Limitation of microbial processes at saturation-level salinities in a microbial mat covering a coastal saltflat

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Abstract (250 words)

Hypersaline microbial mats are dense microbial ecosystems capable of performing complete element cycling and are considered analogs of Early Earth and hypothetical extraterrestrial ecosystems. We studied the functionality and limits of key biogeochemical processes, such as photosynthesis, aerobic respiration, and sulfur cycling in salt crust-covered microbial mats from a tidal flat at the coast of Oman. We measured light, oxygen, and sulfide microprofiles as well as sulfate-reduction rates at salt saturation and in flood conditions and determined fine-scale stratification of pigments, biomass, and microbial taxa in the resident microbial community.

The salt crust did not protect the mats against irradiation or evaporation. Although some oxygen production was measurable at salinity ≤ 30% (w/v) *in situ*, at saturation-level salinity (40%), oxygenic photosynthesis was completely inhibited and only resumed two days after reducing the pore water salinity to 12%. Aerobic respiration and active sulfur cycling occurred at low rates under salt saturation and increased strongly upon salinity reduction. Apart from high relative abundances of *Chloroflexi,* photoheterotrophic *Alphaproteobacteria*, *Bacteroidetes*, and *Archaea,* the mat contained a distinct layer harboring filamentous *Cyanobacteria*, which is unusual for such high salinities.

Our results show that the diverse microbial community inhabiting this saltflat mat ultimately depends on periodic salt dilution to be self-sustaining and is rather adapted to merely survive salt saturation than to thrive under the salt crust.

Importance (150 words)

Due to their abilities to survive intense radiation and low water availability hypersaline microbial mats are often suggested to be analogs of potential extraterrestrial life. However, even on Earth the limitations imposed on microbial processes by saturation-level salinity have rarely been studied *in situ*. While abundance and diversity of microbial life in salt-saturated environments is well documented, most of our knowledge on process limitations stems from culture-based studies, few *in situ* studies, and theoretical calculations. Especially oxygenic photosynthesis has barely been explored beyond 5M NaCl (28% w/v). By applying a variety of biogeochemical and molecular methods we show that despite abundance of photoautotrophic microorganisms, oxygenic photosynthesis is inhibited in salt-crust covered microbial mats at saturation salinities, while rates of other energy generation processes are decreased several fold. Hence, the complete element cycling required for self-sustaining microbial communities only occurs at lower salt concentrations.

Introduction

Microbial mats are densely populated, stratified microbial assemblages performing complete element cycling, perfect for studying microbial interactions and the development of complex microbial ecosystems. Microbial mats are often found in 'extreme' habitats with high UV radiation, high temperatures and high salinity, such as solar salterns (reviewed in 1, 2) and coastal sabkhas, intertidal or supralittoral zones with accumulated evaporites as a result of an arid climate (3). Traces of microbial mats are found early in the rock record and they may also represent the last surviving ecosystems on a future Earth (4) or be potential analogs for surface communities on other planets (5). In these microbial communities, primary production by photosynthesis is balanced by a suite of degradation processes (6), including hydrolysis of organics, aerobic respiration and sulfate reduction. The latter process can drive an active sulfur cycle, where sulfide produced by sulfate reduction is oxidized by phototrophs and aerobic sulfur oxidizers (7, 8).

In hypersaline environments, microbial cells need to actively counter the osmotic stress imposed by high salinity by either accumulating small organic molecules (osmolytes) or potassium ions (reviewed in 9) in order to retain water inside the cell. These stress tolerance strategies are energetically costly and theoretically can impose thermodynamic limits on the type of possible metabolic processes (10) when the cost of growth plus salt adaptation exceed the energy that can be generated by metabolic activity. Processes with low energy yields that were doubted to support growth at high salt concentrations (salinity above 15%) include sulfate reduction, nitrification and methanogenesis (10). However, with more recent reports of sulfate reduction (11, 12), ammonia oxidation (13), and even methanogenesis (14) at high salinities, it has been argued that hypersaline environments can be considered "thermodynamically moderate" allowing for active complete element cycling and thus can represent self-sustaining ecosystems (15).

The key to a truly self-sustaining ecosystem with sunlight as the only perennial energy source is primary production of organic matter by oxygenic phototrophs. While prominent layers of filamentous cyanobacteria can be observed in moderately hypersaline habitats, the

diversity of oxygenic phototrophs diminishes at higher salinity (16) and eukaryotic *Dunaliella* algae are often referred to as the sole primary producer in salt-saturated brines (17). However, detection of microorganisms by microscopy or molecular markers (DNA, pigments, lipids) does not provide information on their metabolic activity and it is well known that microorganisms can survive long periods of unfavorable conditions in dormant states (18–20). While active growth of heterotrophic halophilic archaea and bacteria in saturated brines is well known, less data is available on purely photosynthetic organisms and possible limits of their activity. Even in *Dunaliella* algae, photosynthesis rates have been reported only up to 3M NaCl concentrations (ca. 18% w/v salinity) with some studies showing a decrease of photosynthesis rates (21, 22) and others showing an enhancement (23) at increasing salinities. However, the growth of *Dunaliella* decreases drastically when approaching salt-saturation (24, 25). Although oxygenic photosynthesis is not thermodynamically limited at high salt concentrations (10), salinity shift experiments with microbial mats indicated a kinetic inhibition of oxygenic photosynthesis by decreased oxygen solubility (26, 27).

On the other hand, saturation-level salinity might also provide some life-preserving effect. Already Baas Becking suggested that crusts formed by precipitated salt can protect underlying life against heat and intense light (28). Crystalline halite was further observed to facilitate condensation of water from the air and slow down its evaporation, thus forming a refuge for phototrophic microbial life (13, 29). Although measuring the *in situ* photosynthetic activity in such salt-covered habitats remains a challenge, Davila *et al*. provided indications that in halite-colonizing communities of the Atacama Desert it might be performed by very few unicellular *Cyanobacteria* at low rates (13).

Microbial mats containing filamentous *Cyanobacteria* were found under a layer of crystalline salt at a tidal flat in Oman, with a distinct microbial community composition at the upper tide line suggesting an adaptation of these microorganisms to high salinity (30). We investigated the limitations of metabolic processes to high salt stress in this salt-saturated environment, with special focus on photosynthetic primary production, and tested the hypothesis that salt crust may protect microbial communities against desiccation, heat, and excessive irradiation. We measured light penetration, oxygenic photosynthesis, respiration and sulfate reduction in

salt crust-covered mats at saturation-level salinity and after mimicking a natural flooding event leading to dissolution of the salt crust and reduction of salinity in the mats. The microbial composition of individual mat layers was determined by microscopy, pigment and 16S rRNA gene amplicon analysis.

Results

Geochemistry and mat characteristics

The mats we studied were identified as a cohesive leathery structure easily separable from the sediment. The mats were laminated into five distinct layers, each 0.5-1.5 mm thick, and were (going from the surface downwards) colored orange, green, brown, black and grey (Fig. 1). No gypsum, calcium carbonate or halite precipitates were found inside the mat matrices. The upper two layers (orange and green) were highly gelatinous while the lower two layers (black and grey) contained a higher fraction of sediment particles. The average porosity in the upper 5 cm was 0.35.

During the field trip in February 2018 (Fig. S2) the temperature at midday below the salt crust was 35°C, which was 5°C above the air temperature. In the early morning, the temperature below the crust was 22°C, equal to the air temperature. The porewater salinity in the mats under the salt crust was 30% (Fig. 1). In January 2019, additional mat samples for laboratory experiments (Fig. S2) were collected from the same site at the same salinity and inundation conditions. At the MPI Bremen laboratory, measurements of chloride concentrations by ion chromatography revealed that the pore water salinity of salt crust covered mats without overlaying water was 40%. In order to simulate a tidal event, the cores were inundated in seawater (salinity 3.5%). Three days after inundation the porewater salinity in the mat dropped down to 12%. Below the mat the salinity gradually increased with depth up to 30% at 3 cm. After the inundation experiment, the water covering the mats was left to evaporate (Fig. S2). A new salt crust formed within three days, while the porewater salinity in the mat increased back to 40%. The sulfate concentration in the porewater from the upper 5 cm remained within the same range before (230 and 390 mM) and after inundation (170 to 370 mM).

We performed microscale spectral attenuation measurements to determine the light conditions beneath the crust. In the salt crust rather little (~1%) light attenuation occurred, and attenuation was rather uniform across the spectral range (Fig. 2A). This showed

absence of light harvesting pigments or sedimentary material in the salt layer. Below the salt layer, the light was attenuated exponentially as is typical for sedimentary matrices such as mats. When the salt crust was dissolved, a very similar pattern of spectra was observed (Fig. 2B). Within 3.5 mm into the mat (brown or black layer) PAR light was attenuated to 1% of the mat surface level both, with and without salt layer.

Microbial processes

We measured the essential metabolic processes such as oxygenic and anoxygenic photosynthesis, respiration, sulfate reduction and sulfide oxidation under the salt crust at saturation-level salinity to test if the microbial community can remain metabolically active and sustain itself under this condition. These measurements were compared to measurements performed after inundation of the mats with seawater to mimic flood conditions.

Oxygenic photosynthesis and respiration

In *in-situ* microbial mats and in freshly sampled cores (salinity 30%), elevated oxygen (O_2) concentrations were found in the upper 3-4 mm (corresponding to orange to brown layers, Fig. 1), indicating oxygenic photosynthesis activity. However, when recreating field conditions at SQU in Muscat by inundating the cores with water from the site (salinity 30%) for three days no O_2-production could be measured (Fig. S3). Subsequent tide simulation by inundating the cores in 5% salinity water induced a high O_2 peak in the mat after two days (Fig. S3). When the overlying water was then exchanged back to 30%, lower levels of O_2-production could still be measured for up to 90 min after the change (Fig. S3).

Further cores were transported to MPI Bremen for more detailed analysis. First, O_2 profiles were measured in salt-crust covered cores without overlaying water (pore water salinity was 40% determined by ion chromatography chloride measurements). O_2 profiles measured through the salt crust and into the mats did not show an O_2 peak and O_2 levels did not change upon illumination, indicating complete absence of oxygenic photosynthesis (Fig. 3A). The O_2 profile through the salt crust was flat, indicating absence of significant diffusion resistance. The cores were inundated in seawater and subjected to a 12 h light 12 h darkness cycle while O_2 profiles were continuously measured (Fig. S4). Two days after

inundation by seawater and after the disappearance of salt crust, O_2-production in light conditions commenced (Fig. S4) and increased further on the third day (salinity in the green layer dropped to 12%, Fig. 3D). Two O_2 peaks or shoulders developed, indicating two zones of photosynthesis, close to 2 and 4 mm below the mat surface (Fig. 3D). The respiration rate in the dark increased 9-fold upon decreased salinity (Tab. 1).

Sulfide oxidation and anoxygenic photosynthesis

The sulfide and O_2-profiles (Fig. 3) showed an overlap of the sulfidic- and the oxic zone, where sulfide was consumed aerobically. The pH under the salt layer in the dark decreased with depth, but showed a small peak at 2 mm depth (green layer) under (artificial) illumination (Fig. 3A). Sulfide diffused upwards from 5 mm depth, and under illumination receded slightly and decreased in concentration (Fig. 3B). The pH peak and the sulfide recession during illumination indicated anoxygenic photosynthesis activity below 2 mm depth. The areal sulfide fluxes, where overlapping with the oxic zone, were similar in light and dark. Although from such measurements no areal rates of anoxygenic photosynthesis could be calculated, the minimum distribution of net anoxygenic photosynthesis could be confined to between 2 and 4 mm from the mat surface as the zone of net sulfide consumption under illumination (Fig. 3C).

Sulfate reduction

Sulfate reduction in the mat and underlying sediment (up to 30 mm depth) was detected under the salt crust (40% salinity) and in mimicked flood conditions (12% salinity); several samples were below the detection limit of 0.05 µmol m^{-3} s^{-1} and the rates were rather variable between cores. Under flood conditions average sulfate reduction rates in the mat increased 18-fold (Tab. 1). However, highest rates were found at 7 mm depth (Fig. 4), which, considering not perfectly even mat thickness, could be in the grey mat layer or the sediment directly below the mat. Interestingly, the sulfide fluxes decreased upon reduction of the salinity, indicating a rebalancing between production and consumption processes (an increase in sulfide oxidation).

Microbial community stratification

We determined the fine-scale stratification of the microbial community catalyzing the measured processes based on distribution of photopigments correlated to microscopic observation and quantification of microbial cells. The taxonomic identities of microbial community members in different layers were determined by amplicon sequencing of the 16S rRNA gene.

Distribution of phototrophs based on photopigment stratification

The distribution of photopigments, as assessed by microscale spectral attenuation (Fig. 5) and hyperspectral imaging (HSI, Fig. 6), showed absorption signals corresponding to Chl-*a* (675 nm), phycocyanin (625 nm), BChl-*a* (845 nm, 902 nm) and BChl-*c* (745 nm). The depth distribution of Chl-*a* determined by spectral attenuation showed two peaks separated by ~1 mm (Fig. 5B). With HSI the two peaks were less distinguishable and, based on depth, likely appear as one main upper layer (Fig. 6). The depths of the Chl-*a* bands determined from spectral attenuation profiles (Fig. 5B and C) differed slightly from the depths determined by HSI, probably due to lateral variability in the structure of the mats, which is well depicted in the hyperspectral images (Fig. 6). Additionally, HSI revealed multiple weak layers of chlorophyllic pigments and derivatives in the black and grey layers of the mat and in the sediment below. These lower chlorophyllic layers seen in HSI were also observed in chromatographic analysis of pigments extracted from 2018 mat samples (Fig. S5). In chromatographic analysis Chl-*a* was present throughout the profile peaking in the green layer (~1-2mm depth) and showing a second, lower peak in the grey layer (~4-5 mm). However, chromatography showed that the grey mat layer also contained increased proportions of chlorophyll degradation products such as chlorophyllide-a and pheophorbide-a. Together with the microscopic observation of empty cyanobacterial sheaths at this depth (Fig. S6), it suggests that chlorophyll pigments are not functional in these layers.

A layer of phycocyanin, a water-soluble antenna pigment produced by cyanobacteria, was correlated to the main Chl-*a* layer (Fig. 5, Fig. 6), with spectral attenuation measurements locating it at the depth of the lower Chl-*a* peak (Fig. 5). BChl-*a* was detected below the main

Chl-*a* layer and above the BChl-*c* layer with both methods (Fig. 5, Fig. 6). BChl-*a* was peaking at 3-4 mm and BChl-*c* below 4 mm. The depth distributions of pigments were similar before and after removal of the salt layer by inundation, showing that there was no dramatic change of distribution of phototrophs due to change in salinity (Figs. 5B and C). However, some minor differences were observed after the salt dilution, such as an increase of Chl-*a* and BChl-*a* at the very surface, a minor increase in phycocyanin in the upper 1.5 mm, and appearance of BChl-*c* peak at 5 mm depth.

Distribution of phototrophs by microscopy

Cell numbers per gram mat wet weight were determined by fluorescence microscopy of fixed material (Fig. S6, Fig. S7). The cell numbers in the upper three layers of the mat (orange, green and brown, 1.2×10^{10} to 1.6×10^{10}) were four times higher than in the lower layers (black and grey, 3.5×10^{9} – 4.3×10^{9}) (Fig. S7). *Cyanobacteria* and *Chloroflexi* were distinguished by their morphology and auto-fluorescence (Fig. S6). *Cyanobacteria* cells were large in size but low in numbers (Fig. S6 and S7). Highest number and proportion of filamentous *Cyanobacteria* cells was observed in the green layer (1×10^{9}). Highest numbers of large unicellular *Cyanobacteria* were observed in the brown layer (1×10^{8}). *Chloroflexi* had highest numbers in green (2.2×10^{9}) and brown (2.9×10^{9}) layers.

Besides lower cell numbers, the black and grey layers also contained a higher amount of organic debris, such as empty cyanobacterial sheaths and possibly aggregates of dead microbial cells, visible as a faint fluorescence in the DAPI channel (Fig. S6). The cells in the lower two layers were visibly thinner than in the upper three layers.

Stratification of microbial community by 16S rRNA gene analysis

The sequence analysis supported a community that was clearly structured in layers containing different phototrophs (Fig. 7, File S1). Two groups of primary producers were found - oxygenic phototrophs and potential anoxygenic phototrophs. Remarkably, *Cyanobacteria* constituted a relatively minor proportion of the sequence reads (Fig. 7A). Cyanobacterial sequences in the upper-most orange layer harbored unclassified *Nostocales* (1%), *Dactylococcopsis* (1.4%), and *Geitlerinema* (0.4%) (Fig. 7B), while the green layer

256 contained *Dactylococcopsis*- (2%) and *Coleofasciculus*-related (1.1%) taxa. In the brown
257 layer only unclassified *Nostocales* (0.24%) were detected. Sequences related to
258 *Synechocystis* were only found in the black layer (0.24%).

259 Regarding anoxygenic phototrophs, the brown layer was dominated by sequences classified
260 as *Candidatus* Chlorotrix (29%) (Fig. 7A), an anoxygenic sulfur-oxidizing phototroph from the
261 *Chloroflexi* phylum (31). Other known sulfur-oxidizing taxa, be it phototrophic or
262 chemolithotrophic, were barely present (Fig. 7B). Thus, sulfide oxidation is probably strongly
263 dominated by anoxygenic phototrophic *Chloroflexi*.

264 Sequences attributed to sulfate reducing bacteria (SRB, Fig. 7B) were found mainly in brown,
265 black and grey layers and were dominated by *Desulfovermiculus*. SRB were clearly excluded
266 from the oxic zone.

267 Large fractions of the sequences were assigned to heterotrophic or photoheterotrophic
268 microbial clades neither contributing to primary production nor involved in the S-cycle, such
269 as photoheterotrophic *Rhodovibrio* that were abundant in the upper layers (max. 19% in the
270 green layer). Other highly abundant groups were *Salinibacter* (10% in the orange and green
271 layer) and an unclassified genus of *Rhodothermaceae* (23% and 13% in the orange and
272 green layer, respectively). The lower three layers showed increased proportions of
273 uncultured *Marinilabiliaceae*, likely anaerobic fermenters (13% in the black layer).

274 We noticed high relative abundances of archaeal sequences within the mats, reaching a
275 maximum in the black layer. In this layer, archaeal sequences constituted on average 56% of
276 all reads, with *Halobacteriales* genera *Salinigranum*, *Haloplanus*, and *Natronomonas* being
277 the most abundant (Fig. 7A). Among archaeal sequences we detected only two potentially
278 methanogenic taxa: not further classified members of *Methanosarcinaceae* and
279 *Methanofastidiosales* whose relative abundance did not exceed 0.03% in any of the samples.

Discussion

Investigation of microbial activity at various salinities, ranging from 12% (w/v) simulating a flood, 30% in the field and 40% in mats with fully evaporated overlaying water, revealed that metabolic processes were differently susceptible to salinity stress. At saturation salinity (40%), we were able to measure anoxygenic photosynthesis, aerobic respiration, phototrophic sulfide oxidation and some sulfate reduction which is largely in agreement with current knowledge on microbial communities in salt-saturated environments (15). Oxygenic photosynthesis showed to be more restricted and could only be detected up to a salinity of 30%. Regarding the salt crust, we could not confirm any significant protection against dehydration and irradiation for the microorganisms in the underlying microbial mat. The microprofiles showed that the salt layer forms no resistance to O_2 transport and thus we conclude that gases, including water vapor, can easily exchange and the mats below the salt remain sensitive to desiccation. The temperature below the crust at midday was 5°C higher, while the seawater surface temperature was 9°C higher than air. Therefore, it seems that the salt crust does reduce the mat temperature to some extent as also noticed by Baas Becking (28). Our data further show that the salt transmitted PAR easily (Fig. 5A), and also UV and near-infrared radiation were hardly attenuated (Fig. 2). The idea that microbes below such a salt layer "are, in terms of light intensity, practically living in the deep sea" (28) can thus not be supported. Instead, light can easily penetrate salt crusts, allowing phototrophy in the underlying mat if the microbial community can perform it under salt-saturated conditions.

Primary production

Our results clearly show the absence of oxygenic photosynthesis and cyanobacterial primary production at saturation-level salinities (40%). At salinities of around 30% the results seem more ambiguous at first. In 2018 we were able to measure O_2 oversaturation at 30% salinity *in-situ* and in the field laboratory (Fig. 1). In 2019 however, after transport to the laboratory at SQU, O_2-production in the mat at 30% was only measurable after first inundating the mat with low salinity water (5%) and then returning back to 30% (Fig. S1, Fig. S2). It is important

307 to note that these salinities (5% and 30%) are salinities of the overlaying water, not of the
308 porewater within the mat, which might take longer to equilibrate. This suggests that the upper
309 salinity limit for oxygenic photosynthesis in these mats lies somewhere around 30% NaCl
310 (w/v). Oxygen production at such high salt concentrations has not been measured
311 previously. *Dunaliella* algae are assumed to be the sole primary producer in salt-saturated
312 brines based on their high abundance (17) and unpublished reports on photosynthesis in
313 these environments (32). In culture, *Dunaliella* photosynthesis has only been measured up to
314 a salinity of ca. 18% (3M NaCl) (21–23) while growth is known to occur up to 29% (5M NaCl)
315 (24, 25), albeit at much slower rates. Unicellular *Cyanobacteria* were previously shown to
316 grow in special media saturated with NaCl, but not in salt-saturated natural waters (33).

317 Although *in situ* O_2 production by unicellular cyanobacteria has been indicated in halite
318 crystals from the Atacama desert (13), the very high reported O_2 concentrations even in the
319 dark (4-5 mg/L, exceeding the O_2 solubility of 2.8 mg/L at 20°C, 20% NaCl) suggest that the
320 salinity of the medium was in fact below saturation (based on O_2 concentrations in the dark,
321 probably 10-15% NaCl). The same study also reported pulse-amplitude modulated
322 fluorescence measurements indicating activity of photosystem II (13). However, this method
323 is challenging to apply to cyanobacteria, where it might measure fluorescence of
324 phycobilisome pigments instead of photosystem II (34, 35). Further, presence of functional
325 and active chlorophyll does not necessarily indicate ongoing oxygenic photosynthesis.
326 Phototrophic microorganisms can use their photosystems to generate proton-motive force
327 and ATP via cyclic electron transfer without coupling to CO_2 fixation (36). Such
328 photoheterotrophic mode of operation is also suggested to play a role in desiccation survival
329 by arid surface soils microorganisms (37). Studies monitoring *in situ* O_2 production in
330 hypersaline microbial mats up to 26% NaCl showed a sharp decline of photosynthesis with
331 increasing salinity (26, 27, 38). Extrapolating these data also suggested an upper salinity
332 limit for oxygenic photosynthesis of 30% (26), which is supported by our experimental data.
333 During our fieldwork, the mats were covered with water indicating a recent influx of low-
334 salinity water and that *in-situ* salinity was below saturation. In absence of dilute water, the
335 salinity will rapidly increase to saturation, as it happened within three days in our laboratory

experiment even at room temperature and under artificial light. A transect study of the tidal flat found the same area dry in December 2014 (30) indicating fluctuating water levels. Considering higher temperatures and irradiation in summer and lack of daily tides at the site, the mats will often be exposed to saturation-level salinities under which their photosynthetic activity is halted.

Anoxygenic photosynthesis, on the other hand, was detected even at salt saturation conditions, based on reduced sulfide concentrations under illumination (Fig. 3). This process is thus more robust against salinity stress allowing the anoxygenic phototrophs like *Candidatus* Chlorothrix (31) to prosper. Also some of the *Cyanobacteria* might be capable of anoxygenic photosynthesis (39, 40). Since phototrophic sulfide oxidizers do not produce O_2, they are not limited by decreased O_2 solubility at high salinities as suggested for oxygenic phototrophs (26).

Oxygenic phototrophs

In agreement with a previous study (30), the mat community contained several taxa of *Cyanobactera*, including filamentous genera like *Coleofasciculus* and *Geitlerinema* which is unusual for such hypersaline environment (16). While low relative abundances of *Cyanobacteria* in sequencing data are known from other hypersaline mats (1, 2), they may be an underestimation of cyanobacterial biomass as indicated by our fluorescence microscopy data (Fig. S4 and S5) and supported by pigment analysis. The combination of optical profiling, hyperspectral scanning, microscopy, and 16S rRNA-based analysis provide a coherent picture of a stratified and complex phototrophic community, consisting of two layers of different *Cyanobacteria* below which a zone of anoxygenic phototrophs resides. The double peak in O_2-production aligns with these observations. The spatial separation of two different groups of cyanobacteria could be explained by adaptations to different microenvironments. These microenvironments ca. 2 mm apart differed in several parameters: within 2 mm the light levels attenuated with 1-2 orders of magnitude (Fig. 5A), salinity was likely lower in the upper photosynthetic zone during inundation, and during illumination sulfide never reached the upper zone. Sulfide is a toxic compound that can

364 inhibit photosynthesis in many cyanobacteria, while other species are resistant to sulfide or
365 are even stimulated by it (41). Possibly, the mostly unicellular *Cyanobacteria* in the upper
366 zone are more sensitive to sulfide, whereas the *Coleofasciculus* in the lower layer are more
367 sulfide-tolerant. *Coleofasciculus chtonoplastes* (previously *Microcoleus chtonoplastes*), for
368 example, was shown to be capable of phototrophic sulfide oxidation (42). Unicellular
369 *Dactylococcopsis* seems to have a broad sulfide tolerance range as its sequences were
370 present in both layers. Species of non-motile *Synechocystis,* detected in the brown layer,
371 were reported to contain genes linked to sulfide oxidation and it is speculated that they can
372 perform anoxygenic phototrophy on sulfide (43).

373 In the inundation experiment where high photosynthesis rates were observed, the overall
374 amount of photopigments did not increase strongly (Fig. 5), indicating that the increase in
375 activity was due to a revived community and not due to an increase in phototrophic
376 population or reconstitution of structurally altered pigments (44, 45). This similarity in vertical
377 structure and population size of the phototrophic community in the mat with salt crust and the
378 inundated mat indicated that salinity change induced only minor migration compared to
379 previously studied hypersaline (46, 47) and desiccated (45) microbial mats and likely no
380 substantial growth of the phototrophs.

381 **Anoxygenic phototrophs**

382 Similar to other hypersaline microbial mats (2), we observed a large fraction of *Chloroflexi*
383 sequences in the brown layer below the cyanobacteria-containing layers, which overlaps well
384 with the layering of Chl-*a* and BChl-*c* as determined from the light spectra (Fig. 5) and HSI
385 (Fig. 6). The activity of *Chloroflexi* is hard to assess due to their large metabolic flexibility.
386 They can act as heterotrophic- and autotrophic anoxygenic phototrophs, and can respire a
387 large diversity of compounds with O_2, including sulfide (48–52). The detected *Chloroflexi*
388 (closely related to *Candidatus* Chlorotrix halophila) probably dominated the sulfide oxidation,
389 as other potentially sulfur-oxidizing taxa were barely present in the sequence data. BChl-*a*,
390 which is usually assigned to purple sulfur bacteria, probably stems from photoheterotrophic
391 *Rhodovibrio* (53) whose sequences have high relative abundances in the orange, green and

brown layers. However, it is noteworthy that the peak of BChl-*a* was observed below the cyanobacteria layers, whereas *Rhodovibrio* sequences are also abundant at the very top of the mat. This could mean that the *Rhodovibrio* populations in the orange and green layer rely entirely on organoheterotrophic energy generation, whereas the population deeper in the mat produces bacteriochlorophyll for light harvesting.

Sulfate reduction

Sulfate reduction rates under the salt crust at a salinity of 40% were low but detectable. However, upon decreasing the salinity, sulfate reduction rates in the mat increased 18-fold (Fig. 4, Tab. 1). While originally thought to be thermodynamically unfavorable at high salinities (54), some studies have reported low, yet present sulfate reduction rates in hypersaline sediments and mats (11, 55–57). Other recent reports, however, still come to the conclusion that sulfate reduction is inhibited at salt-saturation (58, 59). Our data suggest that at extreme salinities even if growth of sulfate reducers is stopped, energy generation might still continue, albeit at a lower rate. Unlike in other mats, where SRB and significant sulfate reduction rates were found in oxic layers (60–63), the relative abundance of SRB increased with depth following the classic concept of anaerobic organisms being confined to anaerobic zones. Sulfate reduction in the photic zone remained low even after reducing the salinity. The highest sulfate reduction rates were measured at 7 mm depth, which is at the lower border of the mat or even below the mat itself. Thus, we conclude that the main source of sulfide is the underlying sediment.

Although the sulfur cycling is profoundly influenced by the salinity, using microsensors we found similar sulfide profiles and total sulfide pools in mats at high and low salinities. With a total sulfide pool of 0.2 mol m^{-3} (Fig. 3) and sulfate reduction rate of 2.4 x 10^{-6} mol m^{-3} s^{-1} (Table 1) the turnover rate of sulfide is approximately one day during low salinity periods. Thus, as the sulfate reduction increases with a factor of 20, the sulfide consumption must also increase by the same factor, as otherwise the total sulfide pool would increase with 0.2 mol m^{-3} each day. As sulfide oxidation occurs mainly by anoxygenic photosynthesis, this process must also be strongly stimulated by reducing the salinity.

Note on heterotrophic microorganisms and archaea

Besides microorganisms participating in CO_2-fixation and sulfur-cycling, we detected a variety of heterotrophic or photoheterotrophic microorganisms such as various *Halobacteriales* archaea, *Rhodovibrio* and *Salinibacter* (53, 64, 65), which were previously mostly known to have planktonic lifestyles. Considering the high relative abundance of their sequence reads in the mats, these microorganisms likely have an active role in the mat community and contribute to carbon cycling. The proportion of *Archaea,* represented by members of known mostly photoheterotrophic taxa (various members of *Halobacteriales*), was much higher than the usually assumed 1-20% in mats (1). In fact, with 56% of archaeal sequences in the black layer, this is so far the highest reported proportion for microbial mats (56). Although it has been observed that methanogens can outcompete or live syntrophically with sulfate-reducing bacteria in hypersaline mats (66), this seems not to be the case in the Oman mats as sequences attributed to methanogenic archaea were barely present. However, we cannot exclude the possibility that the methanogenic zone in this environment lies deeper in the sediment.

Conclusion

Although the salt crusts offer little protection in terms of attenuating light or maintaining humidity, the underlying microbial mat community retained the capability to perform all essential tasks for a viable ecosystem with internal recycling of the main elements: primary production and mineralization of organic matter. However, this theoretical capability is not always operational, as oxygenic photosynthesis activity is inhibited during the saturation salinity (40%) periods. As no daily tides were observed at the site, these salinities are likely to occur during summer, whereas inundation occurs either during landward storms or winter rains. At saturation salinities, primary production can be sustained for a while by anoxygenic phototrophy, however, the reducing power needed for CO_2-fixation is provided by sulfide, which is in turn supplied by sulfate reduction fueled by organics. As losses of reduced matter will inevitably occur (organics or sulfide), an ecosystem reliant on anoxygenic phototrophy for primary production and sulfate reduction for mineralization cannot be sustained. The

microbial ecosystems below salt crusts thus ultimately depend on periods of reduced salinity allowing oxygenic photosynthesis or on import of reduced substances e.g. from other ecosystems or from geothermal venting. Additionally, strongly reduced rates of other microbial energy generation processes such as aerobic respiration and sulfate reduction suggest that the microorganisms in the studied mats are adapted to endure and survive the saturated salinity conditions, if sporadically diluted, rather than to thrive in perennially saturated conditions.

Conflict of interest

Authors declare no conflict of interest.

Author contributions

RA, DdB, and DW conceived and coordinated the study. RA, DdB, DM and DW designed the experiments. AC, DdB, MvE, AG, DM, and TM did the experimental work. DdB, DM, and AC analyzed interpreted the data. DdB, DM and DW wrote the manuscript, with contribution of all co-authors.

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Materials and methods

An overview chart of the sampling events and order of conducted experiments can be found in Supplementary Figure 1.

Site description and sampling

Microbial mats were sampled at an irregularly inundated tidal flat in Oman (20°45'39.6"N 58°38'52.3"E), approximately 6.5 km from the low water line in February 2018 (Fig. 1, S1). During our sampling, the area up to 5 km away from the coast was affected by daily tides, whereas stagnant water (30% salinity) and halite crust (composition in Tab S1) covering the bottom was observed further inland (Fig. S1). The whole area is covered by microbial mats with their appearance, thickness and composition changing with the distance to the sea (as described in 30). These mats are naturally exposed to drastic salinity changes. When water evaporates, salinity approaches saturation and accumulating marine salts form a crystalline crust on top of the mats (Fig. 1). Tides, on the other hand, rapidly bring in low salinity water (ca. 5%), dissolving the crust. For this study we sampled microbial mats at the upper tidal line that were covered by several millimeters of crystalline salt and ca. 4 cm of stagnant water. The mats, coherent leathery biofilms, grew on top of permeable sandy sediment and were covered by a 2-3 mm thick salt crust. Mats were easily liftable off the sediment with only a thin layer of loose sediment grains sticking to the downside of the mat. Mats were recovered in February 2018 and January 2019; an overview of conducted measurements is provided in Fig. S2.

Light intensity reached 1799 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at 12:00 and fell to 552 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at 16:30 (Fig. 1D). On site, salinity of the overlaying water (measured throughout the sampling campaign) and porewater extracted from three sediment cores with rhizons (Rhizosphere Research Products, Wageningen, The Netherlands), hydrophilic porous (pore-size 0.15 µm) polymer tubes connected to a syringe by a poly-vinyl-chloride tube (67). was measured with a refractometer after a 1:10 dilution. Crystalline salt was collected and sent for ion composition analysis to the Austrian Agency for Health and Food Safety (AGES) (Tab. S1).

Sediment sampling for porosity determination was conducted in 5 cm intervals from 0 to 20 cm depth, from three different cores. Volumes of 2.5 mL of sediment were collected using cut-off syringes and transferred to exetainers. Porosity was determined by drying a known volume of sample at 60°C to a constant weight.

Three mat patches were collected approximately 1 m away from the O_2 measurement site for DNA, pigment, and microscopic analysis. For these samples, the salt crust was removed from the mat and remaining salt crystals washed off with deionized water. For each of the three mat patches the layers were separated with a scalpel according to their color: orange, green, brown, black and grey (each 0.5-1.5 mm thick, Fig. 1) and stored in separate tubes at -20°C. A fraction of the material was preserved for fluorescence microscopy analysis; it was fixed with 4% paraformaldehyde (PFA) in 1x phosphate buffered saline (PBS) (pH=7.6) for 1.5 h at room temperature, washed twice with 1x PBS and stored in an ethanol:PBS mixture (1:1) at -20°C until further analysis. A second fraction was stored in separate tubes at -20°C until pigment analysis.

Cores from the same site were collected in January 2019, at exactly same salinity and flooding conditions (30% salinity, 2-4 cm water level) and brought to the laboratories of Sultan Qaboos University (SQU) in Muscat, Oman, and to the Max Planck Institute (MPI) in Bremen, Germany, for detailed biogeochemical analyses (Fig. S2). Cores were transported without the overlaying water (but with the salt crust covering the mat) to avoid accumulation of sulfide.

Field experiments

Oxygen (O_2) depth-profiles were measured with microsensors (detailed description of microsensor techniques below) at two different positions *in-situ* and in two cores (consisting of > 10 cm of sediment, mat, salt crust and hypersaline water) in a nearby field laboratory. In the field and field laboratory hardened salt-crust was carefully removed in order not to break the sensor. Loose salt crystals were then added on top of the mat. Separate O_2 measurements in cores were performed with artificial light (~600 $\mu mol \cdot m^{-2} \cdot s^{-1}$, KL1500 halogen lamp, Schott, Mainz, Germany) and natural illumination.

Salinity shift laboratory experiments

For transport, the water was drained from the cores and a head space of one quarter of the core was left above the salt crust. The cores were transported upright in a dark box with coolant packs. The experiments at the MPI Bremen were conducted seven days after the sampling. At SQU, Muscat, cores were inundated with water from the sampling site right after the sampling. The development of oxygen profiles upon salt dilution in the samples send to the laboratory in Bremen were very similar to the samples kept in Oman, both in extent and temporal dynamics (Fig. 3A, Fig. S3) indicating that the microbial community was well preserved during transport.

At the MPI, microbial processes were first measured immediately upon arrival in moist cores that contained salt-saturated porewater and no water above the salt crust (Fig. S2). To study the effect of a strong tide or flood, the mats were inundated with filtered seawater (salinity 3.5%) and kept under 12 h light (600 $\mu mol \cdot m^{-2} \cdot s^{-1}$) - 12 h dark cycling conditions with continuous measurement of O_2 profiles (Fig. S4). The salt crust dissolved after 1.5 days and after three days the salinity in the upper mat (0-3 mm) dropped to 12% (Fig. S2). After all microsensor measurements on the inundated cores were finished, the water was left to evaporate for three days, resulting in 40% porewater salinity and a reformed salt crust (Fig. S2). The effects of these transitions on photosynthesis, respiration and sulfate reduction, as well as photopigment distribution were measured as described in detail below.

At SQU, two cores were inundated with water collected from the site (30% salinity) for three days and O_2 profiles under natural illumination were measured (see microsensor method description below). Subsequently the water was carefully decanted and exchanged for 5% salinity (diluted water from the sampling site) and after two days O_2 profiles were measured again. The water was replaced with 30% salinity again and O_2 profiles were measured immediately and up to 90 min later.

Microsensor measurements

Microprofiles of O_2, pH, and sulfide (H_2S) were measured through the salt crust and into the mats in two cores to detect and quantify metabolic activities. Microsensors for O_2, H_2S and pH were made, calibrated, and used as described previously (8, 68–70). At the MPI laboratory, a thin hole was made in the salt crust with a 0.4 mm needle mounted on the microsensor manipulator, through which the sensors were inserted. The sensors were mounted on a micromanipulator so that they penetrated at an angle of 40° to avoid shading of the studied area by the equipment. The mat surface was set as 0 mm depth.

Local diffusive fluxes of O_2 and H_2S were calculated from three steady state microprofiles, and the change in fluxes with depth was used to calculate local conversion rates as described previously (71). The assumed diffusivities were 1.5×10^{-9} m^2 s^{-1} for O_2 and 1×10^{-9} m^2 s^{-1} for S_{tot} (D_{Stot}=0.64 x DO_2) (7), and were corrected for a porosity of 0.3. Volumetric respiration and photosynthesis rates in Table 1 represent average values across mat depth where O_2 concentrations were above zero.

Geochemical analysis

Sulfate reduction rates were determined by radiotracer incubation of sediments from the same cores as used for laboratory microsensor measurements at the MPI before (in triplicate) and six days after the inundation (in duplicate) (Fig. S1), in 5 mL cut-off syringes that were closed with a butyl rubber stopper. A volume of 25 µL of $^{35}S\text{-}SO_4^{2-}$ tracer solution (200 kBq per syringe core) was inserted in the syringe cores past the butyl rubber stopper. Sediments were incubated for 6.5 hours in the dark at 26°C under an N_2 atmosphere. To stop the reaction, sediment samples were transferred to 2 mL 20% (w/v) ZnAc and stored at -20°C. The top 3.2 mm (volume of 0.4 mL) was sampled, and below this depth subsampling was conducted in 8 mm (volume of 1 mL) intervals. Further treatment of the samples and quantification of the rates were done as described previously (72). Blanks were directly transferred to 2 mL 20% (w/v) ZnAc after addition of 200 kBq of the tracer solution, and were used to calculate the minimum detection limit (MDL) of the measurements.

578 Porewater sulfate and Cl^- were determined on parallel cores. Sampling was conducted as
579 described above for sulfate reduction measurements. Subsamples were transferred to 15 mL
580 centrifuge tubes and stored at -20°C until further processing. When overlaying water
581 evaporated and a new salt crust formed (porewater salinity returned to 40%), sampling was
582 repeated. All samples were transferred to Ultrafree-MC GV filter units with a 0.22 µm
583 Durapore PVDF membrane and centrifuged for 15 min at 14000 rpm. Subsequently,
584 retrieved porewater was transferred to Eppendorf tubes prefilled with 5% (w/v) ZnAc and
585 stored at 4°C until further processing. Both sulfate and Cl^- concentrations were determined
586 using ion chromatography (Metrohm 930 Compact IC Flex). Cl^- concentrations (µM) were
587 converted to salinities (%).

588 **Optical profiling**

589 Optical profiling to determine the layering of photopigments in the mats was performed at the
590 MPI Bremen on two cores sampled in 2019 (Fig. S2). Mat cores were illuminated by artificial
591 light with 600 $\mu mol \cdot m^{-2} \cdot s^{-1}$ intensity (KL1500 halogen lamp, Schott, Mainz, Germany). Scalar
592 irradiance optical microsensors (Zenzor, Denmark) were used to profile the light field within
593 the salt crust, mat and sediment layers (73). The salt layer was first penetrated with the
594 optical fiber retracted into the needle to create a small hole to avoid damaging the spherical
595 tip of the sensor probe. With the sensor extended out of the needle, the irradiance was
596 measured every 400 µm within the salt, mat, and sediment layers using a spectrometer
597 (USB2000+, Ocean Optics USA) attached to the other end of the optical fiber. Each
598 spectrum was smoothened with a linear Savitzky-Golay filter (15 nm window, two passes)
599 and compiled into a depth profile, with the zero-value set at the mat-salt interface. The
600 irradiance spectra were normalized so that values represented the fraction of light at the salt-
601 mat interface. Light intensity profiles were collected into three channels: ultra-violet (UV, 200-
602 400 nm), photosynthetically active radiation (PAR, 400-700 nm) and near infra-red (NIR, 750-
603 950 nm). Spectral attenuation was calculated as the negative gradient between the
604 consecutive log-transformed spectra of each profile. Absorbance peaks corresponding to
605 photopigments chlorophyll *a* (Chl-*a*, 674 nm), phycocyanin (624 nm), bacteriochlorophyll *a*
606 (BChl-*a*, 845 nm & 902 nm) and bacteriochlorophyll *c* (BChl-*c*, 745 nm) were identified in the

attenuation spectra. The abundances of photopigments at a depth level were estimated as the area (if convex) under the attenuation curve within a 10 nm window around the peak absorption wavelengths.

Hyperspectral imaging

Hyperspectral imaging was done on the cores used for experiments at the MPI in 2019 (Fig. S2) in order to assess the lateral variability and depth distribution of photopigments within the mat. After the flooding followed by restoration of the salt crust, the core was subsampled to a depth of 5 cm using a syringe of 1 cm diameter with its tip cut-off. The sample was sliced longitudinally to expose a cut-section of the mat and scanned with a hyperspectral imager, as described previously (74), with a spatial resolution of ~60 µm/pixel. The hyperspectral image, normalized to reflectance, was used to calculate the distribution of photopigment abundances by quantifying the absorption signals of photopigments in second-derivative spectral analysis (45, 75).

Pigment extraction and measurement

Mats sampled in the field in 2018 (Fig. S1) were sectioned in layers according to color, as described above for the DNA and microscopy analyses. Three samples of each layer were freeze-dried and extracted twice with 1 mL ethanol:acetone:water (45:45:10) solution in each step in a 4°C cooled ultrasonic bath for 90 minutes each run. The supernatant was collected by centrifugation for 4 minutes at 16000 x g (Eppendorf 5415C centrifuge) and stored at -20°C until analysis. Pigment extracts were then injected into a Waters UPLC H-Class system (Waters, Milford, MA, USA) equipped with an Acquity UPLC BEH C18 column (1.7 µm, 2.1x150 mm) (Waters Corporation, Milford, USA). Peak separation was based on an existing protocol (76) with gradients modified for UPLC instruments. The gradient started with 96% solvent A (75% methanol: 25% water buffered with 0.5 M ammonium acetate) and 4% solvent B (90% acetonitrile: 10% water) and increased linear to 100% B at 0.75 minutes. At 2.63 minutes, 10% solvent C (100% ethylacetate) was added and slightly increased over 40% (at 6.38 min) to 70% at 10.5 minutes and stayed constant to 12.59 min. Subsequently,

the gradient was made 100% B at 12.97 min, and at 13.34 min, the column was re-saturated with the initial mixture of A and B. The column temperature was 30°C and the flow rate was constant at 0.2 mL/min). Data were analyzed with the software package Empower3 (Waters, Milford, MA, USA). For total concentration, standards with defined concentrations were measured and response factors for all analyzed compounds were calculated.

Microscopy

For microscopic studies, 80 to 200 mg of PFA-fixed materials from different mat layers sampled in the field in 2018 (Fig. S2) was subjected to seven rounds of sonication with a UW 2070 probe (Bandelin Electronic, Berlin, Germany) (30 s at 20% power), after each round 1 ml of supernatant was collected and replaced by 1 ml of ethanol:PBS (1:1) mixture. Collected supernatant was filtered on 0.2 µm pore-size poly-carbonate filters (Sartorius, Göttingen, Germany), 30 µl per filter. The DNA of cells on filters was stained with 4',6-diamidine-2'-phenylindole dihydrochloride (DAPI) and inspected under a fluorescence microscope. The number of cells per gram of mat (wet weight) was calculated based on DAPI signal counts. In filaments, individual cells delineated by visible septa were counted (Fig. S5), not filaments as a whole. *Cyanobacteria* were identified by strong red auto-fluorescence of chlorophyll in the 660 nm channel in addition to strong orange auto-fluorescence of carotenoids in the 550 nm channel. Separate counts were made for cyanobacterial cells arranged in long filaments and large oval single cells. Further, cells with only orange auto-fluorescence in the 550 nm channel and filamentous morphology were counted as "*Chloroflexi*" (bacteriochlorophyll auto-fluorescence peaks at 790 nm and is not visible in the 660 nm channel). Cell area was calculated with ZEN Blue software based on manual identification of cell boundaries (Carl Zeiss, Jena, Germany).

DNA extraction and sequencing

DNA was extracted from each individual layer of three replicate mat patches. For each DNA extraction, ca. 400 mg of mat material from different layers sampled in 2018 was used. Before the extraction, the material was washed twice in 1x PBS (pH=7.4) by resuspending,

vortexing, spinning down at 10000 g, and removing the supernatant. The washed material was then extracted with a phenol-chloroform based protocol as described previously (Angel *et al* 2012), which includes three rounds of extraction with bead-beating at maximum speed and removal of polymeric organic substances. The V4 region of the 16S rRNA gene was amplified by using the universal primers 515F-mod and 806R-mod with a broad-coverage of *Bacteria* and *Archaea* (77) as described previously (78) and sequenced on Illumina MiSeq at the Vienna Biocenter Core Facility. DNA extraction and PCR amplification repeatedly failed for three samples (1x brown layer and 2x grey layer), likely due to failure to remove extracellular polymeric substances or other inhibitors. The obtained sequence reads were error-corrected using the SPAdes assembler (79), merged with BBmerge (80), and analyzed as described previously (81). Briefly, amplicon sequence variants were generated with DADA2 (82) and further clustered into OTUs by SWARM v2 (83). The centroid sequences of SWARM-generated OTUs were classified against the SILVA SSU132 non-redundant database (84) with the SINA aligner (85). All parameters were set as described in (81). Full data on relative sequence abundances is provided in File S1.

Data availability

All sequence reads have been uploaded to European Nucleotide Archive under the project accession number PRJEB37471.

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Table 1: Biogeochemical rates and fluxes

	Under salt crust		Inundated mat	
	Areal (mol m^{-2} s^{-1})	**Average volumetric (mol m^{-3} s^{-1})**	**Areal (mol m^{-2} s^{-1})**	**Average volumetric (mol m^{-3} s^{-1})**
Oxygenic photosynthesis (O_2 production)	0 ± 0	0 ± 0	**Light:** 9.0E-07 ± 3.0E-07	**Light:** 3.11E-04 ± 1.3E-04
Aerobic respiration	**Dark:** 2.1E-08 ± 0.4E-08 **Light:** 2.4E-08 ± 0.1E-08	**Dark:** 1.3E-05 ± 0.4E-05 **Light:** 1.24E-05 ± 0.3E-05	**Dark:** 1.9E-07 ± 0.05E-07 **Light:** 4.7E-07 ± 1.4E-07	**Dark:** 1.1E-04 ± 0.3E-04 **Light:** 1.55E-04 ± 0.45E-04
Sulfate reduction	**Over 3 cm:** 3.4E-09 ± 0.6E-09	**Mat:** 6.2E-08 ± 3.9E-08 **Average 3 cm:** 1.2E-07 ± 0.7E-07	**Over 3 cm:** 6.7E-08 ± 2.6E-08	**Mat:** 1.1E-06 ± 6.2E-10 **Average 3 cm:** 2.4E-06 ± 3.3E-06
Sulfide flux	**Dark:** 8.4E-08 ± 1.2E-08 **Light:** 7.6E-08 ± 0.6E-08		**Dark:** 2.1E-08 ± 0.1E-08 **Light:** 3.2E-08 ± 0.4E-08	

Rates and fluxes are calculated from three steady state microprofiles as described in Gieseke and de Beer (2004), except for SO_4^{2-} reduction rates calculated based on ^{35}S measurements. Volumetric respiration and photosynthesis rates represent average values across mat depth where O_2 concentrations were above zero.

Figure legends

Figure 1: Overview of the sabkha near Shannah, Oman, and the salt covered microbial mats in February 2018. **A)** Image of the sampling site in the salt crust covered tidal flat. **B)** A core including sediment, microbial mat, salt crust and salt-saturated water. **C)** Depth profile of pore water salinity. Squares indicate average values from three cores and error-bars indicate standard deviation. **D)** Light intensity measured over the course of a day with a PAR sensor. **E)** A piece of microbial mat cleaned from the salt and with layers partially scraped off. **F)** Scheme of mat layers based on observations of color and texture. **G)** Representative microsensor profiles of O_2 concentrations in the mats measured during the sampling campaign (at 30% salinity). Profiles were measured in the field and in sampled cores, with artificial and natural illumination. Sunrise and sunset time is indicated to consider differences in light intensity.

Figure 2: Spectral irradiance at different depths in the salt crusts and mats. **A)** Spectra in the mats with salt crust, and **B)** in mats after a simulated flood event. Note the low attenuation of light within the salt layers (blue traces at top) in panel **A**. The depth distribution of the irradiance spectra was normalized to the value at the mat surface. The depth layers are color-coded to approximately correspond to the determined mat layers (Fig. 1F) from orange at the mat surface to grey-green at the bottom of the mat. The spectral traces show the specific absorption of photopigments as well as the overall attenuation of irradiance by the sediment matrix.

Figure 3: **Depth profiles of O_2, pH and total sulfur (S_{tot}) of mats sampled in January 2019. A)** Representative steady state profiles of O_2 (squares) and pH (circles) in a salt covered mat, **B)** Representative steady state profiles of S_{tot} in a salt covered mat, **C)** local conversion rates of Stot in a salt covered mat (arrow indicates sulfide consumption due to anoxygenic photosynthesis), **D)** Representative steady state profiles of O_2 (squares) and pH (circles) three days after inundation with seawater, and **E)** profiles of S_{tot} three days after

955 inundation with seawater. Note the order of magnitude difference in O_2 concentrations
956 between salt-covered **(A)** and flooded mats **(D)**.
957
958 **Figure 4: Sulfate reduction rates (SRR)**, determined by the ^{35}S method, in mats covered by
959 a salt crust (black circles) and in mats after a simulated flood event (white circles). The
960 dashed line indicates an approximate delineation between mat and sediment. Considering
961 the slightly varying mat thickness, the peak of sulfate reduction might lie in the lowest mat
962 layer or in the sediment directly underneath the mat.
963
964 **Figure 5: Photosynthetically active radiation intensity profile and photopigment**
965 **abundances estimated from absorbance peaks.** A) Integrated photosynthetically active
966 radiation intensity profile within the salt crust and the mat, B) photopigment abundances
967 estimated from the absorbance peaks in the attenuation spectra in the salt crust and mats
968 underneath, and C) in mats after a simulated flood event. Pigment abbreviations in B & C:
969 ChlA – chlorophyll *a*, Pcya – phycocyanine, BchlA – bacteriochlorophyll *a*, BchlC –
970 bacteriochlorophyll *c*.
971
972 **Figure 6: The distribution of photopigments across cross-section of a core with the**
973 **mat and underlying sediment.** Photopigment distribution was assessed using
974 hyperspectral imaging. The upper and lower limits of the mat are indicated by horizontal
975 lines. Note that thickness varies between 5 and 8 mm. **A)** Natural color-rendering derived
976 from the rectified hyperspectral image. **B)** Abundance estimates of chlorophyllic pigments
977 based on spectral derivative analysis as a false-color map. Chlorophyllic pigments detected
978 below the mat likely represent debris of phototrophic microorganisms, as supported by
979 presence of chlorophyll degradation products (Fig. S5), absence of phycocyanin (panel C)
980 and phototrophic cells (Fig. S6) in lower mat samples, as well as no light penetrating to these
981 depths (Fig. 6). **C)** Group-specific pigments phycocyanin (*Cyanobacteria*),
982 bacteriochlorophylls A (Purple bacteria, e.g. *Rhodovibrio*) and C (*Chloroflexi*) as composite
983 color map.

Figure 7: Microbial community composition as determined by 16S rRNA gene amplicon sequencing. The relative amplicon sequence abundances were multiplied by total cell counts per gram sediment obtained via fluorescence microscopy (Fig. S7): [Cell number / g sediment]$_{taxon}$ = [Relative sequence abundance]$_{taxon}$ × (Total cell numbers / g sediment) **A)** The composition of the total microbial community, shown for two to three replicates per layer (exception layer 4-5 mm). In the sample name the roman number indicates the mat patch replicate and the arabic number the layer, from "0" being the surface orange layer to "4" being the bottom grey layer. **B)** Estimated cell numbers of photoautotrophs (*Cyanobacteria*), sulfate-reducing bacteria (all *Deltaproteobacteria*), and sulfur-oxidizing bacteria in each layer. Full data on relative sequence abundances is provided in File S1.