



Kinetics of water sorption, EPS swelling and evaporation in biological soil crusts on reclaimed temperate dunes, Brandenburg, Germany

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Abstract: Biological soil crusts (BSCs) play a crucial role in regulating water infiltration into surface substrates; however, their exact hydrological effects remain a subject of ongoing debate. This study quantitatively investigates the kinetics of water immobilization through sorption and the swelling behavior of extracellular polymeric substances (EPS) during the wetting of dry BSCs on recultivated temperate dunes in Brandenburg. Crust samples dominated by the green alga *Zygonium ericetorum* were collected from an inland dune in the Lusatian post-mining area. Following a multi-week drying phase, rehydration experiments were conducted using deuterium oxide (D₂O) to avoid spectral interference with the O–H groups of the EPS. Using diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS), the time-dependent shift of the O–D stretching vibrations was analyzed to determine the sorption kinetics of the biological crusts relative to an undisturbed mineral control. In parallel, gravimetric measurements monitored evaporation, while the EPS composition was characterized via ATR-FTIR spectroscopy. Spectroscopic analysis identified alginate as the primary organic component of the crust EPS. While the uncoated mineral control showed almost immediate water infiltration, the BSC samples exhibited a pronounced plateau in OD vibrations during the first three minutes, indicating an initial phase of free water at the surface. Between the 3rd and 12th minutes, a distinct redshift toward lower wavenumbers followed, reflecting the ongoing functional group reorganization, hydration, and EPS swelling, until thermodynamic equilibrium was reached after approximately 12 minutes. Gravimetric data also revealed a significantly higher and faster evaporation rate for the BSCs compared to the control. These results demonstrate that the alginate and EPS matrix of the biological soil crust acts as a water reservoir that hydrologically seals the surface, drastically limiting vertical infiltration into deeper soil layers while retaining water in a highly accessible surface pool. Furthermore, it is conceivable that this delayed water immobilization by the swelling EPS matrix provides a vital ecological benefit by buffering the crust microbiome against severe hypo-osmotic shock during rapid rewetting. However, direct microbial analyses are required to confirm this effect. By moving beyond conventional visual assessments or indirect macro-hydraulic inferences, this approach provides unprecedented molecular-scale insights into the rapid physicochemical transition from free to matrix-bound water during EPS hydration. To our knowledge, this study represents the first application of time-resolved diffuse reflectance infrared spectroscopy (DRIFTS) combined with deuterium oxide (D₂O) rehydration to directly track water immobilization kinetics in biological soil crusts. By eliminating spectral interference from biopolymer hydroxyl groups, this approach provides unprecedented insights into the rapid physicochemical transition from free to matrix-bound water.

Keywords: Biological soil crusts; EPS swelling; Sorption kinetics; Alginate; DRIFTS; Infiltration; Evaporation.

1 INTRODUCTION

Biological soil crusts (BSCs) control water infiltration, especially in arid regions where they can make up the vast majority of the vegetation cover. The ability of BSCs to facilitate water infiltration or to promote surface runoff, which is critical to water management and the formation of vegetation patterns in arid landscapes, is controversial among scientists because observations vary regionally. As synthesized by (Kidron et al., 2022b), the effects of biocrusts on runoff generation remain highly contradictory, with conflicting conclusions often reported within the same geographical regions. In Australia, for instance, biocrusts have been shown to both increase (Graetz and Tongway, 1986) and decrease runoff (Eldridge et al., 1997). Similar dualities have been documented across the USA (Herrick et al., 2010; Kidron et al., 2012; Whitney et al., 2017; Williams et al., 1999), the Chinese Loess Plateau (Bu et al., 2015; Zhao and Xu, 2013), and

Israel (Kidron and Büdel, 2014). This persistent hydrological enigma has been characterized as both 'site-specific' (Belnap and Eldridge, 2001) and a major 'research challenge' (Pointing and Belnap, 2012). As synthesized by (Belnap, 2006), runoff dynamics are governed by a complex matrix of shifting variables, including antecedent site conditions, soil texture, surface roughness, bulk density, macromorphology, and the specific composition and biomass of the cyanobacterial community.

In mineral soils, total infiltration is the sum of two processes: (i) water moving vertically through the soil pores into the underlying layers, and (ii) water adsorption by capillary forces in the soil matrix and absorption by organic matter, in sum defined as sorptivity. While infiltration for sufficiently large amounts of time is determined solely by hydraulic conductivity (Philip, 1957), capillary sorption occurs rapidly in mineral substrates (Philip, 1957) and microbial EPS isolates have been reported to

rehydrate within 20 minutes (Chenu, 1993). In addition, hydration of EPS in BSCs has been shown to be associated with swelling, reduction in draining soil pores, and infiltration rate (Kidron et al., 1999; Fischer et al., 2010). Because of this sorption kinetics, the amount of water that percolates into the subsurface depends not only on the amount of precipitation, but also on its intensity. Consequently, for identical precipitation volumes, infiltration rates strongly depend on rainfall intensity, as high-intensity events allow more water to penetrate the pore network (meaning the interstitial spaces within the sandy dune substrate where EPS-driven clogging takes place) before structural clogging occurs. The infiltration kinetics during the first 20 minutes thus represent a critical bottleneck dictating the partition between infiltration and surface runoff.

This macro-hydrological behavior is driven by EPS swelling, where the physicochemical bonding of water dipoles to polar polymer groups lowers the matric potential within the EPS sheaths. This mechanism carries significant consequences for the crust microbiome. In general, during rapid rewetting microbial cells either lyse completely or adjust to the water potential shock by releasing intra-cellular osmoregulatory solutes (Halverson et al., 2000), eventually resulting in pulses of CO₂-release (Fischer, 2009; Fischer et al., 2023; Manzoni et al., 2020). We hypothesize that the densely connected alginate matrix delays the rate at which water becomes available for cellular rehydration, thereby protecting them from an hypo-osmotic hydration shock that can lead to cell lysis. While EPS swelling in biocrusts is commonly discussed as a mechanism affecting infiltration and runoff generation (Kidron et al., 2022b, 1999), it may also confer a physiological advantage by buffering the rate of cellular rehydration. Delayed wetting of the EPS matrix could reduce osmotic shock during rapid rewetting events and thereby decrease cell lysis.

Although EPS swelling and its subsequent role in soil pore clogging are widely discussed in biocrust hydrology, conventional investigations have predominantly relied on macroscopic or phenomenological approaches. Traditionally, EPS hydration behavior is either inferred indirectly from macro-hydraulic parameters – such as infiltration rates – or documented through visual assessments under a microscope or by the naked eye. While these classical techniques provide valuable structural context, they are fundamentally limited to capturing the final macroscopic output of morphological changes, offering little to no insight into the underlying physicochemical transitions occurring at the boundary layer.

To address this methodological gap, this study introduces a novel, time-resolved spectroscopic approach operating at the molecular scale. By combining diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) with deuterium oxide (D₂O) rehydration, we directly track the kinetics of water immobilization via the shifting of atomic vibrational bands within the EPS network. Instead of relying on a qualitative observation of volume expansion, this method enables a direct, quantitative differentiation between free surface water and matrix-bound water within the swelling alginate meshwork. This approach not only eliminates spectral interference from biopolymer hydroxyl groups but successfully shifts the analytical paradigm from a static, visual assessment to the real-time quantification of functional group reorganization during hydration.

On a broader scale, resolving these molecular kinetics provides critical insights into near-surface hydrological and ecological processes in arid and semi-arid ecosystems. Hydrologically, quantifying the exact transition from free to matrix-bound water is central to predicting soil sealing, surface runoff generation, and evaporation dynamics at the soil-atmosphere interface. Ecologically, understanding the precise rate of water immobilization

sheds light on the survival strategies of biocrust communities, as the timing of water availability dictates both the metabolic reactivation and the osmotic stress mitigation of the soil microbiome during rapid rewetting events. Ultimately, bridging the gap between molecular-scale hydrogel dynamics and macro-scale soil hydrology offers a mechanistic framework to better predict how dryland ecosystems respond to shifting precipitation patterns.

The aim of this study was to quantify the kinetics of water immobilization by sorption during wetting of dry BSCs to address (I) infiltration kinetics during rainfall onset and (II) the potential role of water immobilization in buffering microbial rehydration. To address this aim, we used infrared diffuse reflectance spectroscopy (DRIFTS), which is sensitive to -OH molecular vibrations. This sensitivity includes chemically bound -OH groups (alcohols, polysaccharides, phyllosilicates), as well as free and bound water molecules, where the vibrational frequencies, or wave numbers, of the -OH groups decrease with increasing strength of water binding to polar surfaces or organic molecules, in particular hydrophilic polymers. It has been reported that physically bound water is less mobile than unbound, free water (Ping et al., 2001; Velazquez et al., 2003).

2 MATERIAL AND METHODS

2.1 Study site

The sampling site is located on restored dunes in the Welzow Süd (Gerwin et al., 2023) mining area in Lusatia, Brandenburg (51°36'53.6' N, 14°13'01.6' E). Due to its predominantly continental climate, Lower Lusatia is one of the driest regions in Germany, with a negative water balance. The nearest weather station, Cottbus (Deutscher Wetterdienst – DWD, ID 880), has an annual average temperature of 10.0 °C (1991–2020), with the average temperature in the coldest month (January) at 0.8 °C and in the warmest month (July) at 19.8 °C. The average annual precipitation is 566 mm (1991–2020), with average precipitation in January at 41.5 mm and in July at 74.8 mm. Recent high-resolution grid data from the German Meteorological Service (DWD Climate Data Center, 2026) reveal that the potential evapotranspiration (PET) over grass in this specific area can escalate to 1000 to 1100 mm yr⁻¹ during highly evaporative years.

In order to restore former moraine landscape features following the cessation of mining, artificial inland dunes (Figure 1a) were constructed using Quaternary sands from the overburden material. The mineral substrate for the development of biocrusts was characterised as carbonate-free pure sand. The sandy substrate consisted of 8% coarse sand (630–<2000 µm), 71% medium sand (200–<630 µm), 19% fine sand (63–<200 µm), and 2% silt and clay (<63 µm). Markers of surface runoff (rills) were not detected. On the nutrient-poor sandy soils, characteristic pioneer acid dry grassland vegetation develops (Ellenberg and Leuschner, 2010), a key feature of which is the formation of biological soil crusts.



Fig. 1. Overview of the sampling site with artificial inland dunes (a) and detail view of example sampling spot No. 3 with green algae biocrusts (b).

The sparse vascular vegetation of the open dune area was characterized by a pioneer community with a total coverage of approximately 10–15%. The dominant grass species was *Corynephorus canescens* (approx. 10% coverage), interspersed with creeping individuals of *Rubus* sp. At the fringes of the dune and in the immediate surroundings, early-successional tree species were present, predominantly consisting of Silver Birch (*Betula pendula*) and Scots Pine (*Pinus sylvestris*), which are highly characteristic of reclaimed temperate sand ecosystems in the Brandenburg region. In the initial phase of biocrust formation, green algae predominate, developing primarily in the direct vicinity of the grass tussocks (Figure 1b); as succession progresses, mosses and ground lichens are added as further components (Fischer et al., 2010; Gypser et al., 2015). The biocrusts were characterized by algal filaments, mainly consisting of *Zygonium ericetorum*, that partially filled the pores in the matrix and entangled sand grains. *Zygonium ericetorum* has been reported as typical soil algae under temperate climate (Gypser et al., 2016a; Lukesova, 2001).

2.2 Sampling

Sampling of the undisturbed biocrusts was conducted in Petri dishes with a diameter of 100 mm and a depth of 15 mm. After extraction, excess soil was scraped off the underside of the dishes with a spatula, and the samples were stored at 5 °C overnight until the drying procedure described below. Five BSC samples were collected on Nov 11, 2021 and prepared for analysis immediately after species inventory in the lab. The sampling was intentionally conducted in November to ensure that the biocrusts were sampled during a phase of high metabolic activity. Unlike vascular plants, the physiological activity of biocrusts in temperate and semi-arid regions is primarily dictated by moisture availability rather than calendar seasons. During the hot and dry summer months, these crusts frequently enter a state of desiccation-induced dormancy, characterized by a brownish appearance and down-regulated metabolic secretion. In contrast, the cooler, more humid conditions of late autumn (November) provided optimal hydration, as evidenced by the vibrant green, metabolically active state of the filamentous *Zygonium* sp. at the time of collection. Sampling during this active window ensured that the extracted EPS matrix reflected its fully functional, hydrogel-like state rather than a degraded or dormant structural conformation. While seasonal variations in polymer composition cannot be entirely ruled out, the shared functional traits of the EPS – such as rapid swelling and high water affinity – remain the central drivers of the observed infiltration-evaporation dynamics regardless of the exact collection month. Uncrusted control samples were collected from open areas between the tussocks and examined under a stereomicroscope to check for the absence of algal filaments.

2.3 Sample preparation

All samples were kept at 20 °C and 60% relative humidity for six weeks for drying and adaptation to drought conditions, which corresponded to an equilibrium soil water potential amounting to -69.1 MPa (pF=5.85).

2.4 Water drop penetration time

Using an adjustable-volume manual micropipette, the water drop penetration time of the BSC was determined after application of 100 µL water after drying at 20 °C and 60% relative humidity for six weeks.

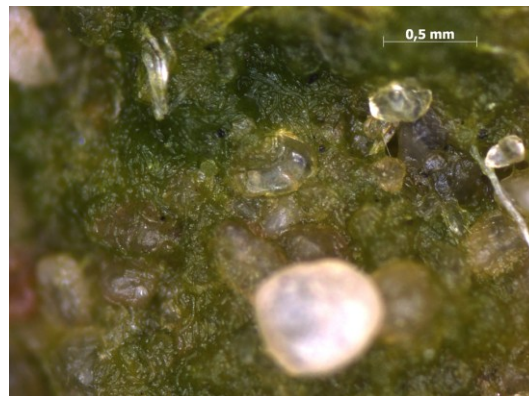


Fig. 2. Stereomicroscopic image of the wet biocrust (example section of replicate 3) with swollen algal biomass on sandy mineral substrate, resulting in clogging of soil pores. The crystalline structures are formed by the quartz grains of the sandy substrate.

2.5 Polymer identification

To identify high molecular weight (MW) carbohydrates, a sub-sample of the biocrust layer was gently homogenized and extracted with a 0.1 M EDTA aqueous solution. The high MW polysaccharides were then precipitated by treating with 70% (v/v) ethanol, the supernatant was discarded and the precipitated sugars re-suspended in deionized water (Rossi et al., 2012). The precipitate was freeze-dried and analyzed with ATR-FTIR spectroscopy.

Table 1. FTIR absorption bands of sodium alginate and the dry BSC sample.

Wavenumber (cm ⁻¹)	Description
~3350 (broad)	Hydroxyl groups (inter- and intramolecular H-bonds)
~2920 (weak)	Aliphatic C–H bonds of the pyranose rings
~1610 (strong)	Carboxylate groups of uronic acids (salt form)
~1415 (medium)	Carboxylate groups of uronic acids (COO ⁻)
~1300 (weak)	C–C–H and C–O–H pyranose skeletal vibrations
~1030 (strongest)	Glycosidic bonds and pyranose ring C–O–C stretching
950 – 800 (weak)	Fingerprint of α-L-guluronic and β-D-mannuronic acids

To understand the mechanisms driving these processes, it is crucial to analyze the specific functional groups of the biocrust exopolysaccharides (EPS). As detailed in Table 1, the chemical matrix of these polymers is characterized by distinct molecular structures that directly dictate soil hydraulic behavior. Specifically, the presence of pyranose ring structures (C–O–C) reflects a stable polysaccharide network capable of structurally entangling loose sand particles. More importantly, the high density of functional hydroxyl (OH) and carboxylate (COO⁻) groups functions as a powerful chemical trap. Specifically, the presence of aliphatic CH bonds and pyranose ring structures (C–O–C) reflects the fundamental, rigid structural backbone of the complex carbohydrate network capable of structurally entangling loose sand particles. Importantly, these skeletal C–H vibrations are tightly locked within the cyclic sugar geometry and do not function as

free, hydrophobic aliphatic chains. Instead, the high density of functional hydroxyl (OH) and carboxylate (COO⁻) groups functions as a powerful chemical trap. Because these groups possess an exceptional capacity for hydrogen bonding, they bind and immobilize water molecules upon wetting. The immediate hydrological implication of these polar properties is the transition of the dry EPS into a swollen hydrogel, creating a self-limiting barrier (bioclogging) that regulates initial infiltration and water retention at the atmospheric boundary layer.

2.6 FTIR microscopy and evaporation

To be able to distinguish between naturally occurring -OH vibrations from polysaccharides, soil minerals or residual moisture remaining after drying, rehydration experiments were conducted with deuterium oxide (heavy water, D₂O), because the -OD vibrational frequencies clearly differ from -OH. It was hypothesized that the -OD stretching frequency decreases after the application of D₂O to BSC surfaces due to progressively stronger adsorption of water to mineral surfaces and during EPS swelling. Temporal shifts in the -OD absorption band were therefore used as a proxy for the kinetics of water sorption in both the mineral substrate and the biological soil crust. The polysaccharide nature of biocrust EPS was confirmed by ATR-FTIR spectroscopy in this study.

100 µL of D₂O (98% NMR grade) were applied to both BSC and mineral surface (Control) after recording a DRIFT spectrum of the dry sample using a Centaurus FTIR microscope (Thermo Scientific). The field of spectral recording (600 µm x 400 µm) was moistened entirely and it was ensured that each sample was kept in place throughout the time of measurement. DRIFT spectra were recorded immediately and 1, 3, 6, 9, 12, 15, 18 and 21 minutes after D₂O penetration into the surface. Sample weight was recorded simultaneously to monitor evaporation at 20 °C and 60% r.h. during a period of 46 minutes after D₂O application.

Background corrected D₂O peak areas, which represent the total amount of D₂O available for spectral recording, were integrated over the spectral range from 1750 to 2780 cm⁻¹.

Evaporation was determined by monitoring the weight loss of D₂O immediately after application and after 1, 3, 6, 9, 12, 15, 18, 21, 26, 36 and 46 minutes at 20 °C and 60% relative humidity.

2.7 Statistical Analysis

To quantitatively evaluate and compare the swelling kinetics of the extracellular polymeric substances (EPS) between the biological soil crust (BSC) and the control group, a non-linear regression approach was applied. Rather than treating time as a categorical factor, the dynamic shift of the D₂O vibrational band was modeled as a continuous function of time using a four-parameter logistic (4PL) model (Dose-Response curve). This model was chosen because its mathematical flexibility accommodates both the immediate exponential decay observed in the control group and the sigmoidal lag-phase characteristic of structured hydrogels like alginate. The 4PL model is defined by equation 1:

$$F(t) = D + \frac{a-D}{1 + \left(\frac{t}{x_0}\right)^B} \quad (1)$$

where: $f(t)$ represents the wavenumber of the D₂O vibrational band at time t . A is the initial upper asymptote (wavenumber at $t = 0$, representing the dry state). D is the final lower asymptote

(wavenumber at maximum swelling equilibrium). x_0 is the inflection point (the time required to reach 50% of the total band shift), serving as the primary kinetic indicator for hydration velocity and penetration barriers. B represents the Hill slope factor, quantifying the cooperativity and abruptness of the structural transition during swelling. All statistical modeling, parameter estimations, and subsequent hypothesis testing were performed in R (version 4.4.2) using the *drc* (Dose-Response Analysis) package. Group-specific parameters (A , D , x_0 , and B) were estimated simultaneously by specifying the experimental group (BSC vs. Control) as a curve identifier. To determine statistically significant differences between the two systems, direct pairwise parameter comparisons were conducted using asymptotic delta-method t -tests via the *compParm()* function. The time axis was treated linearly in all model fits and graphical representations to accurately preserve the initial hydration dynamics at $t = 0$.

To compare the evaporation dynamics, an Analysis of Covariance (ANCOVA) was applied, modeling evaporation as a function of time, treatment, and their interaction. The inclusion of the interaction term allowed us to explicitly test for significant differences between the linear evaporation rates (slopes) of the BSC and the control group. A significance level of $p = 0.05$ was established for all statistical tests.

3 RESULTS AND DISCUSSION

3.1 Water drop penetration time

The water drop penetration time of the BSC was 15±4 s after application of 100 µL water after drying. Water drops immediately penetrated the uncrusted mineral surface of the Control.

3.2 Polymer identification

Fig. 3 displays the FTIR spectra of pure sodium alginate (Na-Alginate) and the dried BSC sample (BSC dry). Both spectra exhibit the characteristic fingerprint of a polysaccharide structure, confirming that alginate is the primary organic component in the BSC sample. A broad and intense absorption band is observed in the region of 3600–3000 cm⁻¹, centered at approximately 3350 cm⁻¹. This band is attributed to the stretching vibrations of hydroxyl groups, participating in both inter- and intramolecular hydrogen bonding. The weak shoulder near 2920 cm⁻¹ corresponds to the aliphatic C–H stretching vibrations of the polymer backbone. The presence of the carboxylate groups COO⁻ in the salt form of the uronic acid residues (mainly mannuronic and guluronic acids) is confirmed by two distinct peaks: a sharp, highly intense peak at 1610 cm⁻¹ arising from asymmetric stretching, and a medium-intensity peak at 1415 cm⁻¹ corresponding to symmetric stretching.

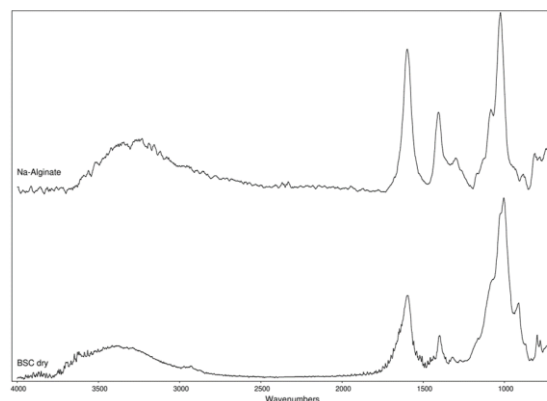


Fig. 3. FTIR spectra of EPS extract of the biological soil crust and commercial sodium alginate (Fisher Chemicals).

The most prominent absorption band in both spectra is located at approximately 1030 cm^{-1} . This peak represents the C-O and C-O-C stretching vibrations characteristic of the glycosidic linkages and the pyranose rings. Finally, the minor bands below 950 cm^{-1} (specifically around 890 and 815 cm^{-1}) are associated with the anomeric region, reflecting the specific skeletal conformations of the β -D-mannuronic and α -L-guluronic acid units.

A comparison of the Na-Alginate spectrum and the BSC dry spectrum (Figure 3) reveals a high degree of conformational similarity. However, the BSC dry sample shows a slight decrease in the relative intensities of the OH band ($\sim 3350\text{ cm}^{-1}$) and the asymmetric carboxylate band ($\sim 1610\text{ cm}^{-1}$) relative to the main pyranose C-O peak ($\sim 1030\text{ cm}^{-1}$), which can be attributed to the dehydration state and minor structural variations within the matrix. IR band assignments for the spectral region of interest of this study are illustrated in Fig. 4.

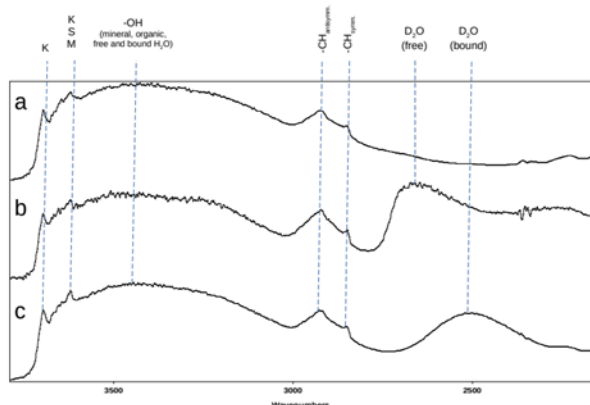


Fig. 4. IR band assignments in the stretching vibration region (a) of the dry BSC, (b) immediately and (c) 21 minutes after application of D_2O to the BSC surface. K, S, M – Inner surface Al-OH of kaolinite, smectite and mica, respectively.

3.3 EPS-driven sorption kinetics

The time-dependent shift of the O-D stretching vibration maximum (Figures 5 and 6) provides clear evidence of the different binding states and interaction kinetics of deuterated water molecules on the biological soil crust (BSC) compared to the mineral control.

The spectral shift from higher wavenumbers ($\sim 2670\text{ cm}^{-1}$) to lower wavenumbers ($\sim 2500\text{ cm}^{-1}$) is fundamentally driven by the strength of hydrogen bonding. In a weakly bound or bulk liquid environment ("free D_2O "), the O-D covalent bond is relatively strong, resulting in higher vibrational frequencies. When D_2O molecules form strong intermolecular hydrogen bonds with polar functional groups, the O-D covalent bond is weakened and elongated, which manifests as a significant redshift to lower wavenumbers ("adsorbed D_2O "). The BSC sample displays a distinct plateau during the first 3 minutes, maintaining a high wavenumber ($\sim 2667\text{ cm}^{-1}$). This indicates that initially, a substantial layer of bulk-like, free D_2O covers the surface. Between $t=3$ min and $t=12$ min, a sharp, progressive decrease in the wavenumber is observed. In contrast, the mineral control surface lacks these polymeric, gel-forming macromolecules. Consequently, the transition from free water to the adsorbed state occurs almost immediately. Within the first 3 minutes, the wavenumber drops drastically from 2642 cm^{-1} down to 2515 cm^{-1} . After approximately 12 minutes, both systems reach a steady-state plateau within the "adsorbed D_2O " zone ($2490 - 2510\text{ cm}^{-1}$, Fig. 6). All fitting parameters according to equation 1 differed significantly between the Control and the BSC (Table 2).

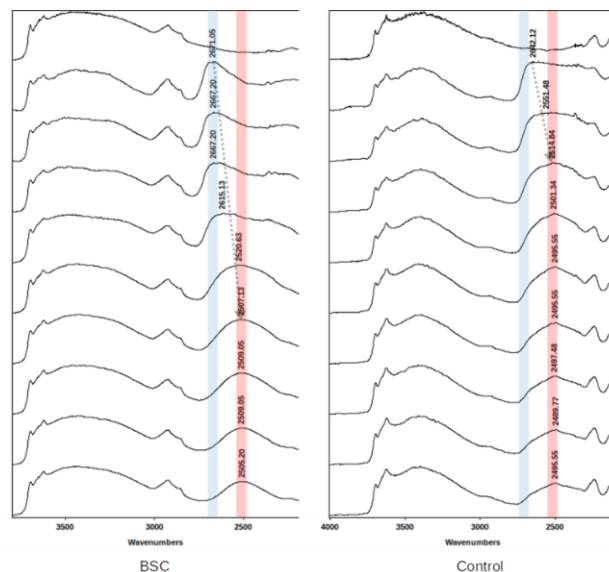


Fig. 5. Time-resolved DRIFTS spectra of a representative green algal biocrust (BSC, left) and the bare mineral control (right) during D_2O rehydration. The stacked spectra track the system from the initial dry state (top) through 0, 1, 3, 6, 9, 12, 15, 18, and 21 minutes after D_2O application (from top to bottom). Numbers indicate the exact peak maxima of the -OD stretching vibration. The blue shaded vertical area highlights the region of weakly bound, bulk liquid water ("free D_2O " at $\sim 2670\text{ cm}^{-1}$), while the red shaded vertical area highlights the region of strongly hydrogen-bonded water molecules ("matrix-bound/adsorbed D_2O " at $\sim 2500\text{ cm}^{-1}$). Dashed arrows trace the distinct kinetic pathways of water immobilization.

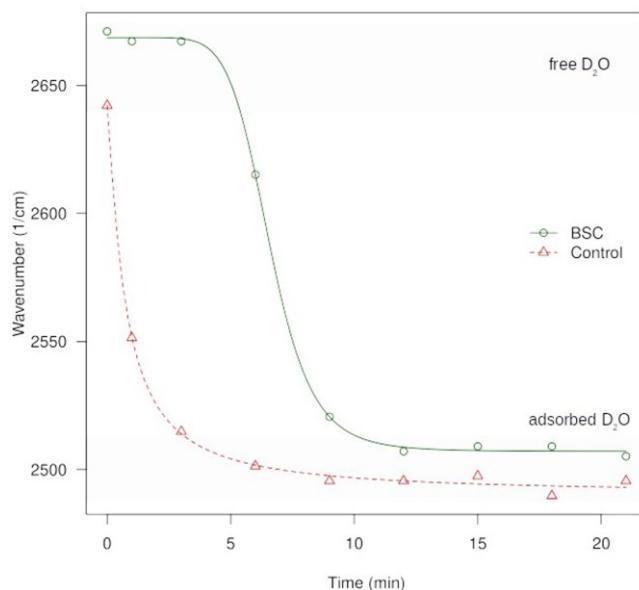
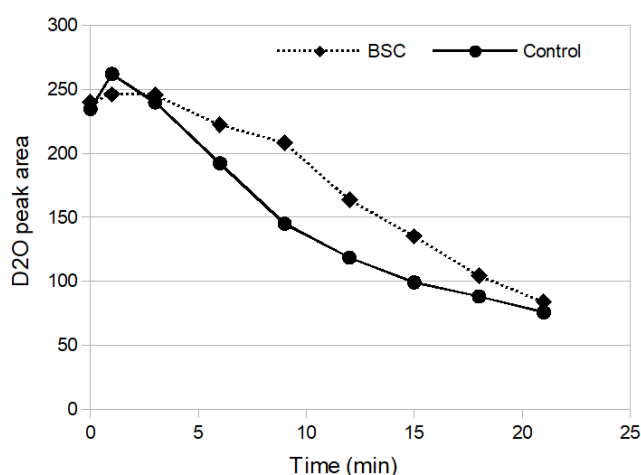
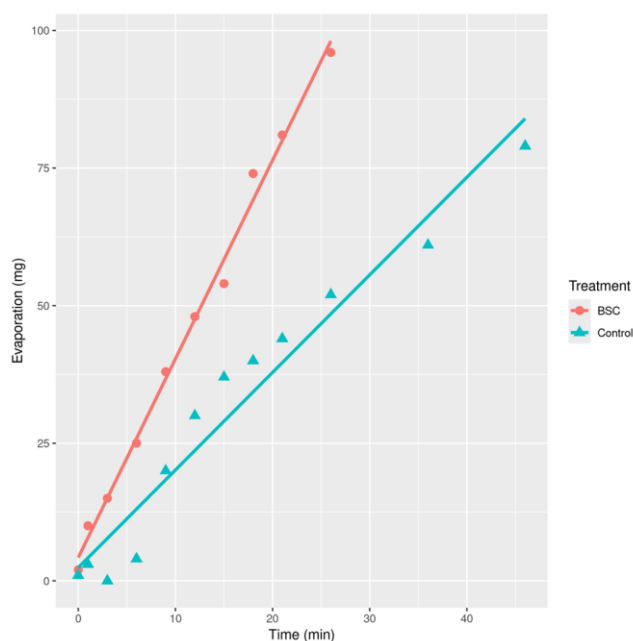


Fig. 6. Kinetics of the -OD stretching vibration peak shift after application of D_2O to dry surfaces of green algal biocrust (BSC) and bare mineral substrate (Control), arithmetic mean of 5 replicates for each data point.

The integrated peak area of the O-D stretching vibration directly correlates with the amount of D_2O located within the sampling area of the DRIFTS microscope ($600\text{ }\mu\text{m} \times 400\text{ }\mu\text{m}$ field at the immediate surface). As shown in Figure 7, the BSC sample maintains significantly higher peak areas over the first 15 minutes compared to the mineral control, which then convergence towards thermodynamic equilibrium.

Table 2. Fitting parameters according to equation 1 and statistical comparison between BSC and Control.

Parameter	Physical Meaning	Group	Estimate	Comparison (p-value)
A	Initial Upper Asymptote (Dry state, $t=0$)	BSC	2669	< 0.001
		Control	2642	
D	Final Lower Asymptote (Maximum swelling)	BSC	2507	< 0.001
		Control	2490	
x_0	Inflection Point (Time to 50% shift / t_{50})	BSC	6,58	< 0.001
		Control	0,72	
B	Slope Factor (Cooperativity / Steepness)	BSC	7,64	< 0.001
		Control	1,19	

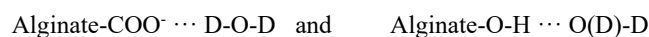
**Fig. 7.** Background corrected D₂O peak areas integrated over the spectral range from 1750 to 2780 cm⁻¹. Lower peak areas in the Control indicate a thin, highly constrained monolayer of D₂O ($p=0.029$, paired t-Test, arithmetic mean of 5 replicates for each data point).**Fig. 8.** D₂O evaporation from BSC and Control after application of 100 μ L D₂O to the surface, $r^2_{\text{BSC}}=0.99^{***}$, $r^2_{\text{Control}}=0.95^{***}$, arithmetic mean of 5 replicates for each data point.

This difference in spatial water distribution directly governs the macroscopically observed evaporation rates (Figure 8). The gravimetric data shows a significantly higher and faster water mass loss (evaporation) for the BSC sample (3.61 ± 0.10 mg/min) than for the control surface (1.77 ± 0.12 mg/min, $p < 0.001$, ANCOVA).

4 DISCUSSION

The EPS of *Zygnematales*, the botanical order to which *Zygogonium* sp. belong to, are composed of multiple polymers that are highly branched, where different species have different EPS backbones and substitution patterns upon the core portions of the molecules. It is predominately composed of a complex matrix of branched, uronic acid containing polysaccharides with ester sulfate substitution and have an almost infinite capacity for various hydrogen bonding, hydrophobic interaction and ionic cross-bridging motifs which characterize their unique function in biofilms (Kiemle, 2010; Rossi et al., 2012). Alginate (Figure 3) is a polysaccharide consisting of the two uronic acids α -L-guluronic acid (GulUA) and β -D-mannuronic acid (ManUA), which are linked 1,4-glycosidically in alternating ratios to form linear chains.

The retarded kinetics in the BSC matrix compared to the Control (Figures 5 and 6) represents the time required for rearrangement of the polar groups of the polymer and their hydration. Upon drying, it is postulated that the hydrophilic ends bond more strongly with each other, leaving an exposed hydrophobic surface, which results in turn in water repellency (Hallett and Gaskin, 2007; Kidron et al., 2022b). Importantly, while the macro-hydraulic infiltration occurs within seconds – as demonstrated by the rapid water drop penetration time (WDPT) of 15 ± 4 s required for the liquid to physically enter the pore network – the subsequent physicochemical hydration and molecular swelling of the internal polymeric structures is a distinct process that takes several minutes to complete. While being repellent, the swelling of polysaccharides in direct contact with water might be characterized by slow hydration of internally bound polar functional groups, thereby increasing surface energy and accessibility to liquid water (Fischer et al., 2013). Subsequently, the polar functional groups of the alginate backbone – namely the highly hydrophilic carboxylate groups (COO^-) and the numerous hydroxyl groups (OH^-) act as strong coordination sites. The dipole molecules of D₂O align and bind via strong hydrogen bonds to these rearranged negative and polar centers:



Because the mineral surface of the Control cannot physically entrap or gelate the water, the bulk liquid infiltrates rapidly, leaving behind only a thin, highly constrained monolayer of D₂O molecules directly adsorbed to the polar mineral sites (Figure 7). Crucially, the BSC sample stabilizes at a slightly higher wave-number (2507 cm^{-1}) than the mineral control (2496 cm^{-1} , Figure 6). This subtle difference suggests that while the alginate matrix excels at retaining a larger volume of water due to its porous, hydrogel-like structure, the energetic binding state or the spatial constraints of the water molecules trapped within the organic polymer meshwork differ slightly from the rigid, flat crystalline adsorption sites of the bare mineral surface.

To quantitatively support the spectroscopic shifts, the integrated D₂O peak areas (Figure 7) and the gravimetric water loss data (Figure 8) were evaluated. The combination of these da-

tasets provides a comprehensive understanding of how the biological soil crust (BSC) alters the hydrologic behavior compared to the mineral control.

The mass loss (evaporation) data (Figure 8) demonstrate that the hydrophilic alginate and EPS matrix of the biocrust acts as a highly effective surface barrier. Due to the high density of polar functional groups (carboxylate and hydroxyl groups), the D₂O molecules are instantly bound and immobilized at the uppermost layer, forming a swollen hydrogel. In contrast, the mineral control exhibits a much faster and more pronounced decrease in the D₂O peak area. Since the bare mineral surface lacks gel-forming organic macromolecules, the applied water is not structurally retained at the surface. Instead, gravity and capillary forces drive a rapid downward infiltration of D₂O into the deeper mineral pore space, moving it out of the detection window of the IR microscope. Therefore, lower peak areas in the control (Figure 7) do not indicate a loss of water from the system at early stages, but rather a rapid physical infiltration into the depth.

Crucially, the strictly linear progression of the mass loss curves in Figure 8 provides direct physical evidence that both systems remained entirely within Phase I (the Constant Rate Stage) of soil evaporation throughout the monitored 46-minute window. Because the total fluid volume was restricted to a micro-scale dose of 100 μ L, vertical drainage out of the 15-mm-deep petri dishes was impossible, ensuring that 100% of the applied fluid infiltrated the sample matrix.

However, the distinct slopes of these linear fits reveal that the constant Phase I evaporation rate is significantly enhanced by the biological matrix. This observation can be explained by two synergistic effects:

- **Water Availability at the Boundary Layer:** Because the BSC traps the D₂O directly at the surface interface, the water remains in constant, direct contact with the surrounding atmosphere. This maximum exposure to the ambient air under constant atmospheric demand (20 °C, 60% r.h.) facilitates continuous and rapid evaporation.
- **Capillary Suppression in the Control:** In the mineral control, the rapid initial infiltration shifts the water body downwards into the sub-surface pores. For evaporation to occur from the control, the water must first be transported back to the surface via capillary action or vapor diffusion through the dry pore network. This tortuous transport path creates a physical resistance that significantly decelerates the evaporation rate, explaining the lower evaporation curve in Figure 8.

No agreement was reached in the literature regarding the biocrust role on evaporation (Kidron et al., 2022a). Our study strongly supports the notion that biocrusts increase evaporation after immediate wetting due to their high capacity of holding water at the very surface of the soil.

This finding contributes a key mechanistic explanation to the ongoing global debate regarding whether biocrusts enhance or reduce evaporation (Kidron et al., 2022a). Historically, this controversy has been driven by a failure to isolate the competing effects of crust type, soil texture, and aridity level. In highly arid environments dominated by cyanobacterial crusts on sandy soils, biocrusts often appear to decrease evaporation over longer cumulative periods because their rapid pore-clogging restricts deep infiltration, keeping the water in a thin surface layer that dries out quickly, leaving the subsurface soil dry and protected from deep evaporative pull. Conversely, in more humid regions or on fine-textured soils where moss- or lichen-dominated crusts create a highly porous, organic-rich surface cover, the crust can act as a physical mulch that shades the mineral soil and keeps the moisture near the soil-atmosphere boundary layer.

Our time-resolved molecular data bridge these conflicting macro-observations by showing that the direction of the evaporation effect is highly dependent on the phase of the wetting-drying cycle and the specific functional traits of the EPS. By demonstrating that the swelling of alginate-like polymers instantly traps water at the immediate atmospheric boundary layer, we show that biocrusts actively accelerate evaporation during Phase I (the constant rate stage). However, this accelerated loss is a direct consequence of the EPS's physiological “choice” to prioritize immediate water availability for the surface-dwelling microbiome over long-term water conservation in the soil profile. Consequently, the apparent global contradictions in evaporation rates can be largely reconciled by accounting for (1) the specific EPS-swelling kinetics of the dominant crust taxa, which determines how long water is held at the boundary layer, and (2) the soil texture, which dictates the strength of the competing capillary forces drawing water downward. The total amount of water (Θ) held at the surfaces and in the contact zones of mineral particles of the uncrusted soil after drainage by forces of gravity (i.e. at a water potential $\Psi \sim -60$ hPa) has been reported to be very much lower than Θ of the algal layer (Fischer et al., 2012; Gypser et al., 2016). The swelling of EPS observed in our study is consistent with the literature data on the swelling of polysaccharides. While in the present study the physicochemical water binding to alginate completed within 12 minutes, Chenu (1993) found that 1% and 5% scleroglucan, as well as 5% xanthan and dextran in association with kaolinite reached their maximum water absorption within 15 minutes. Fischer et al. (2010) reported that soil crusts reduced infiltration to a minimum within 20 minutes and attributed this to EPS swelling and pore clogging. Kidron et al. (1999) synthesized that the wetting of biocrust filaments and sheaths triggers an immediate swelling and expansion of their extracellular polymeric substances (EPS) matrix. This rapid moisture absorption and subsequent structural swelling effectively results in pore clogging, which acts as a mechanism that retards further downward water infiltration. Our results bridge these findings by showing that initial infiltration and EPS swelling act as simultaneous, competing processes; the rapid structural swelling of the alginate filaments creates a self-limiting feedback loop (bioclogging) that seals the pore network, keeping the water at the atmospheric boundary layer and sustaining accelerated Phase I evaporation. Concurrently, this mechanism is suggested to buffer the microbiome from osmotic shock, though direct microbial viability assays are required to confirm this link. Taken together, these findings suggest that the hydrogel-like behavior of biocrust EPS is a key control on near-surface hydrological processes. While the specific polymer composition may vary among taxa and environmental conditions, the shared functional traits – rapid swelling, high water affinity, and the ability to modify pore structure – appear to be central to the regulation of infiltration–evaporation dynamics in biocrusted soils.

4 CONCLUSIONS

In summary, the extracellular polysaccharide matrix (specifically alginate) of the BSC fundamentally modifies the surface hydrology. It acts as a water reservoir that seals the surface, restricts vertical matrix infiltration, and holds the water in a highly accessible surface pool. While this mechanism optimizes immediate moisture availability for the crust's micro-organisms at the very surface, it simultaneously accelerates total evaporative loss under drying conditions due to the unhindered atmospheric exposure. Ultimately, shifting the analytical focus from macroscopically visible volume expansion to time-resolved molecular

diagnostics demonstrates that EPS swelling is not merely a structural state, but a dynamic, chemical optimization of the boundary layer. Beyond these macro-hydrological impacts, the controlled kinetics of EPS hydration and swelling offer a compelling hypothesis for further physiological research. It is conceivable that by regulating the rate at which water becomes available to the cells during rapid rewetting, this hydrogel matrix could buffer the soil microbiome against severe hypo-osmotic shock, potentially protecting the microbial community from cell lysis. Future studies tracking intracellular solute release will be essential to validate this protective ecological role.

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