance deterioration, including freeze-thaw weathering and

517 salt damage. Moreover, biofilms can potentially enhance physical weathering and affect
518 the solubility of salts. However, biofilms might obstruct water absorption and induce
519 hydrophobic conditions, which could protect the building stone.

520 Spectrophotometry allowed to estimate the amount of biofilm, but due to the variability of
521 the water transport properties, biofouling and detachment of the biofilms, it was not
522 possible to quantify the effect of biofilm. It could be solved when future research will
523 determine the water transport properties on the same stone before and after biofouling
524 and if the amount of biofilm could be quantified. Future research should also focus on the
525 WCA as the effect of the biofilms on the wettability was significant. It should measure the
526 WCA of biofouled stones preconditioned at different RH, including both dry and wet
527 biofilms. Future research should study the effect of biofilms in-situ and combine it with lab-
528 based experiments. The effect of environmental biofilms is also understudied: they could
529 increase the water content of a building stone due to sorption or increased water retention
530 or reduce the ingress of water due to the induced hydrophobicity. Depending on the
531 dominant effect, biofilms could thus enhance or reduce deterioration. Moreover,
532 environmental biofilms might also be better attached. Experiments should include
533 heterotrophic bacteria, as they can be dominant on building stones (Adamiak et al., 2018;
534 Tescari et al., 2018; Schröer et al., 2020), and colonize the inner pore space as well.
535 Biofilms on the field are often not as dense as the ones cultured for these experiments.
536 However, due to improved attachment and endolithic colonization, it could be possible that
537 these biofilms have a larger impact on the water-stone relationship compared to laboratory
538 experiments. Moreover, weathered building stones should be included as another behavior
539 is expected, and studies should be performed to assess the effect of biofilms on weathering.
540 At least, experiments should explore the effect of biofilms on the solubility of salts and
541 determine if biofilms can mechanically alter building stones. These should include not only
542 weathering experiments but the developed pressure induced by swelling of the biofilm
543 should be measured as well.

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811 **7. FIGURE CAPTIONS**

812 **Figure 1** Macroscopic image (left) and thin sections under cross-polarized light (right) of
813 (A) Ernzen sandstone, (B) Euville and (C) Savonnières limestone, with the thin sections
814 showing the quartz grains (QZ) cemented by calcite (CA) on Ernzen and the presence of
815 moldic porosity (MP). Within Euville limestone, crinoid fragments (CF) can be identified,
816 overgrown with syntaxial calcite cement and a shell fragment (SH) bordered with thin
817 sparitic cement. The thin section of Savonnières limestone shows ooids (OO) and bivalve
818 fragments (BF). The numbers refer to the pore types with (1) intragranular micropores,
819 (2) intergranular micropores, (3) intergranular macropores and (4) intragranular
820 macropores.

821 **Figure 2** Illustration of the water run-off setup, where the cyanobacteria were constantly
822 flowed over, in this case, cylindrical rocks mounted in a sample holder.

823 **Figure 3** White balanced images of representative biofouled samples Ernzen, Euville and
824 Savonnières used to determine (A) capillarity, drying and permeability (B) water vapor
825 transport, (C) water sorption and (D) wettability. ΔE is written underneath, with ΔE of (A)
826 and (B) the mean of respectively five and nine measurements.

827 **Figure 4** SEM image of biofouled (A) Ernzen, (B) Euville and (C, D) Savonnières covered
828 with elongated cyanobacteria identified as *Phormidium autumnale* embedded in an EPS
829 matrix, which was cracked at some locations. Besides the cyanobacteria, small rod-shaped
830 bacteria were present covering the stone surface, cyanobacteria and EPS. Attached to the
831 cells and EPS, inorganic precipitates were present, most likely salts from the growth
832 medium (based on BSE images, not shown here).

833 **Figure 5** ESEM image of biofouled Euville at a RH of (A) 100% and (B) 10% showing the
834 shrinkage of the cyanobacteria and the EPS. The biofilm in the right corner moves almost
835 out the field of view, during which it takes a potential loose grain with it.

836 **Figure 6** Thin sections under cross-polarized light through the biofouled edge of (A) Euville
837 and (B) Savonnières, illustrating the biofilm covering the stone surface, (A) spanning over

838 the pores or (B) following the surface including local depressions induced by the pores. (C)
839 with a detail (D), indicated by the box, shows the biofilms at the edge of the stone and
840 inside the pores.

841 **Figure 7** Mean capillary water absorption in function of time (left) and mean water content
842 during drying at 23 °C and 50% RH (right) of (A) Ernzen, (B) Euville and (C) Savonnières
843 with the blanks in gray and the biofouled samples in green.

844 **Figure 8** Water droplet immediately after contact with (A) untreated Savonnières
845 limestone and (B) biofouled Savonnières limestone.

846 **Figure 9** Sorption curve showing the mean moisture content at a certain RH of untreated
847 (gray) and biofouled (green) (A) Ernzen, (B) Euville and (C) Savonnières.

848

8. TABLES

Table 1 Overview of the Ernzen, Euville and Savonnières samples biofouled with *Phormidium autumnale*, including the dimensions, shape, number of replicates for each building stone and the process to induce biofouling. For each biofouled sample, a non-biofouled sample was included to allow comparison (except for (E)SEM and the optical petrography studying thin sections).

Experiment	Dimensions	Replicates	Biofilm growth
SEM	2 x 2 x 1.5 cm (cylinder)	2 x	Bench
ESEM	0.6 x 0.6 x 0.5 cm (cylinder)	1 x	Bench
Petrography	4.5 x 2.5 x 3 cm (rectangle)	3 x	Bench + (run-off)
Capillarity, Drying + Permeability	5 x 5 x 5 cm (cylinder)	6 x	Bench + run-off
Water vapor diffusion	8 x 8 x 2 cm (cylinder)	5 x	Bench + run-off
Sorption	3 x 3 x 1 cm (rectangle)	6 x	Bench + run-off
Contact angle	3 x 3 x 1 cm (rectangle)	3 x	Bench

Table 2 Color difference (ΔE) induced after biofouling Ernzen, Euville and Savonnières before the water transport experiments, displaying the mean color difference (ΔE_{mean}) and the sample with the minimal (ΔE_{min}) and maximal color difference (ΔE_{max}). ΔE_{mean} and its standard deviation were retrieved from six samples prepared for the capillarity + drying + permeability (five measurements per sample), five samples for water vapor diffusion (nine measurements per sample), six samples for the sorption experiments (one measurement per sample) and was based on three samples for the wettability (one measurement per sample).

Stone	ΔE_{mean}	$\Delta E_{min.}$	$\Delta E_{max.}$
	Capillary absorption + Drying + Permeability		
Ernzen	27.30	25.23	31.69
Euville	31.36	25.58	36.95
Savonnières	46.01	37.71	51.30
	Water vapor diffusion		
Ernzen	45.71	41.15	48.69
Euville	47.68	44.51	51.16
Savonnières	50.20	45.72	54.42
	Sorption		

Ernzen	46.32	31.20	50.21
Euville	52.50	42.79	58.88
Savonnières	52.80	40.99	57.88
	Wettability		
	Sample 1	Sample 2	Sample 3
Ernzen	9.48	7.88	14.84
Euville	6.75	3.54	10.55
Savonnières	18.70	16.87	20.37

Table 3 Mean coefficient of capillarity, including standard deviation, with minimum and maximum, measured values of each untreated and biofouled stone.

Stone	Treatment	C_{mean} ($gm^{-2}s^{-1/2}$)	C_{min} ($gm^{-2}s^{-1/2}$)	C_{max} ($gm^{-2}s^{-1/2}$)
Ernzen	Untreated	124.63 ± 27.78	80.16	149.70
	Biofouled	105.15 ± 15.97	86.89	124.59
Euville	Untreated	60.56 ± 14.69	42.11	80.79
	Biofouled	50.94 ± 11.26	40.35	65.55
Savonnières	Untreated	306.08 ± 55.87	232.02	377.42
	Biofouled	229.50 ± 27.94	181.68	261.38

Table 4 Mean values and standard deviations of untreated and biofouled Ernzen, Euville and Savonnières describing the duration of the capillary water absorption ($T_{capillary\ abs.}$ (min.)), the capillary moisture content ($M_{capillary}$ (m%)) and the amount of absorbed water by diffusion ($M_{diffusion}$ (m%)) until 24 hours after the start of the experiment.

Stone	Treatment	$T_{capillary\ abs.}$ (min.)	$M_{capillary.}$(m%)	$M_{diffusion}$ (m%)
Ernzen	Untreated	23.74 ± 10.36	4.59 ± 0.45	0.13 ± 0.05
	Biofouled	35.69 ± 8.55	4.61 ± 0.52	0.59 ± 0.06
Euville	Untreated	39.06 ± 16.33	2.56 ± 0.22	0.23 ± 0.04
	Biofouled	78.83 ± 25.21	2.67 ±0.34	0.24 ± 0.02
Savonnières	Untreated	10.52 ± 3.04	9.48 ± 0.98	0.29 ± 0.05
	Biofouled	21.54 ± 2.93	9.71 ± 2.93	1.04 ± 0.12

Table 5 Mean drying characteristics, including standard deviations, of untreated and biofouled and Ernzen, Euville and Savonnières at 23 °C and 50% RH. Constant drying rate is expressed as water loss in mg for each gram stone per hour. The critical moisture content is given in m% of water within each building stone.

Stone	Treatment	Constant drying rate ($mg.g^{-1}h^{-1}$)	Critical moisture content (m%)	Time constant drying period (h)
Ernzen	Untreated	-2.65 ± 0.18	3.44 ± 0.32	6.61 ± 0.06
	Biofouled	-2.00 ± 0.20	2.93 ± 1.31	15.24 ± 9.69
Euville	Untreated	-1.49 ± 0.22	2.10 ± 0.31	6.81 ± 0.22
	Biofouled	-1.34 ± 0.17	2.49 ± 0.24	6.94 ± 0.97
Savonnières	Untreated	-2.20 ± 0.20	4.63 ± 0.59	25.43 ± 4.02
	Biofouled	-2.11 ± 0.13	6.25 ± 0.31	26.07 ± 4.30

Table 6 Mean Tiny Perm (TP) value with the corresponding gas permeability K(mD) and the mean water vapor resistance (μ), with their standard deviations, for the untreated and biofouled Ernzen, Euville and Savonnières.

Stone	Treatment	TP value	K (mD)	μ
Ernzen	Untreated	10.53 ± 0.16	783.3 ± 338.6	21.04 ± 1.66
	Biofouled	10.61 ± 0.12	601.3 ± 185.0	21.24 ± 3.25
Euville	Untreated	10.76 ± 0.26	471.5 ± 345.8	41.53 ± 2.67
	Biofouled	10.76 ± 0.29	481.5 ± 327.7	36.74 ± 5.92
Savonnières	Untreated	10.19 ± 0.13	1976.9 ± 681.4	21.53 ± 0.63
	Biofouled	10.13 ± 0.15	2378.8 ± 844.2	21.27 ± 0.90

Table 7 Measured WCA at the moment when the droplet touched the surface of the untreated and biofouled Ernzen, Euville and Savonnières, including the mean WCA of nine measurements, the minimum and maximal value, mean absorption time ($T_{abs.\ mean}$), including the minimal ($T_{abs.\ min.}$) and maximum ($T_{abs.\ max.}$) time. The mean values are represented with their standard deviations.

Stone	Treatment	WCA_{mean} (°)	$WCA_{min.}$ (°)	$WCA_{max.}$ (°)	$T_{abs.\ mean}$ (s)	$T_{abs.\ min}$ (s)	$T_{abs.\ max.}$(s)
Ernzen	Untreated	53.7 ± 11.3	39.3	73.6	1.56 ± 1.48	0.40	4.48
	Biofouled	85.7 ± 10.2	68.8	105.1	4.21 ± 1.57	1.32	5.56
Euville	Untreated	45.6 ± 7.9	29.4	51.4	1.37 ± 0.89	0.52	2.08
	Biofouled	93.3 ± 8.9	80.3	108.4	3.61 ± 2.90	0.88	10.28
Savonnières	Untreated	47.1 ± 6.5	39.9	57.6	1.31 ± 0.97	0.36	2.76
	Biofouled	103.5 ± 14.6	83.4	128.8	1.16 ± 0.49	0.44	1.96

Table 8 Mean moisture content and standard deviation (m%) acquired by hygroscopic suction of untreated and biofouled Ernzen, Euville and Savonnières at each RH (%). *mean

890 moisture content at a RH of 34.1 instead of 37.1. **measurement after one week as
891 afterwards the biofouled samples were contaminated with mold.

Stone	Treatment	Relative humidity (RH)			
		37.1	60.6	91.5	98.2**
Ernzen	Untreated	0.026 ± 0.004	0.039 ± 0.006	0.080 ± 0.013	0.106 ± 0.019
	Biofouled	0.027 ± 0.002	0.043 ± 0.003	0.096 ± 0.006	0.128 ± 0.008
Euville	Untreated	0.007 ± 0.001	0.010 ± 0.002	0.025 ± 0.004	0.033 ± 0.006
	Biofouled	0.009 ± 0.001	0.014 ± 0.002	0.037 ± 0.008	0.052 ± 0.011
Savonnières	Untreated	0.028 ± 0.006*	0.045 ± 0.008	0.083 ± 0.014	0.113 ± 0.021
	Biofouled	0.040 ± 0.004*	0.063 ± 0.005	0.115 ± 0.038	0.158 ± 0.041

892

893 **Table 9**: Summary of the effect of the cyanobacteria on the studied water transport
894 properties

Property	Impact biofilm	Significance
Capillary coefficient	Decrease	Significant for Ernzen (nearly for Savonnières)
Water absorption at atmospheric pressure	Increase	Significant for Ernzen (nearly for Euville and Savonnières)
Drying rate	Decrease	Only significant for Ernzen
gas permeability	Not measurable	Not significant
Water vapor diffusion	Not measurable	Not significant
Water contact angle	Increase	Significant for all studied stones
Water vapor sorption	Increase	Variable significance

895