**Putative titles:**

* Resilience and persistence of community structure in polyextremophilic endoliths following extreme weather perturbations
* Extremophile microbial community structure rearrangement and resilience following extreme weather perturbations

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**RELEVANCE AND SIGNIFFICANCE**

In this body of work, we characterize the temporal dynamics of endolithic communities for the first time, following the changes in taxonomic and functional composition of halite microbiota at long (year) and medium (months) temporal scales. We also describe the response of this polyextremophilic microbiome to a large-scale weather anomaly, likely resulting from climate change. The isolated nature of these communities allows us to study the effects of such weather changes with minimal interference from other environmental factors. This work has implications on understanding the composition dynamics and functional resilience of highly specialized communities.

**Possible journals for submission**: Microbiome (IF=9.1), ISMEJ (IF=9.3), Nat. Microbiology (Unrated), Trends in Ecology & Evolution (IF=10.5)

**ABSTRACT**

**Background:** The highly specialized halophilic communities residing in halite nodules of the Atacama Desert are adapted to survive decades without rainfall. The majority of members in this halophilic community are salt-in strategists, and have proteomes that are highly adapted to function at high internal salt concentrations. A recent record-breaking rain event across the desert offered a unique opportunity to study the response of endolithic extremophiles to extreme weather perturbations.

**Results:** Our longitudinal study collected microbiota samples before and after the rain, and used amplicon and whole metagenome sequencing to uncover functional adaptations across time. The higher-order taxonomic composition and functional potential of halite microbiomes significantly shifted following the rain event, and gradually recovered to its pre-rain state over the course of the following a year. The rain event favored taxa with higher isoelectric point proteomes, suggesting a decrease in selective pressure from high salt concentrations. Investigation of abundance profiles of individual organisms revealed that the fine-scale community structure not only underwent significant re-shuffling after the rain, but also failed to recover in the following year. This rearrangement is also reflected in the metabolome, where we observe that while the functional potential recovered fully in the year after the rain, the community functions are carried out by a different set of organisms when compared to the pre-rain communities.

**Conclusions:** The overall taxonomic and functional structure as well as individual membership of halite extremophile communities is driven by salt concentration and water availability, and can be significantly altered by large-scale weather perturbations. While the higher order community structure is resilient and more dynamic, the individual membership can may take much longer to recover. However, the exact community membership is not vital to overall community functioning, as we observe persistence of functional niches despite membership rearrangement.

**INTRODUCTION**

Microbial communities exposed to identical environmental conditions often converge on a similar functional potential landscape, as they are subjected to similar selective pressures. Because the functional distance between organisms is at least partially linked to taxonomy, the higher order structure of such communities (i.e. the taxonomic composition at the phylum level or higher) is also expected to converge to a similar composition, and will remain relatively robust given unchanging environmental conditions.

While understanding the taxonomic classification of individual community members is useful for inferring lineages, this fine-scale community composition is often irrelevant to the overall functioning of the community as many taxa can occupy the same functional niche within the microbiome. This functional redundancy ensures that even after major perturbations to community structure, the functional potential of the microbiome persists. Indeed, a community’s taxonomic structure at lower taxonomic ranks may undergo significant rearrangement without an impact on the overall functioning of the community. Therefore, while the higher order taxonomic structure of a microbial community is driven by selective pressures, the individual membership (i.e. individual species constituting the higher order taxonomy groups) of a microbiome may vary greatly across time and space. [1, 2]

Previous studies have shown that higher-order taxonomic composition as well as the functional landscape of microbiomes can be very resilient and can recover after significant perturbations such as temperature changes and antibiotic administration [3-5]. However, while the recovered community may have the same higher order taxonomic composition and functional potential as before the perturbation, but be constituted of a new set of organisms.

The North of the Atacama Desert is one of the driest and harshest places on Earth, receiving rainfall only every few years, exposed to high amounts of ultraviolet radiation, and being subject to extreme temperature and humidity swings throughout the dial cycle. Despite this, polyextremophilic microbiota residing in the desert evolved to exist in these extreme conditions. To survive extreme desiccation, the microbial communities rely on the protection of salt rock nodule formation (halites) found in the salars (salt flats) of the desert. Because of salt’s deliquescent properties, these endolithic microbiomes are able to survive by receiving almost all their water from the humidity in the air. [6-8]

In ecology, highly specialized community members are more vulnerable to change compared to habitat generalists. [9]The unique adaptations of this community to survive under such specific and harsh conditions could potentially render it relatively susceptive to unexpected rare perturbations, making this microbiome an interesting target to study the resilience of microbial community structure. Because the halite nodules are comprised mostly of porous [10]salt, a major rain event could at temporarily alter the external osmotic conditions surrounding the endolithic microbiota, thus creating a major shock and perturbation.

Because the halite nodules are composed mostly of sodium chloride, the halite endolithic microbiomes are also hyper-halophilic [6, 7]. Because of this, the major taxonomic groups of these communities are Halobacteria and Bacteroidetes – two taxonomically different halophiles that throughout convergent evolution share a unique adaptation to high salt concentrations [7, 9, 11]. Instead of actively pumping sodium ions out of the cell (salt-out strategists), they selectively pump potassium ions into the cell instead (salt-in strategists). The high internal potassium concentrations balance out external osmotic pressure from sodium. This strategy is energetically favorable to actively pumping out sodium [12], but the proteomes of these cells must also adapt to function at high potassium concentrations. As a result, their proteomes have an extremely low isoelectric point (pI), which ensures protein surface stability under such conditions. [13-15]

Due to harsh external conditions, the endolithic halite microbial communities receive very little biomass exchange with the outside world. [8] The only major inputs that a halite communities receive are sunlight, atmospheric gases, and humidity from the air. [6, 7] As such, each halite nodule represents a near-closed system, which offers a unique opportunity to investigate the response of microbiomes to external stimuli and perturbations in their natural habitat without other external factors compounding the results.

The North of the Atacama Desert (near Iquique, Chile) received only a handful of recorded rainfall events in the last century. Prior to this study, rain events were documented in 1992 (10.9mm) and 2002 (4.1mm). [16] However, abnormally high Eastern Tropical Pacific sea surface temperatures led to rainfalls reported throughout the Atacama Desert in 2015. [17] A nearby weather station at the Diego Aracena International Airport, located 30.2 miles North of the sampling site, recorded rainfalls on March 25, 2015 (0.3mm) August 8-9, 2015 (4.1mm). [18] The August 2015 rain was the first major precipitation event in the area in 13 years, giving us a unique opportunity to investigate the effects of this rare perturbation on the extremophilic life in the area.

**RESULTS**

**Record-breaking rains in the Atacama Desert allows investigation of the effects of large-scale perturbations on endolithic extremophiles**

Rains were reported in the Atacama Desert throughout 2015, however the location investigated in this study (Salar Grande) likely received only a few rain events. Unfortunately, there were no weather probes placed at the location on interest at the time of the rain. [Need more information about rain]

In order to investigate the taxonomic composition and functional potential dynamics of halite microbial communities in response to the rain, a site at the top of a hill in Salar Grande (SG1) was repeatedly sampled approximately once a year for four years, with pre-rain sampling dates 2014-09 and 2015-06, and post-rain sampling dates 2016-02 and 2017-02.

Following the rain, an alternate nearby site at the bottom of the hill (~300m from the main site) was also sampled at more frequent intervals. Halites were samples in 2016-02 (4 months after the rain), 2016-07 (7 months), 2016-10 (10 months), and 2017-02 (14 months).

The 16S rDNA as well as the gDNA (for the main site only) from the collected samples was amplified and sequenced to estimate the relative taxonomic composition (from rDNA and gDNA) and functional potential (from gDNA) of the communities residing in the halite samples. The changes in community composition over time were investigated at high (domain), medium (phylum), and lowest (OTUs and metagenomic bins) taxonomic ranks.

**Higher-order taxonomic composition of halite communities is sensitive to perturbations, but resilient long-term**

To look at changes in community structure at the highest taxonomic rank, we measured the relative Archaea to Bacteria abundance ratios in the rDNA sequencing. In the samples collected before the rain, the relative Archaea abundance was robust and stable: 83.1±2% in 2014-09 and in 78.8±6.3% in 2015-06. However, the relative Archaea abundance dropped to 60.5±4.3% in 2016-02, indicating a significant drop approximately 4 months after the rain. In the following year, the Archaea abundance increased back to 77.7±3% in 2017-2, returning to the pre-rain baseline.

To investigate the taxonomic shifts between time-points at medium taxonomic ranks, we also looked for dynamics in the relative abundances of different phyla in the rDNA sequences. We found that Cyanobacteria, Green algae (estimated by chloroplast rDNA abundance), and Bacteroidetes significantly increased in relative abundance following the rain, and gradually lowered back to baseline abundance in the following year. On the other hand, the abundance of Halobacteria (the major Archaea phylum in this community) significantly decreased and subsequently recovered following the rain.

At the alternate sampling site, rDNA sequencing of samples collected at various intervals after the rain confirms the recovery in relative Archaea abundance from 41.42±5.3% in 2016-02 to 60.10±6.7% in 2017-02, similar to the main sampling site. Moreover, the Archaea abundances in 2016-07 (50.14±9.2%) and 2017-10 (50.33±5.1%) indicate a relatively gradual and incremental recovery in this metric. At lower taxonomic ranks, we observe similar trends to the original site. Between 2016-02 and 2017-02, Cyanobacteria and Green algae decrease in abundance, while Halobacteria gradually increase.

To further validate the shift and subsequent recovery of halite community composition, the DNA from the main timeline samples was additionally sequenced with whole metagenome sequencing (WMG). Taxonomic profiling of the quality-controlled reads with KRAKEN confirmed that the community composition at higher taxonomic ranks remained largely unchanged in the two years preceding the rain (2014-09 and 2015-06), changed significantly following the rain (2016-02), and returned back to the pre-rain state in the following year (2017-02). At the domain level, we observed the decrease and subsequent recovery in the relative abundance of Archaea (mostly represented by Halobacteria), and the increase and subsequent decrease in the relative abundance of Bacteria (mostly represented by Bacteroidetes).

**Analysis of the community metaproteome after the rain indicates a reduction of selective pressures associated with high-salt adaptation**

To investigate adaptations of halite microbial communities to the rain, we reconstructed metagenomic assemblies of the individual replicates from the time course study, and looked for broad proteome adaptations. In particular, we expected the sudden increase in water availability to affect the salt-related adaptations within this hyper-halophilic community.

The majority of this community is constituted from Halobacteria and Bacteroidetes – two taxonomically distant halophiles that share several key adaptations for living in this poly-extremophilic environment. In particular, they are salt-in strategists – meaning they overcome high external sodium concentrations by actively importing potassium ions, thus balancing out the external osmotic pressure. This approach is energetically favorable to actively exporting sodium ions, but also means that the cell’s proteome must be adapted to function in high-potassium concentrations by having an extremely low isoelectric point (pI).

Then comparing the pI of the individual metagenomic assemblies, it was found that the average pI of the community’s metaproteome significantly increased after the rain, and then recovered to pre-rain levels in the the following year. This major shift results from the different pI of the two major taxa that changed in abundance after the rain – Halobacteria and Bacteroidetes. Looking at the pI of genes encoded on contigs classified as these two taxa reveals that Halobacteria have a much lower average IEP of their proteomes (5.04) compared to Bacteroidetes (5.80). Looking at the functional annotations of the metagenomes, we also found that the average total potassium uptake potential of the community (measured from total Trk protein abundance) significantly decreased after the rain, and then recovered to pre-rain levels in the following year. Together, these results indicate an overall reduced selective pressure coming from high external salt concentrations. This likely resulted from a temporary decrease in salt concentrations during the rains.

These adaptations were also observed within the highly heterogeneous Halobacteria populations found in the halites. Inspection of the average pI of genes encoded on Halobacteria contigs reveals that even within this taxa, the average protein pI increased after the rain. Conversely, the overall potassium uptake potential encoded on Halobacteria contigs decreased, indicating a selection for Halobacteria that were adapted to survive at lower salt concentrations. Interestingly, this adaptation within this taxonomy group persisted in the following year, as the average proteome pI and potassium uptake potential never recovered fully to the pre-rain levels.

**The overall functional potential of the community was temporarily perturbed by the rain**

To evaluate shifts in the overall functional potential of the halite microbiome, the total abundance of functional categories in each sample was estimated from the sum of contig depths that carried those genes. While the majority community functions were present in similar abundances between replicates and time points, we observed a number of gene functions that were significantly differentially present between samples collected in different years (ANOVA test, *pval*<0.01). This analysis was repeated at lower and higher tiers of KEGG functional pathway ontology. The vast majority of these differentially present functional KEGG pathways were differentially abundant in samples collected after the rain (2016-02). Even more strikingly however, all these gene functions recovered to pre-rain levels in the following year (2017-02).

Hierarchical clustering (using Euclidean distance as the metric and the “average” linkage model) of differentially abundant KEGG pathways was used to compare the functional potential distance between replicates and years. While replicates from each time point formed distinct clusters, we observed that the post-rain (2016-02) samples were significantly different from samples collected before the rain, while 2017-02 samples were more similar to the pre-rain samples than to 2016-02 samples. This is consistent with the observation that the community composition at the higher-order taxonomic ranks recovered after the rain.

All of the differentially present pathways recovered to the pre-rain state. However, it should be noted that of these, none showed a trend in abundance recovery when considering only contigs belonging to Halobacteria or Bacteroidetes. When looking at the functional changes within these taxa separately, we find that while many pathways changed in abundance after the rain, they remained in this state even in the following rain. Taken at face value, this would suggest that the functional shift resulting from the rain is linked to the shift in higher-order taxonomy of the community, as well as functional adaptations within individual phyla. On the other hand, the recovery in functional potential in the year following the rain can be explained by only the shift in taxonomic composition of the community, rather than functional adaptation within separate taxa.

The functional potentials of halite communities were also visualized with principal component projections of the vectors representing the abundances of all KEGG functional categories. Consistent with the previous observation, we found that while 2014-09, 2015-06, and 2017-02 samples clustered together on the principal component plane, the replicates from samples collected in 2016-02 (shortly after rain) formed a separate cluster.

**The individual community membership was permanently rearranged by the rain even**

To compare halite samples from the main sampling site at the lowest taxonomic rank, an unweighted Unifrac dissimilarity matrix was constructed from OTUs constructed from the rDNA sequencing. Principal coordinate analysis and as well as hierarchical clustering of the dissimilarity matrix revealed the relative similarity of the replicates to each over in terms of presence or absence of OTUs. We found that the pre-rain replicates from 2014-09 and 2015-06 cluster together and are not significantly different. The samples from halites harvested on 2016-02 (post-rain) form a distinct cluster separately from the pre-rain samples, confirming a composition change resulting from the rain. Most striking however, the taxonomic composition at the level did not recover in the year following the rain. Indeed, the samples form 2017-02 (a year after the rain) are more similar to the samples from 2016-02 than they are to samples collected before the rain. In the context of the community structure recovery at higher taxonomic ranks (domain and phylum), these results suggest that while the taxonomic structure recovered in the year following the rain, the individual community members representing each taxonomic group have been rearranged.

To further investigate this phenomenon, the changes in abundance of individual community members were inspected at the level of metagenomic bins in the WMG data.

The WMG sequences were co-assembled and the resulting contigs were binned to form 94 high-quality (>70% completion, <5% contamination) draft genomes with the help of metaWRAP. The abundances of contigs as well as entire draft genomes were estimated across the replicates from the four time points with metaWRAP’s Quant\_bins module. Hierarchical clustering of the draft genome abundance across the replicates revealed relative similarity between replicates and time points from the longitudinal study.

The clustered heat maps revealed that replicates from 2014-09 and 2015-06 (pre-rain) were highly similar to each other, and clustered away from the post-rain samples. Replicates from 2016-02 and 2017-02 (post-rain) time points formed distinct branches, but were more similar to each other than to the pre-rain samples. Despite high heterogeneity in the draft genome abundance heat map, we observed distinct groups of organisms: those that went up or down in abundance after the rain, and those that were largely unaffected. These results were also recapitulated in the hierarchical clustering of samples based on individual contig abundances.

This samples were also compared on a PCA projection of individual contig abundances in each sample. The resulting PCA revealed that samples from each year form distinct clusters on the PCA projection, however samples from before the rain appeared to be closer to each other together than to samples harvested after the rain. Indeed, this result was further confirmed by performing hierarchical clustering of a Pearson Correlation matrix comparing all the samples, which revealed that the pre-rain and post-rain replicates formed distinct clusters. These results confirmed that while the higher order taxonomic order (at the domain and phylum levels) shifted after the rain and then recovered in the following year, the individual members of the community were reshuffled and stayed relatively unchanged, suggesting a permanent rearrangement of the halite communities at the lower taxonomic ranks.

**Community functioning persisted, but members constituting functional niches were rearranged**

In order to assess if the community functions were being carried out by the same organisms between samples, a gene product representation metric was computed. This change in individual community member representation of functional categories was evaluated by computing the changes in abundance of contigs that carried genes belonging to those gene functions (KEGG ontology). This metric evaluates the change in the individual organisms that are representing a given gene function between two replicates or time points, and represents the "rearrangement" in community member composition at the functional scale.

When comparing two samples, a high average rearrangement index indicates that the community’s gene functions are performed by different community members, while a high spread (variance) of the fold changes in total function abundances indicates that many functions changed in abundance and that the functional potential of the community changed. It is important to note that a high a rearrangement index does not necessarily imply a high total fold change for that gene function, as it is possible that the function is present in equal abundances between two samples, but carried by different organisms.

When looking at the variance in the fold change of total abundance of gene functions, we observe that there was relatively little functional potential change between the pre-rain samples (2014-09 to 2015-06), but there was a significantly higher spread of the fold changes in gene functions following the rain (2015-06 to 2016-02). In the year following (2016-02 to 2017-02) there was also a high variance in the fold changes of gene function abundances, indicating a recovery of the community’s functional potential. This was further confirmed by comparing the 2014-09 samples to the 2017-02 samples. This appears to be consistent with the observation that the overall functional potential of the halite communities shifted following the rain event and subsequently recovered in the following year.

The average shuffling index between 2014-09 and 2015-06 was relatively low, indicating that prior to the rain there was relatively little rearrangement in the individual members constituting functional categories. However, between before (2015-06) and after (2016-02) the rain, the average shuffling index was quite high, indicating that's the genomic functions were being performed by different community members after the rain. Finally, despite significant functional potential shifts between 2016-02 and 2017-02, we observed a low reshuffling index between these dates. These results suggest that after the rain there was a significant change in the organisms that carry the genes of each functional category in this community's metabolome, and that this composition did not recover in the year following the rain. This is striking, considering our observation that the overall functional potential recovered in the year after the rain. Based on this, we conclude that while the community functions the same as it did prior to the rain, its gene functions are being carried out by different organisms. This could be explained by high functional redundancy of the microbial genomes constituting this microbiome.

**DISCUSSION**

The highly specialized nature of halite microbiomes renders them sensitive to changes in the environment, particularly to changes in water availability, as it is a major limiting factor for life in such a desiccated environment. The rain that's the northern Atacama Desert experienced in 2015 was the first major rain event in over 13 years. Normally, the endolithic communities within the halites receive water from atmospheric moisture, so the water made immediately available from the rainfall would be much greater than normal. The rain likely temporarily decreased the salt concentrations within the colonized pores of the halites, which could have significantly disrupted the micro niches found within the halites. Such a drastic change in environmental conditions would likely favor some taxa over others. In this study, Bacteroidetes were selected for, while Halobacteria was selected against. This is possibly due to Bacteroidetes being more resistant to the sudden hypotonic conditions, or alternatively were better adapted to take advantage of the newly available water, temporarily outcompeting Halobacteria.

What is also striking is that the higher-order structure change in the community composition was observed six months after the rain. This makes it possible that the original composition shifts immediately following the rain was much more drastic that reported in this study, which would also explain the rearrangement of individual community members. However, despite the major alterations in the community’s taxonomic structure, as well as its functional potential, the resilient halite communities were able to recover in approximately 18 months. The fact that it took so long for this community to recover suggests that its members are very slow-growing in nature. This makes sense considering the scarcity of resources and harsh nature in this environment, which would limit the growth rates of microbiota. Additionally, evolution likely favor survivability as opposed to high growth rates, which would lead to more conservative, slow-growing microbiomes. On the other hand, this also renders them slow to recover from catastrophic events such as the the rain.

The majority of the halite microbial communities is comprised of two taxonomically distant salt-in strategists – Halobacteria and Bacteroidetes. The unique adaptations that allow them to survive high salt concentrations can also make them potentially susceptible to a sudden reduction and salt concentrations. However, they also have drastically different isoelectric points of their proteomes meaning that they likely have different survival thresholds. In particular, Bacteroidetes has a much higher pI of their proteomes when compared to Halobacteria, making it more suitable to survive in lower salt concentrations. Therefore, it is not surprising that Bacteroidetes were able to survive the sudden reduction in salt concentrations following the rain better than Halobacteria. This type of adaptation is further seen whithin the finer taxa in the Halobacteria phylum, where organism with a higher pI were selected for following the rain.

The overall functional potential of the community temporarily shifted following the rain. This makes sense considering the change in selective pressures resulting from lower salt concentrations and much higher water availability. With previous conditions restored, the functional potential of the communities gradually recovered. When considering the linkage of functional potential and taxonomy in the two major shifts – the response to the rain and the subsequent recovery – we find strikingly different trends. In the first shift after the rain, we find that many of the pathways that changed in abundance on a community-wide scale also changed in abundance within the two major individual taxa – Halobacteria and Bacteroidetes. This would suggest that the community underwent a significant remodeling, and that the fitness of the community members was at least partially driven by their functional potential. However, in the second shift during the community’s recovery, we find that the functional potential of the individual major phyla remains largely unchanged. This indicates that this second functional shift can be explained by the change in the relative abundance of taxa, rather than taxa-wide functional adaptations.

These observations illustrate the two possible functional shifts that this type of microbial community can undergo. The first is in response to a sudden change in conditions, and requires all the taxa to adapt to survive in the new environment. In this type of response, the changes in the community’s overall functional potential are driven by changes in higher order taxonomic composition, as well as functional changes within individual taxa. The second type of shift results from a much more gradual adjustment of the community. In this case, the functional potential of the community is driven by the overall higher-order taxonomic composition.

We found that the individual community membership was permanently rearranged by the rain. This is interesting interesting, considering the functional potential and higher order taxonomic structure of the halite communities was virtually indistinguishable between 2014 and 2017, as were the overall conditions surrounding the community. This suggest that this drastic membership rearrangement was largely unlinked from functional potential, and instead resulted from a stochastic process. One explanation is that the halites were so significantly perturbed during the rain that they had to effectively re-colonize the nodules. The rain could have created a great osmotic stress that wiped out the majority of the population, allowing a new set of organisms to occupy the old niches. Indeed, the individual membership rearrangement and virtually identical higher-order taxonomic and functional structure we observe between the samples from 2014 and 2017 is reminiscent of the differences we find between individual halites. The inter-halite variation we observe is likely driven by a similar stochastic selection process to the one responsible for the community “reshuffling” following the rain.

The rearrangement in the individual contigs carrying each gene function allows us to examine the effects of the rain perturbation on the organism constituting the functional niches of the community. We found that while the total abundance of most gene functions is similar between the 2014 and 2017 samples, these gene functions are carried on a different set of contigs. The further supports the idea that after the recovery from the rain the old functional niches are occupied by a new set of organisms. Considering the functional redundancy of many individual members of microbial communities, it is not surprising that the halite microbiome underwent such a drastic composition rearrangement while retaining its former functional potential.

It is interesting to note how the community was able to recover its former functional potential after the rain without significant taxonomic rearrangement compared. This constrasts with the drastic rearrangement in taxonomy and functional potential that followed the rain event itself. This further highlights the drastic nature of perturbation and its effect on this highly specialized microbiome.

Our findings show the that highly specialized communities are highly sensitive to environmental change, but their higher-order taxonomic structure and functional potential are very resilient despite this. We found that just 4mm of rainfall induced a temporary drastic change the higher-order community structure structure, and a permanent rearrangement of the lower-order taxa. These findings suggest that the endolithic poly-extremophilic microbiomes found in the Atacama Desert are hyper-sensitive to major weather perturbations, making them an interesting model to study in tracking and investigating the effects of climate change to environmental microbiomes.

**METHODS**

**Sample collection**

In this study, halites from two sites were harvested in the Atacama Desert – the main site, located at the top of a hill at 123’4W, 321’0S, and the supplementary site, located at the bottom of that hill at 456’67W, 543’6S. At each site, an area approximately 50m by 50m was randomly sampled. With a sterilized hammer, halite nodules were randomly broken into smaller pieces (<20cm), and pieces of halite with visible green coloration (indicative of colonization), were stored in sterile bags. Due to low available colonization zones, samples were pooled such that each collected biological replicate contained pieces from 3 halite nodules. The halite samples were stored in dark, dry conditions until cell extraction in the lab.

**Cell extraction**

Using a dull sterilized knife, the halite pieces with visible green colonization were manually scraped to extract a green powder. Because the halites are comprised of primarily NaCl salt, the powder could be dissolved in water to release the cells. In a 50ml falcon tube, 2g of halite powder was mixed with 2ml of 20% NaCl. After a 5-minute acclimation period, 6ml of ultrapure water was added with a syringe, adding the water drop wise over the course of 2 minutes while vigorously mixing the halite mixture to prevent cell lysis due to osmotic shock. The mixture was left for two minutes to allow larger debree to settle, and then the supernatant was transferred to a new 50ml tube. The cells in the suspension were then spun down in a centrifuge at 8000g for 10 minutes. The cell pellet was suspended again by using 500ul of the supernatant, and transferred to a 1.5ml tube, where the cells were spund down one more time at 13000g for 10 minutes. The supernatant was discarded and the resulting pellet was stored at -20°C.

**DNA extraction**

The DNA was extracted from the cells by using the DNAeasy Powersoil DNA extraction kit from QIAGEN. 300ul of the liquid in the bead tubes was used to re-suspend the cell pellet and transfer to the bead tube. After addition of 60ul of the C1 buffer, the rest of the DNAeasy protocol was followed without alterations. The final DNA was eluted in 50ul of C6 elution buffer, and quantified by using the Qubit dsDNA HS Assay Kit from Invitrogen.

**Ribosomal amplicon library preparation**

First, the V4 region of the community 16S ribosomal DNA was amplified with 515F and 926R primers using the Phusion High-Fidelity PCR kit from New England BioLabs. The PCR mixture was put together according to the kit specifications with a total reaction volume of 50ul, and using the recommended DMSO component. 40ng of environmental DNA was used as the template. The PCR was performed with the following cycle: 30 seconds at 98°C, followed by 20 cycles of 10 seconds at 98°C, 15 seconds at 55°C and 15 seconds at 72°C, and with a final step of 5 min at 72°C. 10ul of the reaction was run out on a 2% agarose gel using the 1kb Plus DNA Ladder kit from New England BioLabs to verify that the reaction worked. If viable ~411bp amplicons were produced, the product was then cleaned up with DNA-binding Sera-Mag SpeedBeads from GE Healthcase Life Sciences. 40ul of Sera-Mag beads were added to the remaining 40ul of PCR product and incubated 5 minutes at room temperature. The tubes were then placed on a magnetic rack for 3 minutes and then the beads were washed gently 2 times with 200ul of 80% ethanol. After drying the beads for 5 minutes, the DNA was eluted in 20ul of UltraPure water for 3 minutes. MiSeq sequencing adapters and unique barcodes were then added to the purified rDNA amplicons with a second PCR reaction. Finally, the samples were quantified again with the Qubit dsDNA HS Assay Kit from Invitrogen, pooled together to equal molarity, and sequenced on a MiSeq sequencer.

**Amplicon sequencing analysis pipeline**

The de-multiplexed and quality trimmed 16S amplicon reads from the MiSeq sequencer were processed with MacQIIME v1.9.1 [19]. The samples from the two sites described in this paper (main and supplementary) were processed separately. The reads were clustered into OTUs at a 97% similarity cutoff with the pick\_open\_reference\_otus.py function (with --suppress\_step4 option), using the SILVA 123 database [20] release as reference and USEARCH v6.1.554 [21]. The OTUs were filtered with filter\_otus\_from\_otu\_table.py (-n 2 option), resulting in a total of 624 OTUs for the main site (top of hill) and 173 OTUs for the supplementary site (bottom of hill). The taxonomic composition of the samples was visualized with summarize\_taxa\_through\_plots.py (default options). The beta diversity metrics of samples in the two sites were compared by first normalizing the OTU tables with normalize\_table.py (default options), and then running beta\_diversity.py (-m unweighted\_unifrac, weighted\_unifrac). The sample dissimilarity matrices were visualized on PCoA plots with principal\_coordinates.py (default options, but providing the mapping file with sample information) and on heat maps with Seaborn v0.8 [22].

**WMG library preparation**

The whole genome sequencing libraries of the halite DNA were prepared with the KAPA HyperPlus kit from Roche. The fragmentation was performed on 5ng of input gDNA for 6 minutes to achieve peaks around 800bp. Library amplification was done with dual-index primers for a total of 7 cycles, and the product library was cleaned up 3 times with XP AMPure Beads from New England BioLabs with the following bead ratios: 1X ratio (discard unbound), 0.4X (discard beads), and 0.6 (discard unbound). The other steps were performed according to the kit’s recommendations. The final 35 libraries were quantified with Qubit dsDNA HS kit, inspected on a dsDNA HS Bioanalizer, pooled to equal molarity, and sequenced on the HiSeq 2000.

**WMG sequence data analysis pipeline**

The de-multiplexed WMG sequencing reads were processed with the complete metaWRAP pipeline on a UNIX cluster with 48 cores and 1024GB of RAM available. Read trimming and human contamination removal was done by the metaWRAP Read\_qc module (default parameters) on each separate sample. The taxonomic profiling was done on the trimmed reads with the metaWRAP Kraken module (default parameters, standard KRAKEN database). The reads from all samples were co-assembled with the metaWRAP Assembly module (--use-metastades option). For improved coverage of low-abundance organims, sequences from all the halite samples sequenced in the HiSeq run were pooled together, including samples from an additional site located at 432.43W, 423.54S, which were otherwise unused. The co-assembly was then binned with the metaWRAP Binning module (--maxbin2 --concoct --metabat2 options) while using all the available samples for differential coverage information. The resulting bins were then consolidated into a final bin set with metaWRAP’s Bin\_refinement module (-c 70 –x 5 options). The bins and the contig taxonomy were then visualized with the Blobology module (--bins option specified), classified with the Classify\_bins module (default parameters), and quantified with the Quant\_bins module (default parameters). Gene prediction and functional annotation of the co-assembly was done with the JGI Integrated Microbial Genomes & Microbiomes (IMG) annotation service.

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