Investigating the effects of phosphate mining in the Negev Desert on biological soil crust communities

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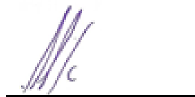
Prof. Osnat Gillor  **15-10-2022**

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Abstract

Anthropogenic disturbances cause large scale destruction and fragmentation of natural systems, leading to loss of biodiversity and ecosystem functioning. Such disturbances include mining, which involves clearing large areas of land, effectively destroying the soil structure, and killing most organisms. In hyper-arid regions of the Negev Desert, phosphate has been mined for over six decades, altering the natural landscape over large spatial scales. In recent years, restoration-

oriented practices were mandated in mining sites, however, the impact of such practices on the ecosystem, particularly the biological soil crust (biocrust) layer, have not been tested.

The biocrust is the upmost layer of soil in arid environments, containing a variety of microbial groups. They provide important ecosystem services in drylands by fixing carbon and nitrogen, stabilizing the soil, and influencing hydrological regimes. Given the scarcity of plants in desert systems and the resilience of biocrusts to harsh environments, biocrusts are the primary producers, thus highly important to maintain a 'healthy' and fully functioning soil ecosystem. To meet these challenges, I aimed to identify the dynamics and patterns that drive biocrust restoration in hyper-arid phosphate mines.

In my research, I had three specific aims: (1) Characterize the bacterial communities of post-mining and nearby reference ('natural') biocrusts to understand the effect of mining restoration practices on the biocrust community over space and time; (2) Examine which bacterial groups become active in post-mining and reference biocrusts after hydration, using water marked by a stable isotope; (3) Conduct a restoration experiment testing various treatments including increased hydration of the soil and inoculation of biocrust (natural and enriched communities), aimed to enhance establishment of biocrust primary producers in bare topsoil.

To address the first aim, we collected samples from four restored mining sites (each restored at a different year) and their corresponding reference sites. We hypothesized that post-mining bacterial communities would differ significantly from reference communities, given the slow regeneration of the biocrust. We also hypothesized that bacterial communities would vary among post-mining plots based on their restoration age. To test these hypotheses, we assessed the abundance and diversity of bacterial communities by sequencing the 16S rDNA and the abundance of cyanobacteria and Chlorophyl *a* (Chl *a*). Our results showed that the bacterial diversity was lower, and community composition differed significantly between post-mining and reference biocrusts. In addition, cyanobacteria abundances and Chl *a* content were lower in post-mining biocrusts, indicating lower photosynthetic potential. However, no significant changes in bacterial communities were detected, regardless of the restoration age. To address the second aim, we selected one mining site to identify active bacterial groups in post-mining and reference biocrusts by applying DNA stable isotope probing (DNA-SIP). Given that biocrust organisms become active after wetting, we incubated the biocrusts with $H_2^{18}O$ for 96 hrs. We also evaluated soil properties, Chl *a* concentration and abundance of functional genes in the biocrusts. The DNA-SIP assay revealed low bacterial activity and no significant differences in the active community

composition when comparing post-mining and reference biocrusts. We further found no significant differences in the function potential, soil properties, or Chl *a* content.

To address the third aim, we collected topsoil from an excavated pile near Zin mining sites and applied three treatments – hydration; hydration & inoculation with natural biocrusts; hydration & inoculation with enriched biocrusts. We tested the effect of the applied treatments on the establishment of biocrusts on the excavated topsoil. The topsoil was packed in mesocosms, inoculated with natural or enriched biocrusts and kept at ambient temperature under day/night cycles. The mesocosms were hydrated to the maximum water holding capacity every week for 20 weeks. Mesocosms were sacrificed after 10 and 20 weeks and the bacterial community in the biocrust and topsoil was evaluated, as well as photosynthetic potential and physico-chemical parameters. Our results showed no changes in community composition of the biocrust in all treatments regardless of the duration of the experiment or treatment. Also, the concentration of Chl *a* decreased significantly over the experiment, suggesting a decrease in photosynthetic activity.

The results of this thesis show that the bacterial communities in post-mining biocrusts differ from reference biocrusts, regardless of the time since restoration. The lower abundances of cyanobacteria and Chl *a* content suggest a decrease in photosynthetic potential of post-mining biocrusts. The low proliferation of bacteria in reference and post-mining biocrusts suggest that even when activated through hydration, biocrust bacteria do not replicate or grow. Continues hydration and biocrust inoculation were also not effective, and the biocrust community did not show any signs of restoration even after 20 weeks. We conclude that the restoration practices currently implemented in the hyper-arid Negev mines cannot support the recovery of the biocrust communities, particularly the cyanobacteria. We hypothesize that the recovery of the biocrust communities is extremely slow in hyper-arid environments, due to the harsh climatic conditions imposing low proliferation rates on the biocrust bacteria. Therefore, further experiments are needed to test soil amendment treatments in post-mining biocrusts, since active restoration measures are still the best potential solution for restoring the biocrust and primary production after mining.

Abbreviations

Adonis – Permutational multivariate analysis of variance

ASV – Amplicon sequence variant

Ca – Calcium

CaMg – Calcium—magnesium

Cl – chloride

Chl *a* – chlorophyll *a*

CsCl - Caesium chloride

DW – Distilled water

DDW – Double-distilled water

DNA / rDNA – Deoxyribonucleic acid / ribosomal deoxyribonucleic acid

dNTPs – Nucleoside triphosphate

EC – Electrical conductivity

EPS – Extracellular polymeric substances

JM - Jaworski Media

K – Potassium

Na – Sodium

NH_4 – Ammonium

NMDS – Non-metric multidimensional scaling

NO_3 – Nitrate

P – Phosphorus

PAR – Photosynthetic active radiation

PCoA – Principal Coordinate Analysis

PCR – Polymerase chain reaction

pH – Potential of hydrogen

RNA / rRNA – Ribonucleic acid / ribosomal ribonucleic acid

ROS – Reactive Oxidative Species

SIP – Stable isotope Probing

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1. Introduction

1.1. Restoration ecology

In the last decades there has been a dramatic increase in the utilization of biotic and abiotic natural resources to meet growing demands as the human population continues to increase rapidly. Many of earth's ecosystems have been altered or damaged, often beyond repair, by human activities. As more and more ecosystems are being overexploited and degraded, the services they provide on which all living organisms rely, will be lost (Hobbs & Harris, 2001).

Out of an understanding that active measures could ameliorate the environmental damage caused by human activities, the field of restoration ecology has emerged to become increasingly important in recent years (Hobbs & Cramer, 2008, Palmer et al., 2016). It aims at assisting the recovery of compromised ecosystems, thus restoring at least some of the system's structure and function (Clewell et al., 2004). Hence, restoration offers a solution towards the ecosystem's recovery following environmental disturbances whether they are natural or anthropogenic (Gann et al., 2019).

1.2. The effect of mining on drylands

Disturbances can occur in aquatic or terrestrial systems and are described as discrete events in space and time, altering the structure of populations, communities, and ecosystems (Walker et al., 2007). One example of a severe anthropogenic disturbance to terrestrial environments is the practice of mining. Mining is the process of extracting minerals and metal resources which provide vast industrial and agriculture services (Walker et al., 2007). Phosphate mining is practiced globally, mainly for fertilizers production (Carvalho, 2017), using large-scale surface techniques such as open-pit mining (UNEP et al., 2001). The direct impact of mining disturbances is the complete removal of soil surfaces, including all plants and animals, thus destroying the natural system at the mining site. Indirect effects include fragmentation of the natural environment and pollution of soil and ground waters by mining waste (UNEP et al., 2001, Cooke & Johnson, 2002). Consequently, restoration actions are required in mining sites (Hobbs & Cramer, 2008, Prach & Tolvanen, 2016, Shackelford et al., 2018).

Phosphate mining has been carried out in hyper-arid regions of the Negev Desert for more than six decades, over an area of 200 km^2, led by Rotem ICL company. In the past,

restoration efforts focused merely on redesigning the landscape. However, during the past decade, the focus has shifted towards ecological restoration. After the extraction of phosphorus, the excavated topsoil including waste rocks and overburden soil layers are used to backfill the site according to a pre-defined protocol – the overburden is returned first, followed by the topsoil. The surface is then covered with small rocks and is shaped by a rototiller to fit the local topography. The procedure can take months to years to complete. Despite being restoration oriented, this protocol has a few drawbacks; (i) The topsoil layer is excavated at a depth of 50-80 cm and cannot be considered as biological topsoil (typically defined as the top 10 cm), thus, the seedbank of microorganisms and plants is either buried or mixed with other layers; (ii) Piles of overburden and topsoil are exposed for a few years before they are used as backfill, which decrease soil biodiversity and health (Cooke & Johnson, 2002); and (iii) There are no active restoration measures being implemented to accelerate the restoration process.

1.3. Biocrusts in arid environments

One of the ecosystem components that is destroyed in the mining process is the biological soil crust (biocrust) layer. Biocrusts are the top layer of soil established by an association between soil particles and a biofilm of photoautotrophic and heterotrophic microorganisms (Weber et al., 2022). This biofilm contains various groups of organisms including bacteria, archaea, mosses, lichens, and fungi. Biocrusts occur globally in areas with scarce vascular plant cover and are especially common in ecosystem with limited water (Weber et al., 2022). Biocrusts play a pivotal role in ecosystem functioning (Belnap, 2003, Elbert et al., 2012), by stabilizing the soil surface (Kidron & Zohar, 2014) and providing nutrients by fixing carbon and nitrogen (Lange et al., 1992, Barger et al., 2016, Sancho et al., 2016). In arid ecosystems, such as the Negev Desert, cyanobacteria are key to biocrust formation through photosynthesis (Mazor et al., 1996, Grishkan & Kidron, 2013, Grishkan & Kidron, 2016) and the binding of soil particles by producing extracellular polymeric substances (EPS) (Hagemann et al., 2015, Hagemann et al., 2016).

While biocrust microorganisms developed various adaptations to withstand the harsh desert environment (Makhalanyane et al., 2015), they are extremely sensitive to mechanical disturbances. Such a disturbance, especially over large scales (for example, mining activity), kills biocrust organisms, subsequently disrupting their metabolic activities and often resulting in the destruction of the biocrust communities (Belnap & Eldridge, 2003, Steven et al., 2015). Previous studies indicated that the bacterial communities' composition and abundance

vary through biocrust succession stages (Zhang et al., 2016, Liu et al., 2017, Zhang et al., 2018).

1.4. Biocrust recovery following perturbations

It is difficult to accurately predict biocrust recovery times following a mechanical disturbance, given that various factors, such as the characteristics of the disturbance (intensity, timing, frequency), the level of degradation, soil stability, site characteristics, water and nutrient limitations and the availability and dispersal of biocrust propagules, are unpredictable and may influence the successional process of biocrust communities (Belnap & Eldridge, 2003, Bowker, 2007). Also, biocrust recovery is not linear over time, making it further challenging to estimate accurate recovery times (Weber et al., 2016). Regardless, it is widely agreed that natural rehabilitation of biocrusts is slow and could take anywhere from tens of years in more mesic habitats or under favourable conditions, to hundreds or even thousands of years in arid and hyper-arid habitats (Belnap & Warren, 1998, Belnap, 2003, Pointing & Belnap, 2012). Biocrusts in habitats with greater precipitation, high soil stability, fine-textured soils, and low disturbance frequency or intensity tend to recover more quickly (Weber et al., 2016). Thus, mechanical disturbances to biocrusts are expected to be especially severe in hyper-arid systems, characterized by very low and unpredictable rainfall (Noy-Meir, 1973), strong radiation and low nutrient availability (Reynolds et al., 2007). Consequently, restoration efforts are crucial in such environments and could introduce a unique set of challenges.

1.5. Biocrusts restoration practices

Biocrusts are often used as a tool to combat land degradation and desertification. Given biocrusts' important ecosystem functions, they have become a focal group in dryland restoration efforts (Antoninka et al., 2020). Effective biocrust restoration techniques have been long studied. As the field expands, further advances and improvement of current applied practices are emerging (Antoninka et al., 2020). Previous studies regarding biocrust restoration following anthropogenic disturbances focused on the manipulation of the main factors that limit biocrust re-establishment such as propagules proximity, particularly following disturbances in large areas (Belnap, 1995). In turn, inoculation was identified as an efficient method to actively introduce biocrust propagules (particularly strains of cyanobacteria, given their role as primary producers) to speed up biocrust recovery (Belnap & Eldridge, 2003, Zhao et al., 2016, Mugnai et al., 2018). Inoculation may also benefit the

soil ecosystem by optimizing soil physico-chemical properties and increasing soil stability (Zaady et al., 2017, Rossi et al., 2022). Water availability is another important factor affecting biocrust restoration. In arid environments, hydration induces the establishment and growth of biocrust organisms. Therefore, restoration of biocrust following a disturbance greatly depends on the amount and frequency of hydration events (Bowker et al., 2006, Zhang et al., 2018).

Though each factor on its own has the potential to ameliorate damages to biocrusts following disturbances, further optimization of rehabilitation practices can be achieved by combining various treatments. Velasco Ayuso et al. (2017) conducted a microcosm experiment to grow biocrust inoculum under various treatments and found that high watering frequency and low light exposure were key to biocrust establishment and growth. Likewise, Chock et al. (2019) found that a combination of shading and inoculation resulted in successful rehabilitation in fine-textured biocrusts.

1.6. Knowledge gap and research goal

There is a large body of evidence regarding the effects of mechanical disturbances such as human traffic and trampling on the diversity and functioning of biocrust communities (Barger et al., 2006, Kidron et al., 2008, Williams et al., 2008, Steven et al., 2015, Faist et al., 2017) (Barger et al., 2006, Kidron et al., 2008, Williams et al., 2008, Steven et al., 2015, Faist et al., 2017). However, despite posing a severe mechanical disturbance, the effects of mining on biocrust communities have been rarely studied (Zaady et al., 2016). Also, biocrusts have been rarely studied in hyper-arid deserts, thus examining the effects of mining on the Negev Desert biocrust communities pose a novel, yet challenging research system. Biocrust rehabilitation does not only serve to restore soil functionality, rather it is pivotal for restoring the whole arid ecosystem functioning (Belnap, 1995, Bowker, 2007). Thus, scientific research-based evaluation and action is needed. In this study, I aimed to study biocrust bacterial communities following a mining disturbance, understand how the mining disturbance affects biocrust community composition and function, and identify potential restoration treatments to enhance biocrust recovery.

2. Aims and hypotheses

2.1. Aim 1 - Examine changes in biocrust bacterial communities in phosphate mining sites.

We aimed to examine the spatiotemporal changes in biocrust bacterial communities in post-mining sites and adjacent natural (hereafter, 'reference') sites, to test the efficiency of the mining company's current restoration practices. We evaluated biocrust bacterial community measures (abundance, diversity, composition, and photosynthetic potential) on two scales: (i) Spatial, comparing biocrust communities from post-mining and reference plots within the same site; and (ii) Temporal, comparing biocrust communities from post-mining sites that were restored in different years. We hypothesized that biocrusts from post-mining plots differ in community measures at both scales: at the spatial scale we predicted that diversity would be lower in post-mining biocrust communities while at the temporal scale we predicted that diversity would increase with restoration age. We also predicted that primary production would be higher in reference biocrust communities, reflected in higher cyanobacterial abundance and Chl *a* concentration.

2.2. Aim 2 – Examine the active biocrust bacterial communities in post-mining biocrusts.

We aimed to investigate the active biocrust bacterial groups by hydrating post-mining and reference biocrusts. To that end, the biocrust samples were marked with a stable isotope of water ($H_2{}^{18}O$) and DNA-SIP was performed. We hypothesized that growth patterns and taxonomic identity of active bacterial groups will differ significantly when comparing reference and post-mining biocrusts. Specifically, we expected to see higher bacterial growth (more active groups) in reference biocrusts, as well as higher primary production in the reference biocrusts, based on our previous findings (Gabay et al., 2022).

2.3. Aim 3 – Examine the role of biotic and abiotic factors in accelerating establishment of biocrusts on burden soil.

We aimed to apply relevant biotic and abiotic treatments and examine their effect on biocrust establishment in topsoil excavated during mining. We monitored biocrust establishment in mesocosms of soil collected from a stockpile in Zin mining sites. the mesocosms underwent the following treatments: two inoculation methods – cyanobacteria enriched biocrusts and field-collected (natural) biocrusts – and applied a weekly hydration regime in a climate-controlled setting. We hypothesized that natural biocrusts with hydration will be the most effective treatment, since natural biocrust organisms are adapted to life on the hyper-arid soil

surface of Zin Valley compared to enriched biocrust communities. We further hypothesized that weekly hydration alone wouldn't enhance the establishment of the biocrust community, since the topsoil used in the experiment likely lacks both nutrients and biocrust propagules, given that it was mixed during its excavations and was exposed to the elements for at least two years.

Note:

Study 1 has been published:

Gabay, T., Rotem, G., Gillor, O., & Ziv, Y. (2022). Understanding changes in biocrust communities following phosphate mining in the Negev Desert. Environmental Research, 207, 112200. DOI: https://doi.org/10.1016/j.envres.2021.112200

3. Methods

3.1. Study 1

3.1.1. Study Area and sampling

Phosphate mining is carried in three separate locations in the Negev Desert – Oron, Mishor Rotem and Zin. In this study, we focused on Zin Valley mining sites (30.53°N, 35.07°E, elevation 100 m; Fig. 1). Zin valley is a hyper-arid environment with 50 mm annual rainfall average (unpublished data, Zin factory meteorological data). The soils also receive moisture in the form of dew and fog. However, dew amounts in Zin valley were not measured. The local soils contain phosphate rock deposits, covered by chalk and marlstone as well as flint rocks that contribute deposits of manganese and iron to the soil due to erosion (Nathan et al., 1997). They are composed of variable amounts of sand, silt, and clay and are highly saline (Table A1; Levi et al., 2021).

Sampling took place in four sites in the Zin Valley mining area – Afik, Hagor, Saif and Gov – during the spring of 2017. Two reference plots and two post-mining plots were sampled in each site (Fig. 1). Post-mining plots varied in years since restoration – 2007, 2010, 2012 and 2015, for Gov, Hagor, Afik and Saif, respectively. These plots and their adjacent reference plots were chosen based on similar geological layers, soil characteristics and topographic structure. The biocrusts in both reference and post-mining plots are thin, smooth, and light coloured (Fig. A1), typical to hyper-arid deserts (Bowker et al., 2016; Chamizo et al., 2016). They appear to be mainly bacterial biocrusts, with no visible growth of mosses, lichens, or fungi (Fig. A1). In the lab, we observed cyanobacterial growth on biocrust samples within hours of wetting (Talia Gabay, unpublished data). Each sampling plot consisted of a rectangular area of 100 × 50 m, divided into fifty 10 × 10 m squares (Fig. 1). Five subsamples of biocrust were composited from three squares in each plot resulting in 48 samples (= 3 squares × 4 plots × 4 sites). Samples were collected using a sterile spatula to separate between the biocrust and the topsoil. The hyper-arid biocrust is 1-3 mm thick, thus collection was carefully performed, and samples were placed in 5 cm diameter petri dishes and transported to the laboratory on ice within five hours of sampling. In the laboratory, a portion of soil from each petri dish was immediately sieved through a 0.5 cm sieve, placed into 2 mL tubes (Eppendorf) and stored in -80°C for molecular analyses. The rest of the soil was left intact in petri dishes and stored under dark, cool conditions for Chl *a* extraction. We note that

the physico-chemical characteristics of the topsoil of three of the sites studied here (Afik, Hagor and Gov) were thoroughly investigated (Levi et al., 2021) and are discussed here.

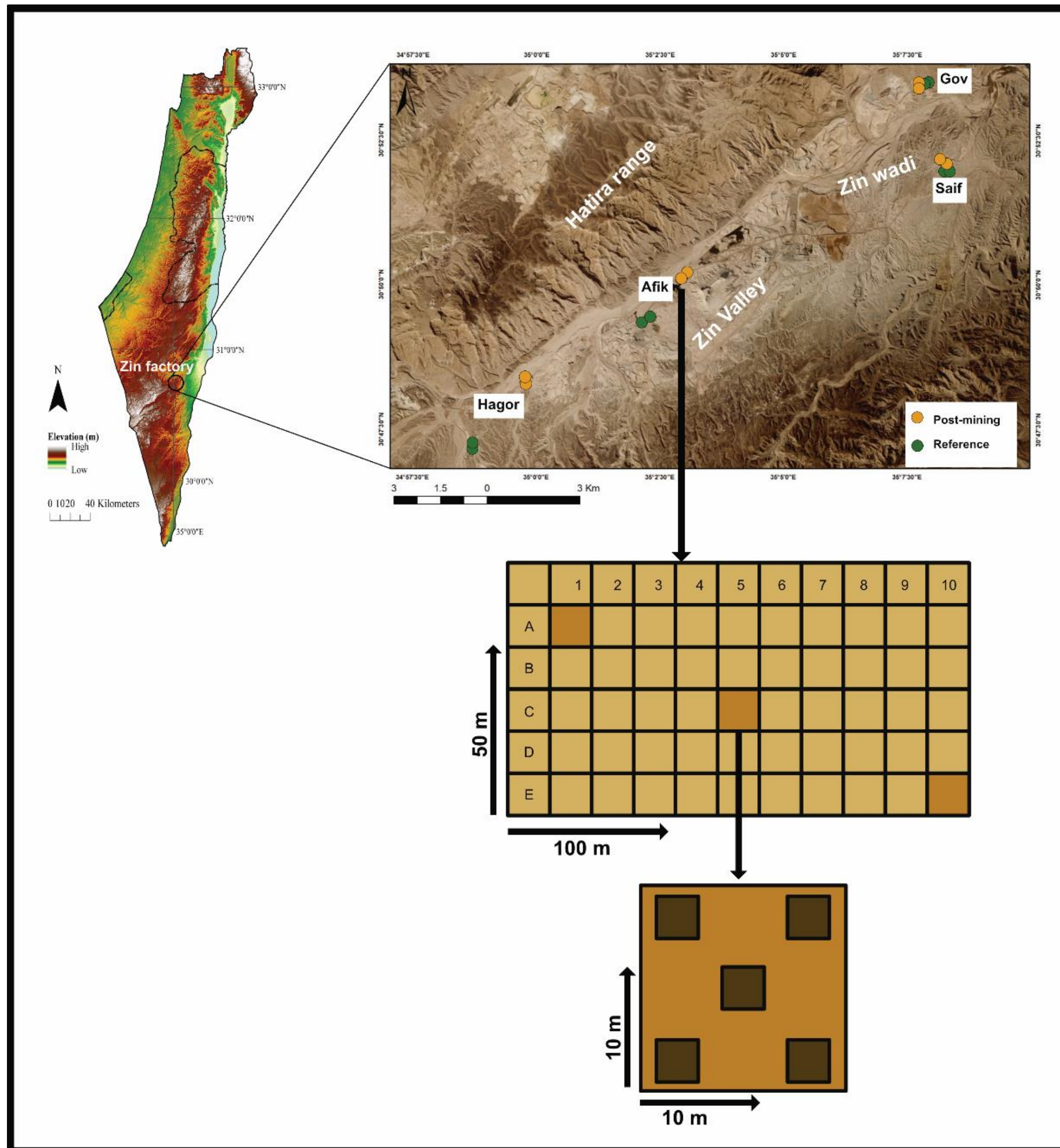


Figure 1. Map of research area and scheme of sample collection. Each sampling site consisted of two reference (green dots) and two post-mining (yellow dots) plots. In each plot was a rectangular area of 100×50 m marked and divided into 50 squares of 10×10 m. In three squares, five subsamples of biocrust were collected and composited.

3.1.2. Chl *a* extraction

Chl *a* was extracted from biocrust samples using an adjusted protocol based on Wetzel & Westlake (1969); Lichtenthaler & Wellburn (1983); Dere et al. (1998) and Castle et al. (2011). Briefly, 3 g of soil was placed in a glass tube with 9 ml of 100% Methanol, heated at

65°C for 15 min, and then cooled for 2 h in 4°C. The samples were covered throughout the extraction to prevent Chl *a* degradation. The extracts absorption was measured in a spectrophotometer (TECAN, Männedorf, Switzerland) at 665 nm. The concentration of Chl *a* was calculated according to Ritchie (2006) and normalized per g soil.

3.1.3. DNA extraction and amplification

Total DNA was extracted from 0.4 g of homogenized biocrust samples, using Soil DNA Extraction kit (GeneAll, Seol, S. Korea), according to the manufacturer's instructions. The DNA was quantified (Nanodrop, Thermo Scientific, Waltham, MA, USA) and the V3-V4 region of bacterial 16S rRNA encoding gene was PCR amplified using 341F and 806R primers (Table A2). Each reaction contained: 2.5µL of BSA, 2.5µL of the Taq polymerase reaction Buffer, 2µL of dNTPs, 0.4µL of DreamTaq DNA Polymerase (Thermo Scientific), 1µL each of 8µM stock solution of forward and reverse primers, 5-10 ng of template DNA and DDW was added to adjust to 25 µL. The reaction was run for 26 cycles in T100 Thermal Cycler (Bio-Rad, Hercules, CA, USA) using the following program: denaturation at 95°C for 30 seconds, annealing at 60°C for 30 seconds, extension at 68°C for 30 seconds and one cycle of final extension at 68°C for 5 minutes. The amplicons were visualized on 1% agarose gel electrophoresis to ensure successful amplification. In total, 46 samples were sequenced.

3.1.4. Total bacterial abundance

qPCR amplification of the V3 region was used to quantify the general bacteria 16S rRNA encoding gene copy, using 341F and 515R primers (Table A2). Each 20 µL reaction contained: 10 µL of Absolute QPCR SYBR Green Rox Mix (ABGene, Portsmouth, NH, USA), 1 µL each of 8 µM stock solution of forward and reverse primers, 5µL of total DNA template (10 ng/µL) and 3 µL of RNase-free DDW. All reactions were conducted in CFX-9600 thermocycler (Bio-Rad) using the following protocol: denaturation at 95°C for 5 minutes followed by 40 cycles of denaturation at 95°C for 30 seconds, annealing at 60°C for 30 seconds and hold at 72°C for 30 seconds. At the end of each reaction, a melt curve was done in increasing temperature from 65°C to 95°C at 0.5°C increments every 5 seconds. Concentrations were estimated based on 10-fold dilutions of pJET plasmid (Takara, Shiga, Japan) containing the entire 16S rRNA encoding gene (~1500 bp) amplified from Escherichia coli DNA template.

3.1.5. Cyanobacterial total abundance

For quantification of the cyanobacterial 16S rRNA encoding gene, qPCR amplification of the V3-V4 region was performed using the cyanobacterial primers CYA359F, CYA781R(a) and CYA781R(b) (Table A2). Each 20 µL reaction contained: 10 µL of qPCR-BIO SYBR Green Blue Mix Hi-ROX (BioSystems, Barcelona, Spain), 1 µL of 8 µM forward primer, 0.5 µL of 8 µM from each reverse primer, 5 µL of total DNA template (10 ng/µL) and 3 µL of RNase-free DDW. All reactions were conducted in CFX-9600 thermocycler (Bio-Rad) using the following protocol: denaturation at 95°C for 3 minutes followed by 40 cycles of denaturation at 95°C for 5 seconds and annealing at 60°C for 30 seconds. Melt curve was done in increasing temperature from 55°C to 95°C at 0.5°C increments every 5 seconds. Concentrations were estimated based on 10-fold dilutions of pJET plasmid (Takara) containing cyanobacterial 16S rRNA encoding gene amplified from arid soil enriched sample dominated by Nostophycideae and Synechococcciphicideae.

3.1.6. Sequencing and bioinformatics

The amplified fragments were sequenced using NextSeq (Illumina, San Diego, CA, USA) at the Genome Research Division, Genome Research Core, the University of Illinois in Chicago (https://rrc.uic.edu/cores/genome-research/genome-research-core/). The generated reads were processed using NeatSeq Flow workflow (Sklarz et al., 2017) which utilizes QIIME2 (Bolyen et al., 2018, Bolyen et al., 2019) and DADA2 (Callahan et al., 2016) pipelines for illumina amplicon data analysis. SILVA 128 database (Quast et al., 2012) was used as the reference database for sequence alignment, amplicon sequence variant (ASV) identification at 97% sequence similarity threshold and taxonomic assignment. Rarefaction curves were calculated at a sampling depth of 5,604 reads per sample, for all samples, based on the lowest sample read (Table A3). Raw sequences are available on NCBI Sequence Read Archive, Accession numbers SAMN16965356 through SAMN16965401, under bioproject ID PRJNA681698.

3.1.7. Statistical analyses

All statistical analyses were performed in R environment (R core team, 2022). Chl *a* concentrations were compared using t-tests. Diversity indices were calculated using Phyloseq (McMurdie & Holmes, 2013) and Vegan (Dixon, 2003) packages. Alpha and beta diversity indices as well as total abundance measures were compared between reference and post-mining plots using non-parametric Mann-Whitney U tests. Kruskal-Wallis test was used to compare alpha and beta diversity between post-mining plots of different mining sites

(temporal scale). NMDS multivariate analysis of biocrust bacterial communities was based on Bray-Curtis dissimilarity matrices and group significance was tested using PERMANOVA.

3.2. Study 2

3.2.1. Study site and Sample collection

Sampling was conducted during June 2020 at the Gov Mining Site, located in the Zin Valley (30.84°N, 35.09°E, elevation 98 m), where restoration was completed in 2007. The study area is described above. Biocrusts were sampled either from the post-mining mining or adjacent natural sites. In each sampling site, we measured a 100 m strip and sampled along the strip at approximately 10 m intervals (Fig. 2). In total, we sampled 20 biocrust samples (10 from each site). For the SIP assay, we chose 5 of the 10 samples from each site containing the highest Chl *a* concentrations.

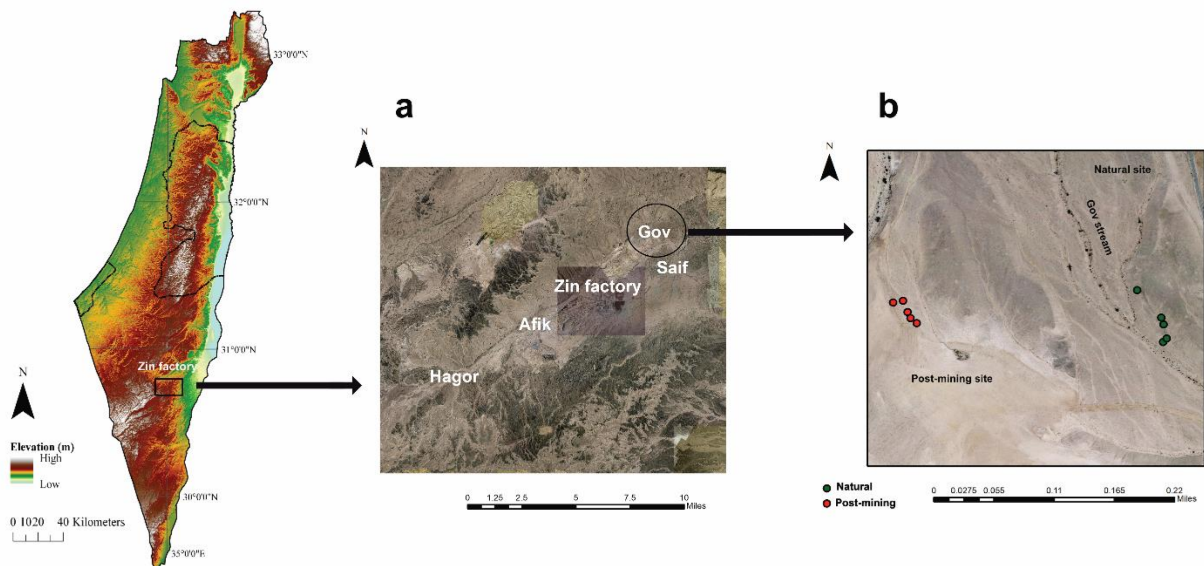


Figure 2. Map of the research area. Map a shows the different post-mining sites around Zin factory. Map b shows the biocrust sampling points in gov mining site used for this research. Green dots represent the natural biocrusts and red dots represent the post-mining biocrusts.

3.2.2. Soil properties

Five biocrust samples from each plot type (post-mining and reference) were sent for analysis of soil properties (pH, EC, NO_3 concentration, and soil organic matter). The analysis was performed at the Gilat Soil Lab (Gilat Research Center, Gilat, Israel).

3.2.3. Chl *a* extraction

Chl *a* was extracted from biocrust samples as described above (3.1.2.). Extractions were performed before (dry biocrusts) and after 96-hr incubation with DW under identical conditions to the incubation with $H_2{}^{18}O$.

3.2.4. Stable isotope probing

Stable isotope probing (SIP) is a culture-free approach that allows the detection of active microbial groups by labelling them with stable isotopes such as ^{15}N, ^{14}C and ^{18}O (Radajewski et al., 2000, Dumont & Murrell, 2005). Labelled substrates are introduced into the sample, metabolized by active microbial groups, and incorporated into their DNA and RNA (Neufeld et al., 2007, Coyotzi et al., 2016). The subsequent extraction, separation in a density gradient, and sequencing of the cells' DNA or RNA, allow phylogenetic, metagenomic and functional gene analysis of active groups (Dumont & Murrell, 2005).

SIP can utilize the incorporation of water containing the stable isotope of oxygen ^{18}O into actively replicating DNA or transcribing RNA. The analysis of stable isotope marked nucleic acids elucidates the growth and function of microorganisms that activate in response to wetting (Aanderud et al., 2015). Desert biocrusts make an ideal study system for $H_2{}^{18}O$ SIP experiments, as they become active quickly following hydration, resuming processes of growth, nutrient cycling, and excretion of EPS (Garcia-Pichel & Belnap, 1996, Belnap & Lange, 2003).

3.2.4.1. Soil incubation

To test the incorporation of ^{18}O into biocrust communities, a microcosm was designed to control for incubation conditions. Each microcosm consisted of a 10 mL glass vial in which 1 g of biocrust sample was placed. To achieve field water-holding capacity, 0.15 mL of $H_2{}^{18}O$ was added. The glass vials were then sealed with butyl rubber stoppers to prevent evaporation. Each labelled sample had a non-labelled control, incubated under identical conditions but with DNase-free water instead of ^{18}O water. Both labelled and unlabelled controls were incubated in duplicates, for a total of 40 vials. Samples were incubated under a 12-hr photoperiod for 96-

hr in a 25°C incubator with a LED lamp. Following incubation, the microcosms were sacrificed, and each biocrust sample was divided into four and each 0.25 g of soil was placed in a bead beating tube (QIAGEN) and stored at -80°C until further analysis.

3.2.4.2. DNA extraction

DNA was extracted from all biocrust samples using DNeasy PowerSoil Pro Kit (QIAGEN), according to the manufacturer's instructions. Since biocrusts in the research area tend to have minute amounts of DNA, each sample of soil was extracted in batches of 0.25 g, and the extracts were later consolidated to increase DNA yield.

3.2.4.3. Fractionation

The light and heavy fractions of the labelled DNA were separated by fractionation using a CsCl gradient as described in Neufeld et al. (2007).

3.2.4.4. PCR and sequencing

Following fractionation, all samples (labelled and unlabelled) were amplified using the 16S rRNA primers 515F_mod and 806R_mod (Apprill et al. 2015, Parada et al. 2016). Each reaction consisted of 2.5 µL Green Taq Buffer, 2.5 µL of dNTP set (Biotechrabbit), 0.1 µL of BSA (Thermo Fisher), 0.625 µL of each primer (10 µM), 0.125 µL DreamTaq Hot Start Green DNA Polymerase (Thermo Fisher) and 17.5 µL of PCR water. The PCR ran for 38 cycles using the following program: denaturation at 94°C for 45 sec, annealing at 52°C for 45 seconds, extension at 72°C for 45 sec, and a final cycle of extension at 72°C for 10 min. The amplified fragments were sequenced using Miniseq (Illumina) at the UIC sequencing core, University of Illinois, Chicago, Illinois (https://rrc.uic.edu/cores/genome-research/genome-research-core/). PCR negative controls and a mock community (ZymoBIOMICS Microbial Community Standard II Log Distribution; Zymo) sample were also sequenced to allow the removal of contaminants from sequencing results.

3.2.5. Bioinformatic analyses

All the bioinformatic and statistical analysis was done in R V4.1.1 (R Core Team, 2013). Labelling of bacteria was detected using differential abundance analysis as described in Angel et al. (2018). Briefly, the sequences were processed using the DADA2 V8.8 (Callahan et al., 2016) for quality filtering, denoising, read-merging, chimera removal, constructing amplicon sequence variants (ASV) tables, and taxonomic assignment. Detection and removal of potential contaminant sequences were performed using the decontam V.1.12.0 (Davis et al., 2017).

Prevalence filtering of rare ASVs was done using Phyloseq V1.36.0 (McMurdie & Holmes, 2013). ASVs that appeared in less than 2.5% of the samples were removed. A maximum-likelihood phylogenetic tree was calculated using IQ-TREE2 V 2.1.1. (Minh et al., 2020). Finally, differential abundance analysis was performed using DESeq2 V1.32.0 (Love et al., 2014) to compare the relative abundance of each ASV in the heavy fractions of labelled DNA to the unlabelled heavy fractions, which allows identifying the bacterial taxa that incorporated the oxygen isotope into their DNA. The results were filtered to include only ASVs with a 2-fold log change and a significance value $p < 0.1$.

3.2.6. Predictions of genomic functions

Abundances of functional genes based on 16S rRNA gene abundances was performed using Picrust2 (Douglas et al., 2019). Abundances were predicted using a filtered ASV table, containing only ASVs that were identified as active based on the differential abundance modelling. The resulting output is functional ids that were annotated using KEGG database, to infer functional gene families. Each gene was then classified to 11 function categories based on Meier et al. (2021). The abundances of the different genes within each category were averaged.

3.2.7. Statistical analyses

Chl *a* concentrations were visualized as an estimation plot using the dabestr V0.3.0 (Ho & Tumkaya, 2020). The effect size was calculated as a bootstrap 95% confidence interval. Abundances of functional genes and soil properties were compared between natural and post-mining biocrusts using Mann-Whitney tests.

The community composition of natural and post-mining biocrusts was assessed using sequences identified as active based on DESeq2 modelling. The weighted UniFrac distance metric (Lozupone et al., 2011) was used to calculate the similarity between the natural and post-mining communities, and adonis model was used to assess whether communities differ significantly from each other.

3.3. Study 3

3.3.1. Topsoil collection

Topsoil was collected from a stockpile near Hagor in August 2021 (Fig. 3). The stockpile was excavated approximately two years before collection. Ten bags of topsoil were collected and stored in dark, dry, cool conditions until the start of the experiment.

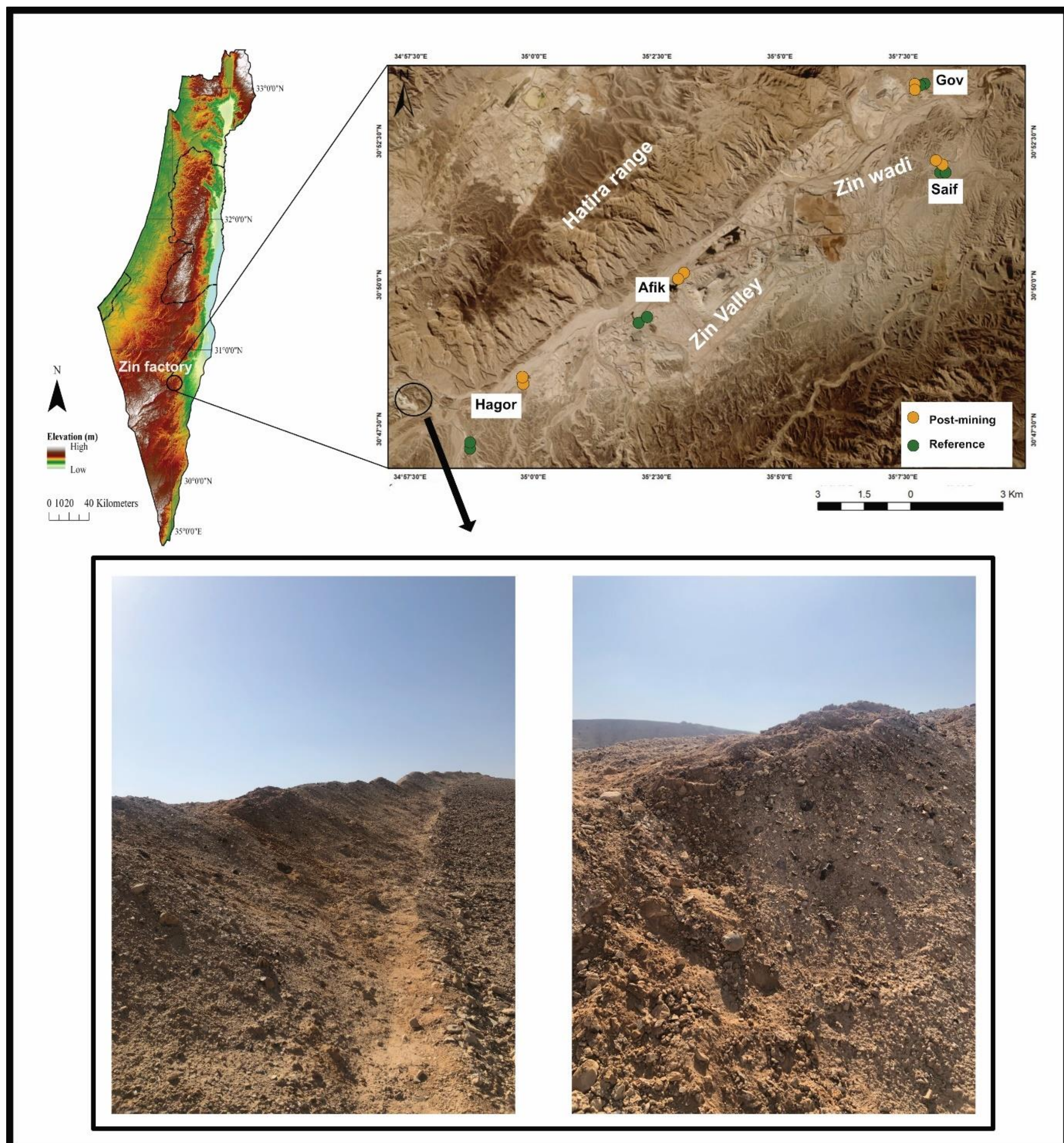


Figure 3. Map of research area. Our research plots within 4 mining sites, restored in different years, are indicated in green (reference - natural) or orange (post-mining). Topsoil was collected near Hagor mining sites, from a topsoil stockpile (bottom pictures).

3.3.2. Experimental setup

The experiment took place in a climate-controlled room with an average temperature of 25°C. A dehumidifier was placed in the room to decrease humidity levels, resulting in an average relative humidity of 33%. Soil was placed in mesocosms (13 cm length, 9 cm width and 5 cm depth). Mesocosms were filled up with soil at 4.5 cm depth and placed in plastic trays (6 mesocosms per tray) under LED lights (2580 Lumen, Photosynthetic active radiation (PAR) – 74 µMol/m^2/s on average; (Fig. 4).

The experiment included four treatments: hydration; hydration & inoculation with natural biocrusts; hydration & inoculation with cyanobacteria enriched biocrusts; and negative controls (no treatment). Hydration to maximum water holding capacity was applied at one-week intervals for 20 weeks. The soil in microcosms dried approximately 48 hours after hydration. Each treatment was performed with 16 replicates; eight replicates were sacrificed after 10 weeks and the rest after 20 weeks. Treatments were randomly assigned to mesocosms.

3.3.3. Inoculation treatments

3.3.3.1. Natural biocrusts

Natural biocrusts used for inoculation were collected from reference areas near Gov and Hagor mining sites in the fall of 2018 (Fig. 3). The samples were kept in petri dishes lined with cotton in dark, dry, cool conditions.

3.3.3.2. Cyanobacteria enriched biocrusts

Cyanobacteria were enriched from wetted biocrusts collected from a natural area near Gov mining site in the winter of 2020 (Fig. 3). The enriched cyanobacteria were grown in liquid Jaworski Media (JM), which targets non nitrogen fixing cyanobacterial strains. The cultivation protocol used was based on Giraldo-Silva et al. (2019) with slight modifications. Following enrichment, the cyanobacterial biomass was used to grow a cyanobacterial crust on bare soil using the following protocol: 150 g topsoil samples collected from natural areas in Zin Valley were placed in 500 mL Erlenmeyer flasks and supplemented with 150 mL of JM and 50 mL of the enriched culture of cyanobacteria. The flasks were sealed and incubated for 10 – 14 days under 12-hr photoperiod and agitation at 78 rpm. The soil was incubated until significant cyanobacterial growth was observed on the soil surface (Fig. C1). Following

the incubation, the JM was removed from the flasks, and they were left to dry for 3 days inside the flasks, then transferred to two petri dishes: one containing the biocrust and the other the rest of the soil column (Fig. C1). The plates were left to dry in a climate-controlled room under 12-hr photoperiod. When the soil was completely dry, the biocrusts were kept in petri dishes under dark, cool condition until they were used as inoculates.

3.3.4. Inoculation and hydration

15 g of dried natural or cyanobacteria enriched biocrusts were powdered and distributed evenly on top of the mesocosms right before the first hydration event. Each mesocosm was manually wetted once a week with a spray bottle to ensure minimal disruption to the soil surface. Each mesocosm was hydrated with 100 mL (±5 mL) of autoclaved DW until equal to maximum water holding capacity.

3.3.5. Chl *a*

Chl *a* was extracted from the top layer of experiment soil samples after 10 and 20 hydration events, as well as from natural and cyanobacteria enriched biocrusts used as inoculants. Chl *a* was measured as described above (3.1.2.).

3.3.6. Soil properties

Four samples of the stockpile soil and 12 soil samples collected after 20 hydration events (4 samples for each treatment) were sent to Gilat soil laboratory (Gilat Research Institute, Gilat, Israel) for soil property analyses (Table 5).

3.3.7. DNA extraction and amplification

DNA was extracted from the natural and cyanobacteria enriched biocrusts using DNeasy PowerSoil Pro Kit (QIAGEN). Each sample underwent four extractions of 0.25 gr each that were then consolidated and concentrated. The DNA of the soil samples taken during the experiment was extracted using Presto™ Soil DNA Extraction Kit (Geneaid, New Taipei City, Taiwan). DNA was extracted from 0.5 gr of soil samples taken from the top few millimetres of soil (crust) and the bulk soil column (topsoil).

For all extracts, the 16S rRNA gene was amplified using primers 431F and 806R supplemented with CS1 and CS2 linkers. Each reaction contained 1.25 μL of BSA, 1.25 μL of PCR buffer, 1 μL of dNTPs, 0.25 μL of each primer and 0.2 μL of Taq polymerase (Takara). 1.5 μL of water and 7 μL of sample were added for a total volume of 12.5 μL. PCR ran for 35 cycles in T100 Thermal Cycler (Bio-Rad) using the following program:

denaturation at 95°C for 45 sec, annealing at 60°C for 30 seconds, extension at 68°C for 30 sec and final extension at 68°C for 5 min.

In total, 148 samples were sequenced (20 samples of natural and cyanobacteria enriched biocrusts and 128 experiment sampled soil – 8 replicates × 4 treatments × 2 time points × 2 soil strata).

3.3.8. Sequence analyses

The amplified fragments were sequenced using MiSeq (Illumina) at the Genomics and Microbiome Core Facility, Rush University, Chicago, Illinois (https://www.rushu.rush.edu/research/rush-core-laboratories/rush-genomics-and-microbiome-core-facility). The generated reads were cut using cutadapt (Martin, 2011) and processed using DADA2 (Callahan et al., 2016). SILVA database version 138 (Quast et al., 2012) was used as the reference database for sequence alignment, ASV identification at 97% sequence similarity threshold, chimera removing and taxonomic assignment. Rare taxa were filtered out of the sequence tables using Phyloseq (McMurdie & Holmes, 2013). Sequences that appeared in less than 10% of the samples were removed.

3.3.9. Statistical analyses

3.3.9.1. Inoculation treatments

Alpha diversity was calculated using Chao1 index. Beta diversity was calculated using Bray-Curtis dissimilarity index. Community compositions were plotted using NMDS ordination. Community composition was compared between natural and cyanobacteria enriched biocrusts using an Adonis model. Chl *a* concentrations, alpha diversity and relative abundances were compared between natural and cyanobacteria enriched biocrusts using Mann-Whitey tests.

3.3.9.2. Experiment samples

Chl *a* concentrations and soil properties were compared between treatments and time points using two-way ANOVA and post-hoc Tukey's tests. Alpha diversity was calculated using Chao1 index. Beta diversity was calculated using Morisita-Horn dissimilarity index and was plotted using a CAP ordination. Community composition was compared between treatments and time points using an Adonis model.

All statistical analyses were performed in R environment (Version 4.2.1; R Core Team, 2013)), using Vegan (Dixon, 2003) and Phyloseq (McMurdie & Holmes, 2013) packages.

4. Results

4.1. Study 1

4.1.1. Total bacterial abundance

The 16S rRNA gene copies in reference plots were, on average, three orders of magnitude higher than post-mining plots (10^9 and 10^6 gene copies per g soil, respectively). The mean abundance estimates were significantly higher in reference compared to post-mining biocrusts across three of the four sampling sites (Saif: W=30, p=0.004; Hagor: W=33, p=0.01; Gov: W=36, p=0.002; Table 1).

4.1.2. Total cyanobacterial abundance

The cyanobacterial 16S rRNA gene copies in reference plots were, on average, at least one order of magnitude higher than post-mining plots (10^7 - 10^8 and 10^5 - 10^7 gene copies per g soil, respectively). The mean abundance estimates are significantly higher in reference compared to post-mining biocrusts across two of the four sampling sites (Saif: W=28, p=0.01 and Gov: W=34, p=0.008; Table 1, Fig. 5a).

4.1.3. Chl *a* concentration

Chl *a* concentrations were lower in post-mining compared to reference biocrusts across all sampling sites. The differences were most significant for Saif (t=4.54, p=0.005) and Gov (t=2.14, p=0.05), marginally significant for Afik (t=2.18, p=0.06), and not significant for Hagor (t=0.37, p=0.71; Fig. 5b)

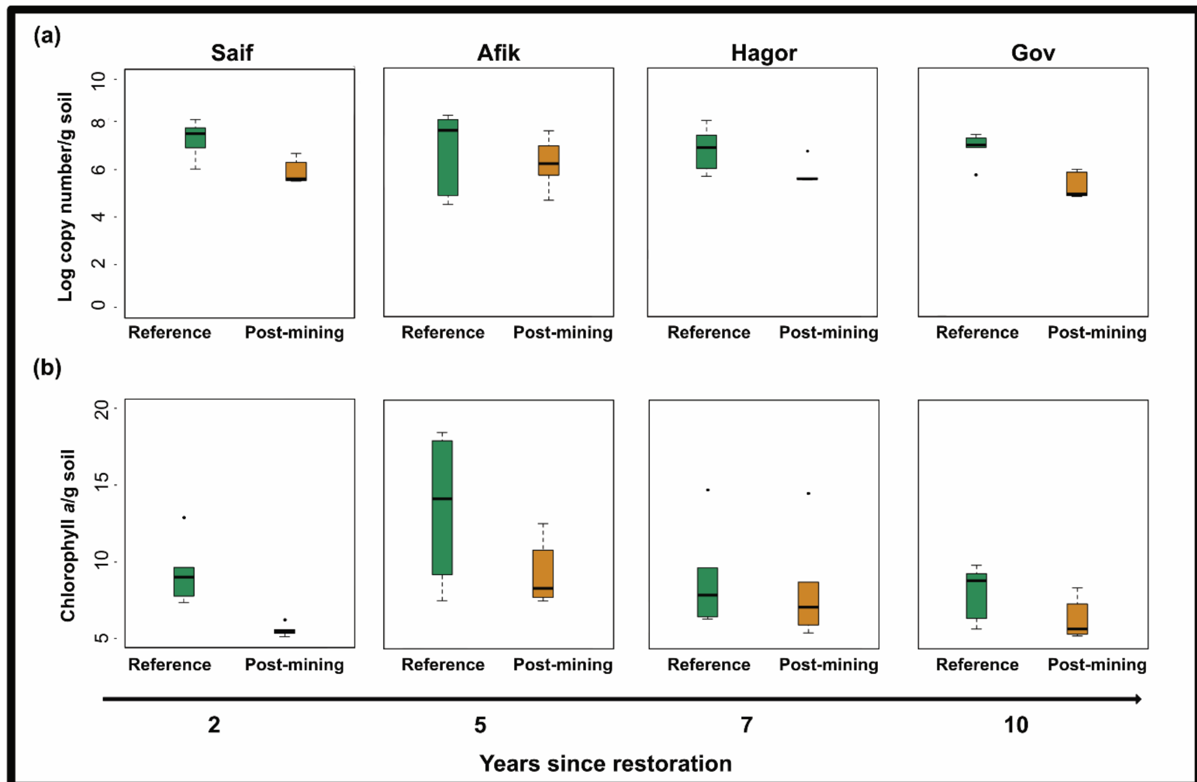


Figure 5. Boxplots of cyanobacterial total abundance in log scale (a) and Chl *a* concentrations per g soil (b). Whiskers represent range and black line represents median. The plots are ordered according to the year since restoration (2, 5, 7 and 10 corresponding to Saif, Afik, Hagor and Gov).

4.1.4. Alpha diversity

Three diversity indices were calculated – observed ASVs, chao1 and Fisher's alpha diversity. The mean values for all indices were higher in reference compared to post-mining plots across all sites (Table A4). However, the differences were significant only for Hagor (Observed ASVs: W=5, p=0.04; Chao1: W=4, p=0.02, Fisher's alpha: W=5, p=0.04; Table A4) and marginally significant for Gov (W=6, p=0.06 for all indices; Table A4).

4.1.5. Beta diversity

NMDS clustering analysis shows distinct bacterial communities in the post-mining and reference plots across all four sampling sites. PERMANOVA tests on the Bray-Curtis matrices indicated that the clustering is statistically significant for all sites (Saif: F=5.61, R^2=0.41, p=0.003; Afik: F= 5.53, R^2=0.35, p=0.003; Hagor: F=4.17, R^2=0.29, p=0.004; Gov: F=4.63, R^2=0.31, p=0.003; Fig. 6).

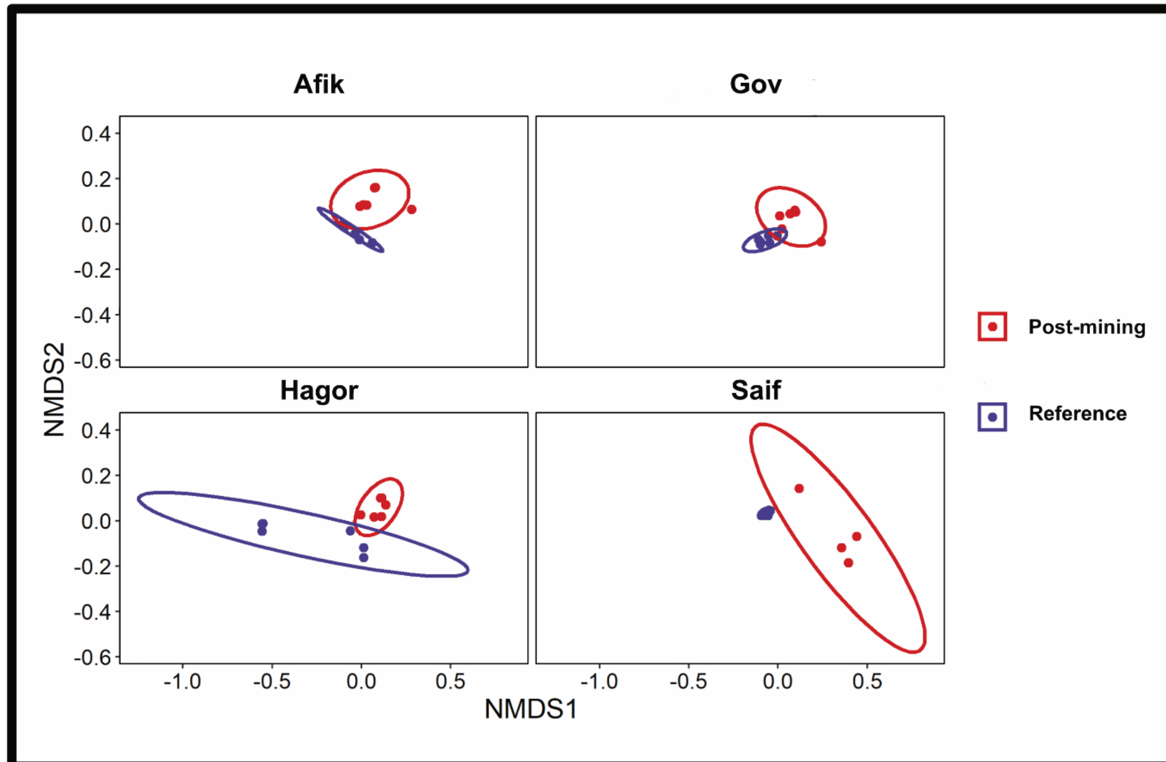


Figure 6. NMDS plots of samples from reference (blue dots) and post-mining (Red plots) plots. Ellipses are 95% confidence interval ellipsoids. Stress values for all sites < 0.1.

4.1.6. Community composition

Phylum level – Cyanobacteria and Chloroflexi were the most dominant phyla across all reference biocrusts. The relative abundance of Cyanobacteria was significantly higher in reference compared to post-mining plots in three sites (Saif, Afik and Hagor; Table 2), and the relative abundance of Chloroflexi was likewise significantly higher in reference plots across all the sites (Table 2). In post-mining biocrusts, Proteobacteria and Actinobacteriota were the dominant phyla. The relative abundance of Actinobacteriota was significantly higher in two of the post-mining plots (Afik and Hagor) while, the relative abundance of Proteobacteria was significantly higher across all the sites (Table 2).

Table 1. qPCR results for total 16S and Cyanobacterial 16S for all mining sites. Statistically significant results are highlighted. Values are means and standard errors.

Study site	**Saif**			**Afik**			**Hagor**			**Gov**		
Plot type	Reference	Post-mining	*p*-value	Reference	Post-mining	*p*-value	Reference	Post-mining	*p*-value	Reference	Post-mining	*p*-value
16S rRNA gene (copies g^{-1} Soil)	**$3.12x10^9$ $\pm3.56x10^9$**	$1.14x10^4$ $\pm9.64x10^3$	**0.004**	$4.93x10^9$ $\pm5.51x10^9$	$9.64x10^8$ $\pm1.37x10^7$	0.3	**$1.79x10^9$ $\pm2.24x10^9$**	$9x10^7$ $\pm1.73x10^8$	**0.01**	**$3.42x10^9$ $\pm1.92x10^9$**	$8.73x10^7$ $\pm1.27x10^8$	**0.002**
Cyanobacterial 16S rRNA gene (copies g^{-1} Soil)	**$4.34x10^7$ $\pm4.70x10^7$**	$1.41x10^6$ $\pm1.79x10^6$	**0.01**	$8.16x10^7$ $\pm9.26x10^7$	$1.09x10^7$ $\pm1.96x10^7$	0.3	**$2.66x10^7$ $\pm4.30x10^7$**	$1.33x10^7$ $\pm1.35x10^7$	0.81	**$1.27x10^7$ $\pm9.92x10^6$**	$2.99x10^5$ $\pm2.56x10^5$	**0.008**

Table 2. Percent relative abundances at the Phylum level (phyla with abundance of < 1% are not presented). Statistically significant results are highlighted.

Site	**Saif**			**Afik**			**Hagor**			**Gov**		
Phylum/Plot type	Reference	Post-mining	*p*-value	Reference	Post-mining	*p*-value	Reference	Post-mining	*p*-value	Reference	Post-mining	*p*-value
Actinobacteriota	17.74	28.93	0.91	**18.86**	**43.21**	**0.004**	**23.33**	**46.8**	**0.04**	19.32	29.79	0.39
Bacteroidota	4.1	2.52	0.11	6.9	7.14	0.58	**3.31**	**6.92**	**0.02**	5.27	4.03	0.3
Chloroflexi	**10.7**	**1.64**	**0.01**	**13.25**	**<1%**	**0.002**	**23.88**	**1.04**	**0.002**	**21.34**	**1.97**	**0.002**
Cyanobacteria	**48.94**	**2.44**	**0.01**	**38.99**	**10.53**	**0.002**	**18.89**	**8.32**	**0.04**	30	12.18	0.13
Deinococcota	1.05	<1%	0.23	2.54	3.49	0.24	8.71	5.69	0.58	3.01	4.22	0.39
Desulfobacterota	**<1%**	**2.13**	**0.01**	<1%	<1%		<1%	<1%		<1%	<1%	
Firmicutes	**<1%**	**1.45**	**0.01**	<1%	<1%		**<1%**	**1.4**	**0.01**	<1%	<1%	
Gemmatimonadota	**1.83**	**<1%**	**0.01**	**2.57**	**<1%**	**0.002**	5.46	3.21	0.24	4.07	3.42	0.48
Myxococcota	<1%	<1%		**1**	**<1%**	**0.008**	<1%	<1%		<1%	<1%	
Patescibacteria	1.99	1.25	0.47	**<1%**	**1.84**	**0.02**	**<1%**	**1.88**	**0.004**	**1.06**	**3.22**	**0**
Proteobacteria	**12.79**	**51.68**	**0.01**	**13.98**	**30.31**	**0.002**	**12.99**	**23.96**	**0**	**13.5**	**38.53**	**0.002**
Verrucomicrobiota	<1%	4.5	0.17	<1%	<1%		1.79	<1%	0.37	<1%	<1%	

Table 3. Percent relative abundances at the Order level (orders with abundance of < 1% are not presented). Statistically significant results are highlighted.

Site	Saif			Afik			Hagor			Gov		
Order/Plot Type	Reference	Post-mining	*p*-value	Reference	Post-mining	*p*-value	Reference	Post-mining	*p*-value	Reference	Post-mining	*p*-value
Cyanobacteriales	**18.29**	**<1%**	**0.009**	**26.7**	**1.62**	**0.002**	**15.36**	**4.17**	**0.04**	**26.62**	**7.52**	**0.008**
Oxyphotobacteria Incertae Sedis	**28**	**<1%**	**0.01**	9	7	0.8	**3**	**3**	**0.37**	**3**	**3**	**0.93**
Kallotenuales	**8.77**	**<1%**	**0.01**	**8.28**	**<1%**	**0.01**	**15.76**	**<1%**	**0.004**	**13.73**	**<1%**	**0.002**
Thermomicrobiales	<1%	<1%		**3.79**	**<1%**	**0.002**	**4.46**	**<1%**	**0.002**	**4.13**	**<1%**	**0.002**
Frankiales	6.2	11.18	0.2	**7.54**	**21.02**	**0.008**	**7.23**	**28.51**	**0.002**	**4.54**	**21.95**	**0.002**
Rubrobacterales	**<1%**	**8.39**	**0.01**	**6.26**	**<1%**	**0.002**	1.82	3.96	0.39	**5.82**	**2.16**	**0.002**
Micrococcales	**<1%**	**7.19**	**0.01**	**<1%**	**18.47**	**0.002**	**3.39**	**9.6**	**0.01**	**<1%**	**1.85**	**0.002**
Solirubrobacterales	<1%	<1%		**2.23**	**<1%**	**0.002**	4.2	<1%	0.3	3.15	1.48	0.06
Burkholderiales	**<1%**	**30.59**	**0.009**	**1.1**	**12.89**	**0.002**	**<1%**	**5**	**0.002**	**<1%**	**9.11**	**0.002**
Sphingomonadales	4.69	8.01	1	4.69	3.95	0	**2.96**	**9.24**	**0.002**	**5.08**	**15.84**	**0.01**
Rhizobiales	<1%	0.03	0.4	2.21	2.35	1	**4.14**	**<1%**	**0.008**	**3.2**	**1.95**	**0.04**
Rhodobacterales	<1%	<1%		2.89	5.27	1	2.14	6.33	0.13	**1.95**	**6.92**	**0.002**
Cytophagales	3.9	<1%	0.11	6.33	5.99	0.93	**2.55**	**6.81**	**0.04**	4.14	3.76	0.39
Deinococcales	<1%	<1%		2.56	3.5	0.24	8.87	5.75	0.58	3.07	4.25	0.39

Saccharimonadales	<1%	<1%	<1%	1.79	0.06	**<1%**	**1.9**	**0.002**	1.01	1.34	0.06
Longimicrobiales	<1%	<1%	**2.3**	**<1%**	**0.002**	5.3	3.16	0.24	4.04	3.32	0.48

Order level – In accordance with the phyla relative abundances, the order Cyanobacteriales (belonging to the phylum Cyanobacteria) as well as the orders Kallotenuales and Thermomicrobiales (belonging to the phylum Chloroflexi) were significantly more abundant in reference plots compared to post-mining plots across all mining sites (Fig. 7, Table 3). In post-mining plots, the orders Burkholderiales (Beta-Proteobacteria) as well as the orders Frankiales, Rubrobacterales and Micrococcales (Actinobacteriota) were significantly more abundant across all sampling sites (Fig. 7, Table 3).

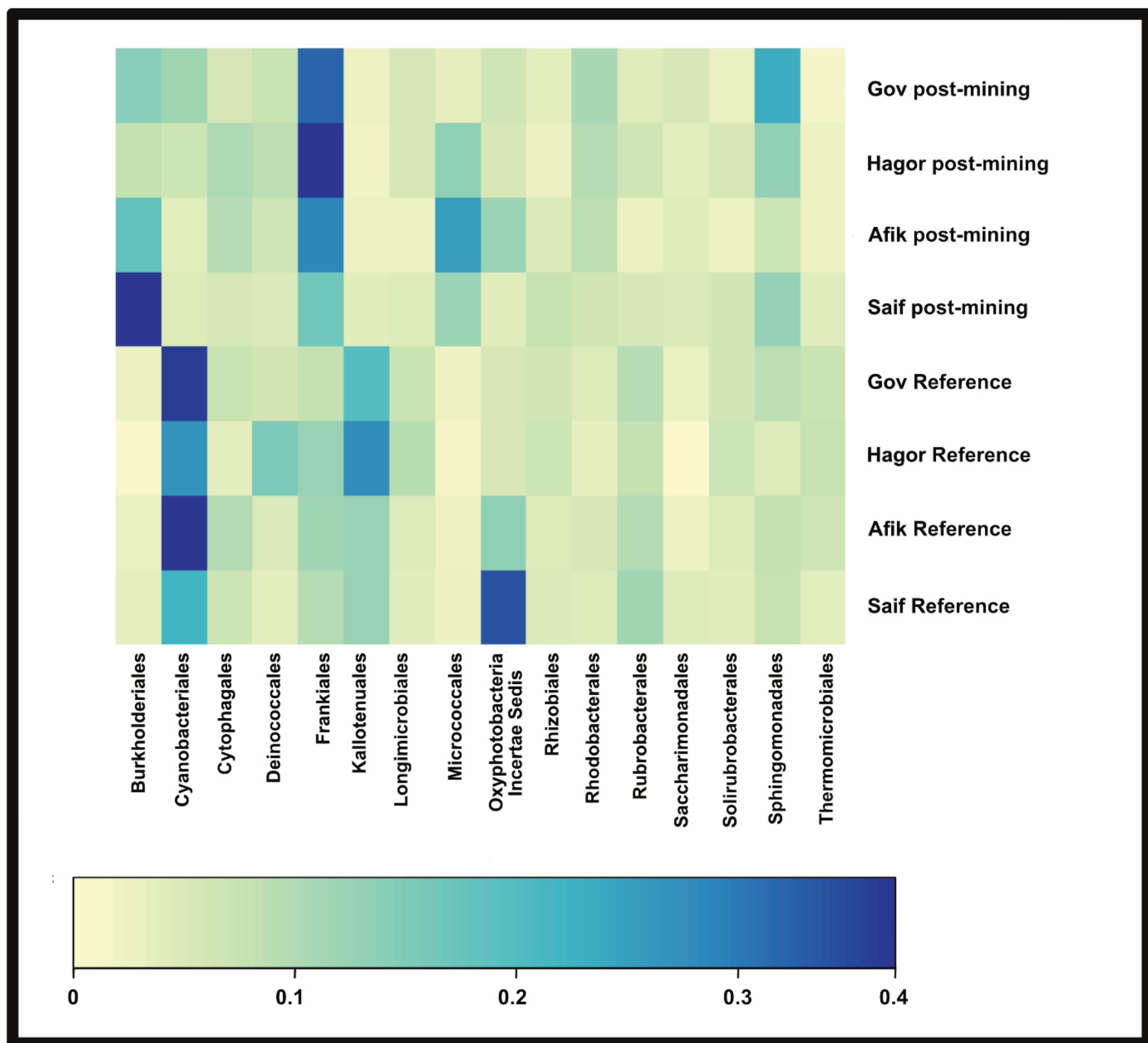


Figure 7. Heatmap of bacterial orders with relative abundance of over 1%. Each row represents a different mining site and plot type. Columns represent different orders.

4.1.7. Comparisons of post-mining biocrust communities on a temporal scale

Chl *a* concentrations vary significantly when comparing post-mining sites restored at different years ($\chi2=12.44$, $p=0.006$). However, a post hoc Dunn's test revealed significant differences only between Afik and Saif ($Z=3.22$, $p=0.007$; Fig. 5b).

Diversity indices did not vary significantly when comparing post-mining sites restored at different times (Table A5). The NMDS clustering shows a random clustering of samples rather than by different mining sites (Fig. A2). However, pairwise PERMANOVA comparisons revealed that the community composition of Afik differs significantly from Hagor and Gov ($F=3.18$, $R^2=0.24$, $p=0.024$ and $F=2.98$, $R^2=0.23$, $p=0.04$ respectively).

Relative abundances at the phylum level did not vary significantly between post-mining sites among the most dominant phyla (Cyanobacteria, Chloroflexi, Actinobacteriota and Proteobacteria, Table A6). Kruskal-Wallis test revealed that Bacteroidota varies significantly between sites, however, a post hoc Dunn's test revealed no significant differences (Table A6). There were significant differences in the phylum Deinococcota between Hagor and Saif (Dunn's test - $Z=2.94$, $p=0.01$; Table A6). Saif differed significantly from all other sites in relative abundance of Desulfobacterota (Dunn's test - Afik-Saif: $Z=2.83$, $p=0.02$; Gov-Saif: $Z=2.74$, $p=0.024$; Hagor-Saif: $Z=2.88$, $p=0.02$). The relative abundance of Patescibacteria differed significantly between Gov and Saif (Dunn's test - $Z=2.82$, $p=0.02$; Table A6). Gemmatimonadota relative abundances differed among all sites except for Afik and Saif (Afik-Gov: $Z=2.71$, $p=0.04$; Afik-Hagor: $Z=2.62$, $p=0.04$; Gov-Saif: $Z=2.58$, $p=0.03$; Hagor-Saif: $Z=2.50$, $p=0.03$; Table A6).

4.2. Study 2

4.2.1. Sample wetting and greening

Most biocrust samples (both reference and post-mining) showed greening 36-48 hrs into the 96-hr incubation. By the end of the experiment, most samples displayed varying degrees of greening, indicating cyanobacterial activity (Fig. 8). Yet, post-mining biocrust showed less greening compared to reference biocrusts (Fig. 8).

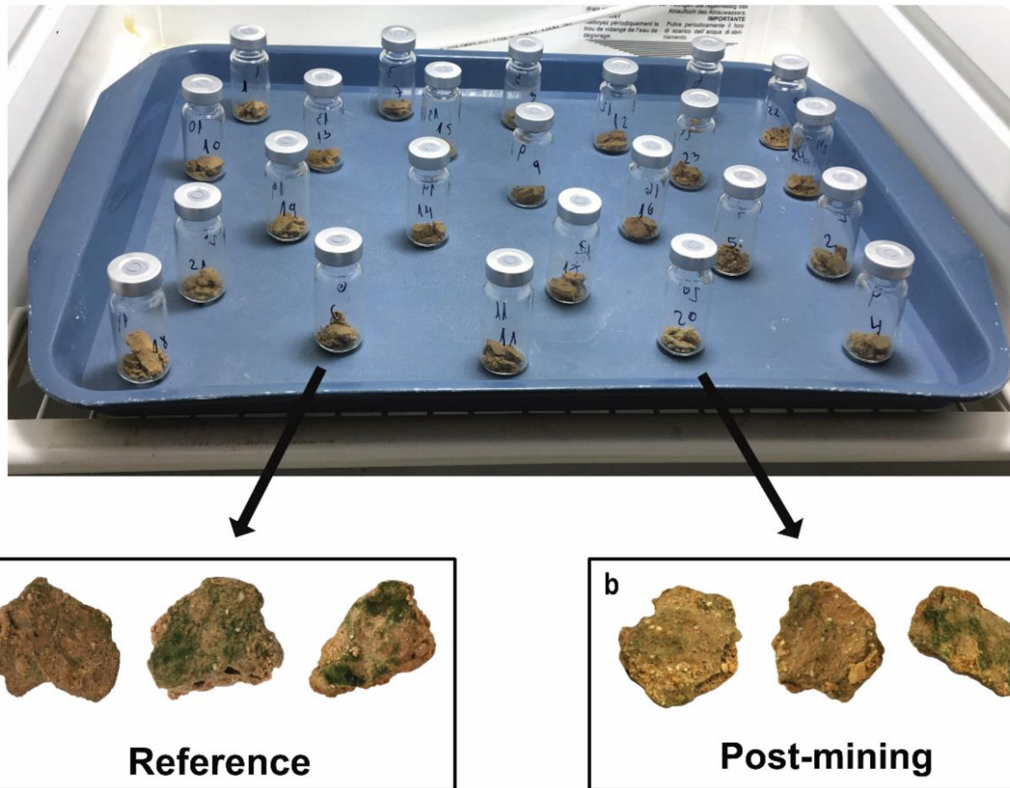


Figure 8. Incubation setup. Top picture – biocrusts in sealed, glass vials in the incubator. Bottom picture – reference (a) and post-mining (b) biocrusts following the 96-hr incubation.

Table 4. Soil properties for reference and post-mining biocrusts. The numbers represent the means for each property. Significant differences are marked with asterisk (* = $p < 0.05$; ** = $p < 0.01$).

Plot type/Soil property	Reference	Post-mining
pH	7.6	7.5
EC	26.22*	9.94
NO3	84.82**	14.75
Soil organic matter	1.2**	0.81

4.2.2. Soil properties

EC and NO_3 were significantly higher in reference biocrusts comparted to post-mining biocrusts (EC: $t = 2.89$, $p < 0.05$; NO_3: $t = 4$, $p < 0.01$; Table 4). Soil organic matter was also

significantly higher in reference biocrusts ($t = 3.77$, $p < 0.01$; Table 4). pH was slightly higher in reference biocrusts; however, the differences were not statistically significant ($t = 1.41$, $p = 0.19$; Table 4).

4.2.3. Chl *a*

The estimation plot revealed an effect size estimate of 1.42 (95CI -0.432; 3.03; Fig. 9). In the reference samples, there was no clear clustering of the samples according to the state of the soil (dry or hydrated for 96-hr). There was a larger variance between the samples after incubation (Fig. 9). In the post-mining biocrusts, the hydrated biocrusts showed consistently higher Chl *a* concentration compared to dry biocrusts. It was also apparent that the variance between samples was smaller in the post-mining biocrusts (Fig. 9).

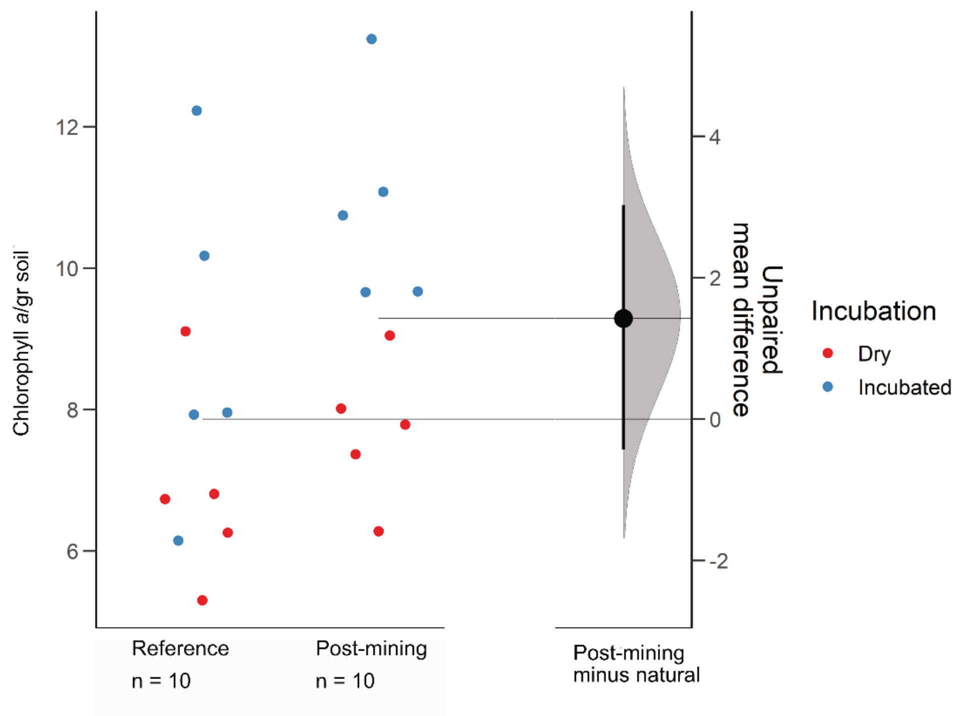
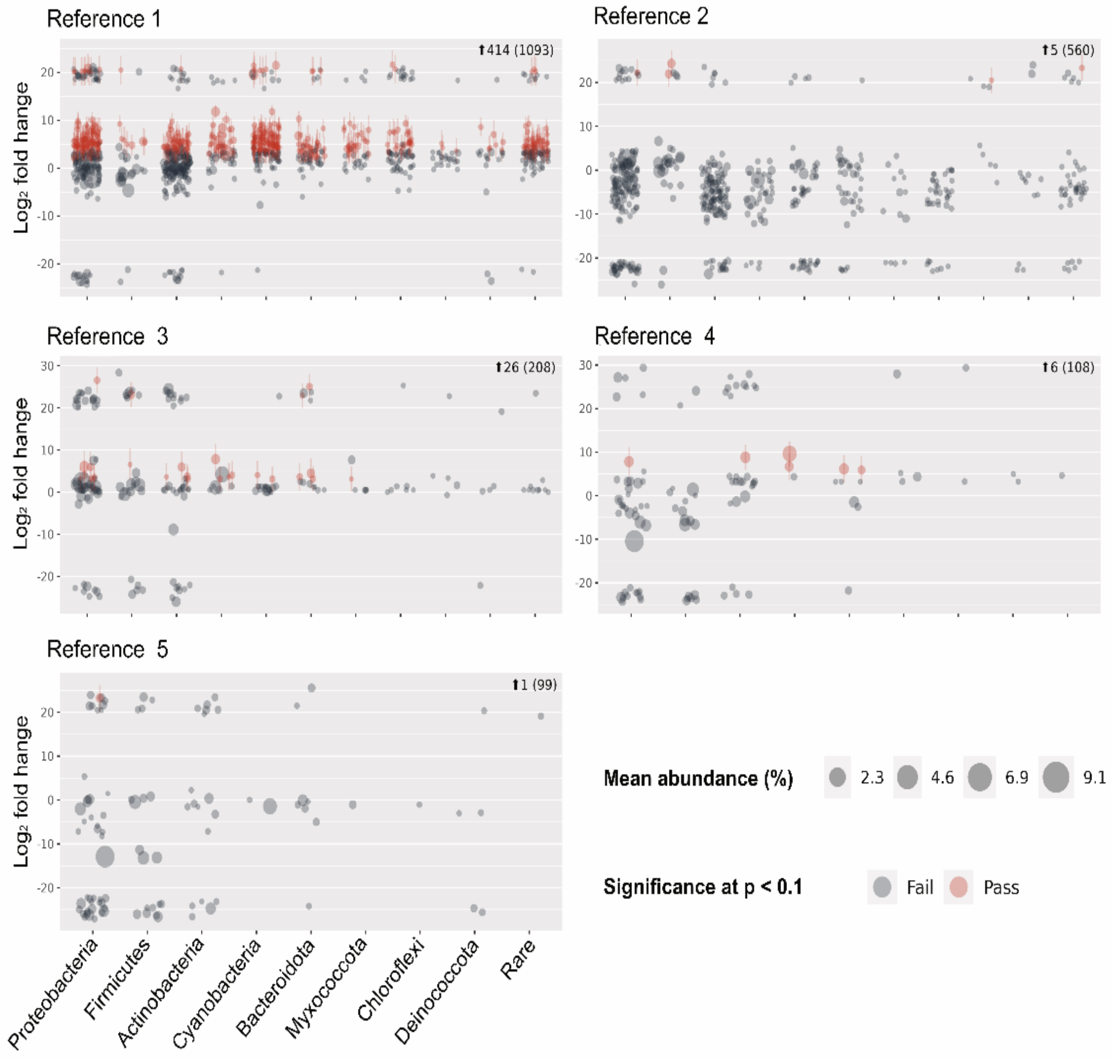


Figure 9. Estimation plots of Chl *a* concentrations. Dots represent the biocrust samples and colors represent either dry or incubated soil.

4.2.4. Sequencing and differential abundance modeling

Sequencing resulted in an average of 47,311 reads per sample (Table B1) and 10,275 ASVs (Table B2). Following decontamination and prevalence filtering, 86% of ASVs were

removed (Table B2). However, they account for only 16% of the total reads. Out of the remaining 1,404 ASVs, 1,266 were labelled and used for the differential abundance modelling (Table B2). Each sequence in the labelled samples was compared to its corresponding negative control and the Log_2-fold change in labelled sequences was evaluated to determine whether an ASV was considered active (significance threshold). One of the reference biocrust samples (no. 1, Fig. 10) displayed a much higher labelling than the other four samples (414 ASVs passed the significance threshold out of a total of 1,093, Fig. 10). Thus, it was excluded from further analyses. In the rest of the reference samples, 38 out of 975 ASVs passed the significance threshold for Log_2 fold change. In post-mining samples, the number of active reads was more consistent among the different samples (Fig. 10); 68 out of 874 ASVs total passed the threshold for Log_2 fold change. The number of active ASVs Log_2 did not differ significantly when comparing natural and post-mining samples (reference sample 1 was excluded, reference mean = 9.5, post-mining mean = 13.6, $W = 9$, $p = 0.9$).


a
Reference 1
Reference 2
Reference 3
Reference 4
Reference 5
Log₂ fold hange
↑414 (1093)
↑5 (560)
↑26 (208)
↑6 (108)
↑1 (99)
Mean abundance (%)
2.3
4.6
6.9
9.1
Significance at p < 0.1
Fail
Pass
Proteobacteria
Firmicutes
Actinobacteria
Cyanobacteria
Bacteroidota
Myxococcota
Chloroflexi
Deinococcota
Rare

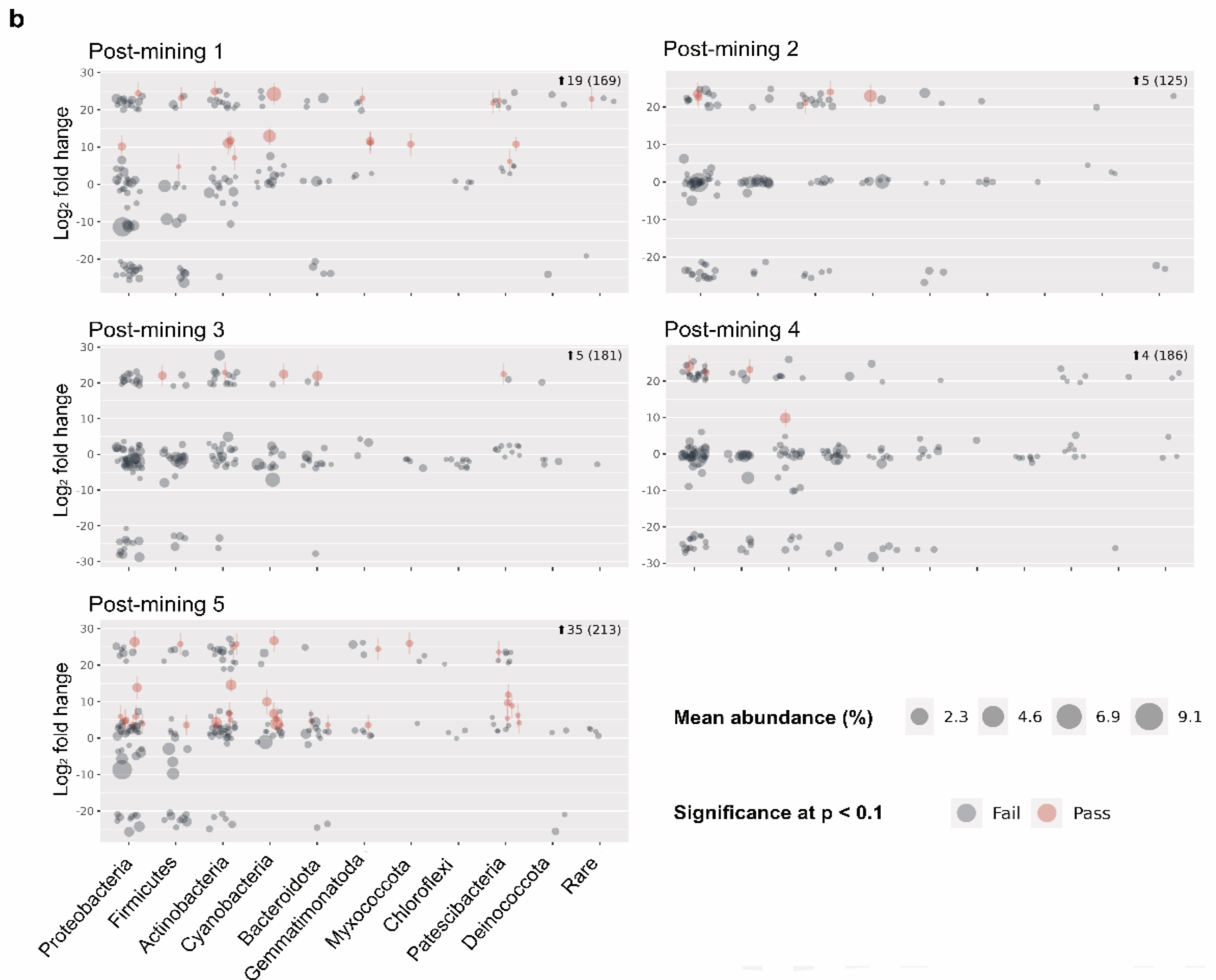


Figure 10. Community composition of active bacteria in reference (a) and post-mining (b) biocrusts. Each graph represents a different sample. Red dots indicate active ASVs, and grey dots indicate ASVs that were not identified as active, based on Deseq2 modelling.

4.2.5. Composition of active community

PCoA ordination based on weighted UniFrac metric showed that samples did not cluster according to plot type (Fig. 11). Furthermore, adonis test revealed no significant differences in community composition between natural and post-mining plots (Weighted UniFrac ~ Plot type; $F = 1.06$, $R^2 = 0.13$, $p = 0.37$). However, a Venn diagram of unique and overlapping sequences revealed that only 8 out of 83 sequences appeared both in reference and post-mining samples (Fig. B2). Despite the small overlap, it is likely that the unique sequences to each plot type are phylogenetically similar. This is supported by phylogenetic trees of

different bacterial groups, which indicated that for the most part, the same orders/classes appeared in natural and post-mining biocrusts; In the phylum Cyanobacteria, active sequences belonged to two classes, and most sequences in both natural and post-mining samples belonged to the class Cyanobacteriia, with a slightly higher prevalence in post-mining samples (Fig. B1). The class Bacteroidia, belonging to the phylum Bacteroidota, had a similar prevalence for natural and post-mining samples (Fig. B1). The trend was similar in the class Bacilli, belonging to the phylum Firmicutes (Fig. B1). In Alphaproteobacteria, the orders Rhodobacteriales, Rhizobiales and Sphingomonadales appeared in both natural and post-mining samples (Fig. B1). Gammaproteobacteria appeared only once in post-mining samples and was more prevalent in natural samples (Fig. B1). The phylum Actinobacteria was more prevalent in post-mining samples, but the orders Frankiales, Micrococcales and Propionibacteriales appeared in both natural and restored samples (Fig. B1).

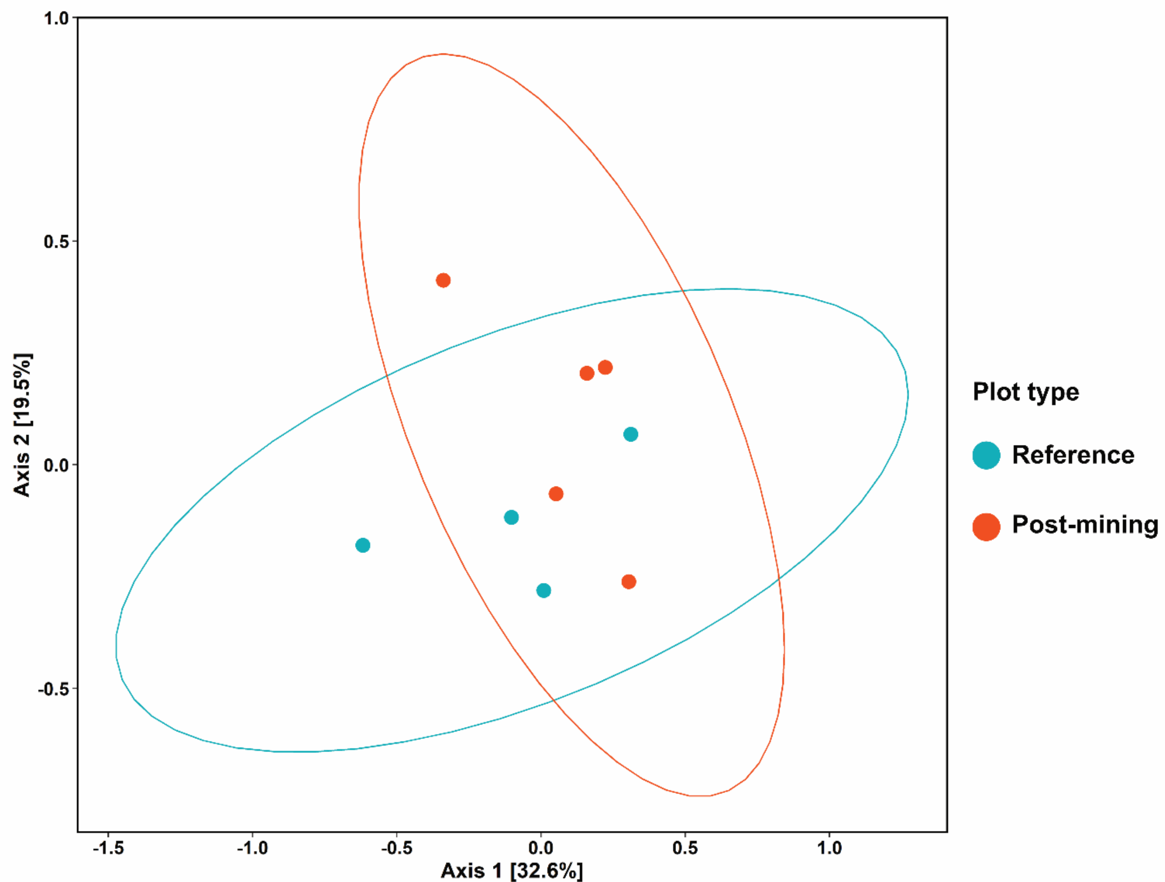


Figure 11. PCoA ordination of community composition based on weighted UniFrac similarity metric. Blue dots are reference samples and pink dots are post-mining samples. The ellipses represent 95% confidence intervals.

4.2.6. Predictions of genomic functions

Abundances of 11 function categories (listed in Table B3) were compared between natural and post mining samples. Abundances were generally higher in natural biocrusts compared to post-mining biocrusts (Table B3). Also, the variance between samples is larger in natural biocrust (Fig. 12). Genes related to phototrophy were more abundant in post-mining biocrusts; however, the differences were not statistically significant ($W = 94$, $p = 0.14$; Fig. 12, Table S3). The only significant differences were observed in genes related to light energy or sensing, which were more abundant in post-mining biocrusts ($W = 75$, $p < 0.05$; Fig. 12, Table B3).

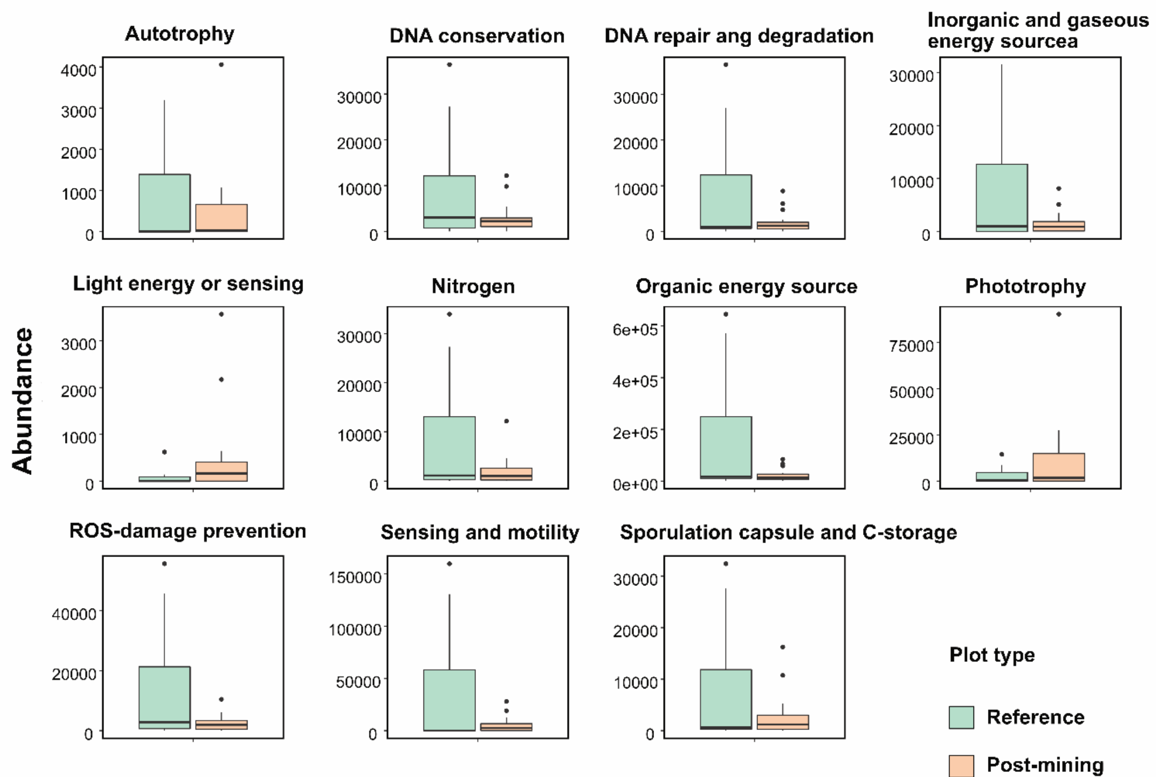


Figure 12. Boxplots of abundances of functional gene categories. The Y axis represents abundance. The line represents the median and the whiskers represent the range.

4.3. Study 3

4.3.1. Natural and cyanobacteria enriched biocrust communities

4.3.1.1. Chl *a*

Chl *a* concentrations were slightly higher in cyanobacteria enriched (mean = 12.75) compared to natural biocrusts (mean = 11.86). However, the differences were not statistically significant ($W = 58$, $p = 0.47$). Estimate plot supports the statistics, as Chl *a* concentrations were similar in natural and cyanobacteria enriched biocrusts (effect size estimate = 0.883 [95CI: -0.139; 2.54], Fig. C2)

4.3.1.2. Alpha diversity

Chao1 index revealed higher richness in cyanobacteria enriched biocrusts, however, the difference was marginally significant (Chao1: $W = 72$, $p = 0.06$). An estimation plot of Chao1 values showed higher values and a larger variance between samples for cyanobacteria enriched biocrusts (effect size estimate = 70.1 [95CI: -33.4; 141], Fig. C3).

4.3.1.3. Beta diversity

Adonis model (Bray-Curtis dissimilarity ~ inoculation type, permutations = 999) revealed significant differences in community composition when comparing natural and cyanobacteria enriched biocrusts ($F = 13.6$, $R^2 = 0.43$, $p < 0.01$). The NMDS ordination supports these results, as sample clustered according to inoculation type (stress = 0.04, Fig. 13).

-

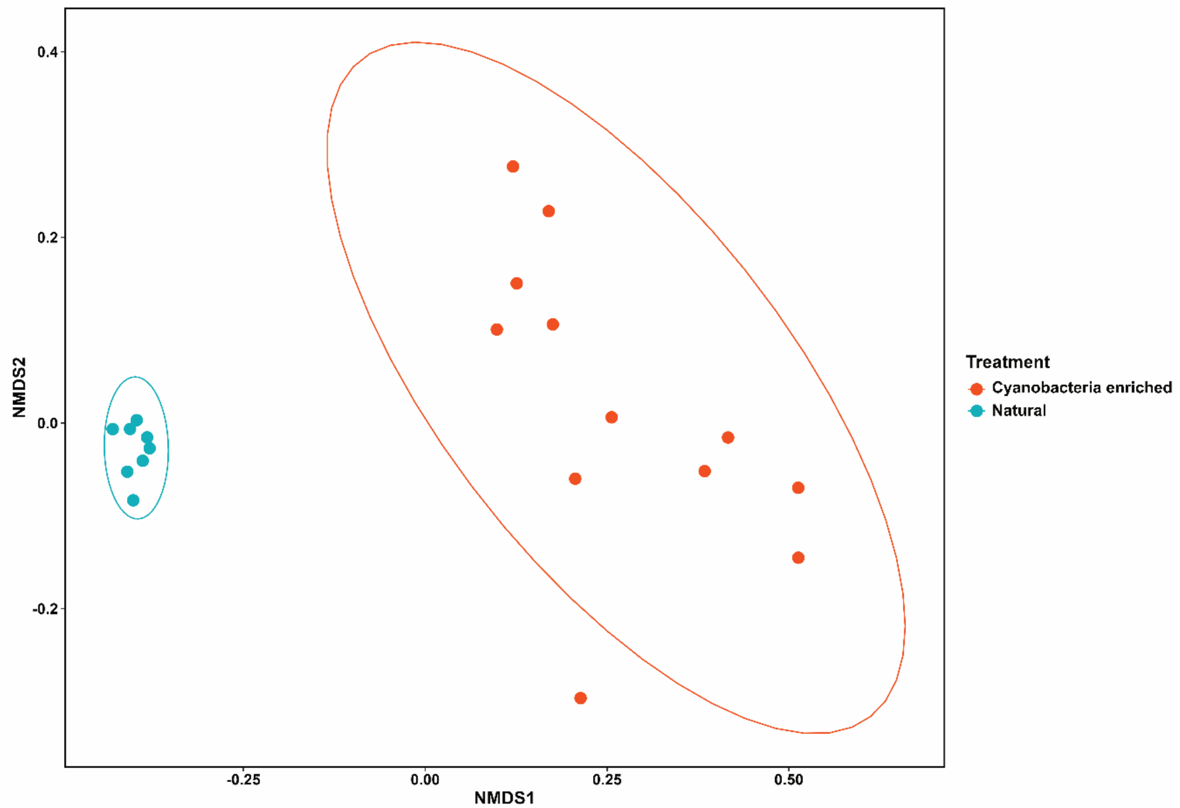


Figure 13. NMDS ordination of composition of natural and cyanobacteria enriched biocrusts, used as inoculations in experiment. Ellipses represent 95% confidence interval.

4.3.1.4. Taxonomic composition – phyla

Natural biocrusts were dominated by Actinobacteria (38.25%), Cyanobacteria (24.28%) and Proteobacteria (18.07%) (Table C1, Fig. 14). Whereas cyanobacteria enriched biocrusts were dominated by Proteobacteria (31.01%), Cyanobacteria (29.01%) and Firmicutes (10.48%) (Table C1, Fig. 14).

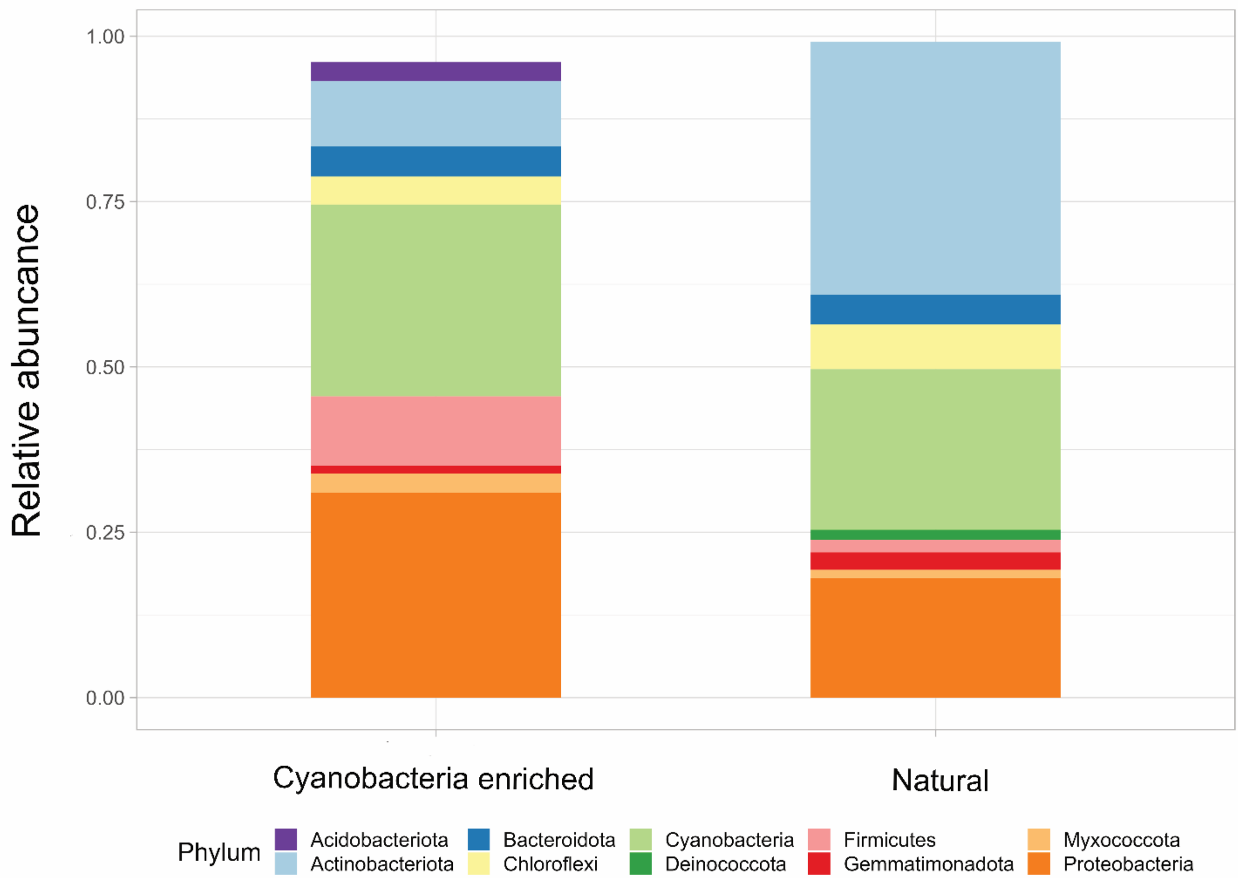


Figure 14. Taxonomic composition of natural (a) and cyanobacteria enriched (b) biocrusts, at the phylum level. The Y axis represents relative abundances. Each bar represents a different sample.

4.3.2. Experiment samples

In most mesocosms, salt crystals began to appear after two to three hydration events (Fig. 15a). Mesocosms supplemented with both natural and cyanobacteria enriched biocrusts turned green after the first hydration event. However, as the weeks progressed, the green pigments disappeared (Fig. 15b).

Figure 15. Pictures taken during the experiment. (a) examples of salt accumulation on the soil surface. (b) An example of disappearance of green pigment in a sample inoculated with cyanobacteria enriched biocrusts, after the first hydration event (left), 10 hydration events (middle) and 20 hydration events (right).

4.3.2.1.Soil properties

NH_4 and P concentrations could not be compared since some measurements were below the limit of detection (Table 5). EC significantly decreased in treated soils compared to soil

retrieved from the stockpile ($F = 8.83$, $p < 0.01$; post-hoc natural biocrusts – $p < 0.05$; post-hoc hydration treatment – $p < 0.01$; Table 5). Chloride (Cl) differed significantly between treatments ($F = 8.83$, $p < 0.01$; Table 5). A Tukey post-hoc test revealed that treatment with natural biocrusts ($p <0.05$) and hydration ($p < 0.01$) decreased Cl concentrations compared to untreated soil. Cl was also significantly lower in hydration treatment compared to cyanobacteria enriched biocrusts ($p < 0.05$; Table 5). Sodium (Na) concentrations were significantly lower in the hydration treatments compared to untreated soil ($F = 3.71$, $p < 0.05$; post-hoc - $p < 0.05$; Table 5). Calcium (Ca) differed significantly between treatments ($F = 7.21$, $p <0.01$). A post-hoc revealed a decrease in natural biocrusts ($p < 0.05$) and hydration ($p < 0.01$) treatments compared to untreated soil (Table 5). Magnesium (Mg) significantly decreased in natural biocrusts, and hydration treatments compared to untreated soil ($F = 6.08$, $p < 0.01$; pots hoc for natural biocrusts and hydration treatments – $p < 0.05$, Table 5). Lastly, NO_3 concentrations were significantly lower in natural biocrusts, and hydration treatment compared to untreated soil ($F = 7.89$, $p < 0.01$; post-hoc for both – $p < 0.01$; Table 5). Other properties did not significantly differ between treatments.

Table 5. Soil properties for experiment topsoil samples after 20 weeks of hydration and bare topsoil. Measurements that were below the limit of detection are marked with less than (<). P-values of one-way ANOVA are presented, significant values are highlighted.

Treatment/Soil property	Bare topsoil	Lab-grown biocrust	Natural biocrust	Watering	P value
pH	7.45	7.48	7.48	7.50	0.13
EC	78.61	70.21	64.39	60.95	**< 0.01**
Cl	33132.50	31192.35	26600.38	24741.23	**< 0.01**
Na	457.92	397.92	389.58	363.54	**< 0.05**
Ca	8016.13	6840.05	5776.70	5301.88	**< 0.01**
CaMg	1895.63	1773.95	1463.53	1374.83	**< 0.01**
Sodium Adsorption Ratio	27.47	25.63	27.42	26.50	0.76
NO3	115.58	81.17	61.47	66.82	**< 0.01**
NH4	7.01	<	<	<	N/A
P	<	11.00	16.88	21.00	N/A
K	65.53	59.40	61.25	64.20	0.78
Soil Organic Matter	1.52	2.23	1.70	2.13	**0.05**

4.3.2.2. Chl *a*

Chl *a* concentrations were measured only in the crust, given that photosynthetic activity is expected to occur primarily in the light exposed soil surface. Differences between treatments were only marginally significant ($F = 2.6$, $p = 0.06$). A Tukey post-hoc test revealed that in

after 10-week samples, Chl *a* concentrations differed between hydration and cyanobacteria enriched biocrust treatments ($p < 0.01$; Fig. 16). Chl *a* concentrations differed significantly between 10- and 20- week samples and were generally higher after 10-week incubation ($F = 55.13$, $p < 0.01$; Fig. 16; Table C2). The interaction between treatment and time was also significant ($F = 3.06$, $p < 0.03$; Table C2).

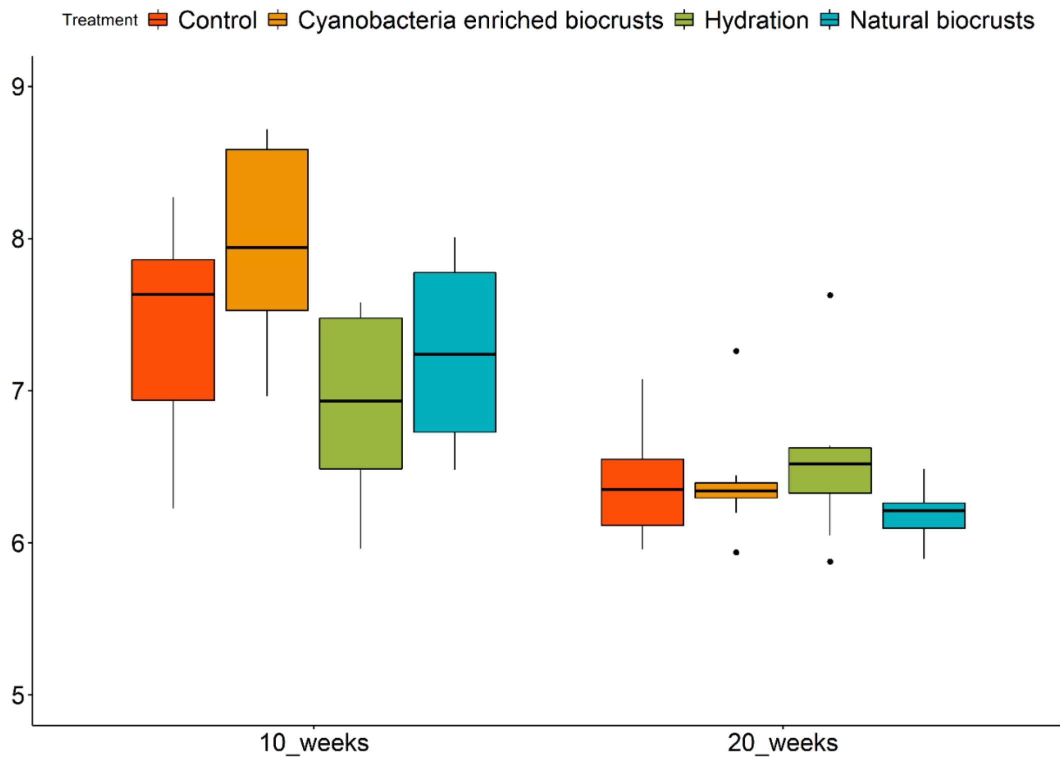


Figure 16. Boxplot of Chl *a* concentrations of experimental biocrust samples after 10 and 20 weeks of hydration. Lines in the boxplot represent the median and whiskers represent the range. Circles represent outliers.

4.3.2.3. Alpha diversity

4.3.2.3.1. Biocrust layer

Richness estimated by Chao1 index, did not differ significantly between treatments ($F = 1.2$, $p = 0.31$). However, Chao1 diversity was significantly higher after 20-week compared to 10-week incubation ($F = 19.36$, $p < 0.01$; Table 6). The interaction between factors was not significant ($F = 0.58$, $p = 0.62$).

4.3.2.3.2. Topsoil layer

Here, the trend is opposite; the Chao1 index was significantly higher after 10-week incubation ($F = 84.9$, $p < 0.01$; Table 6). There were no significant differences between treatments ($F = 1.6$, $p = 0.2$), and the interaction was not significant ($F = 0.64$, $p = 0.58$).

Table 6. Mean chao1 values for different treatments after 10 and 20 weeks of hydration. Top part is the biocrust layer and bottom part is the topsoil layer.

Biocrust	**10 weeks**	**20 weeks**
Control	50.13	54.54
Cyanobacteria enriched biocrust	53.31	58.69
Natural biocrust	47.81	57.45
Hydration	50.42	58.29
Topsoil	**10 weeks**	**20 weeks**
Control	57.34	20.50
Cyanobacteria enriched biocrusts	55.31	30.50
Natural biocrusts	51.69	16.63
Hydration	52.69	14.88

4.3.2.4.Community composition

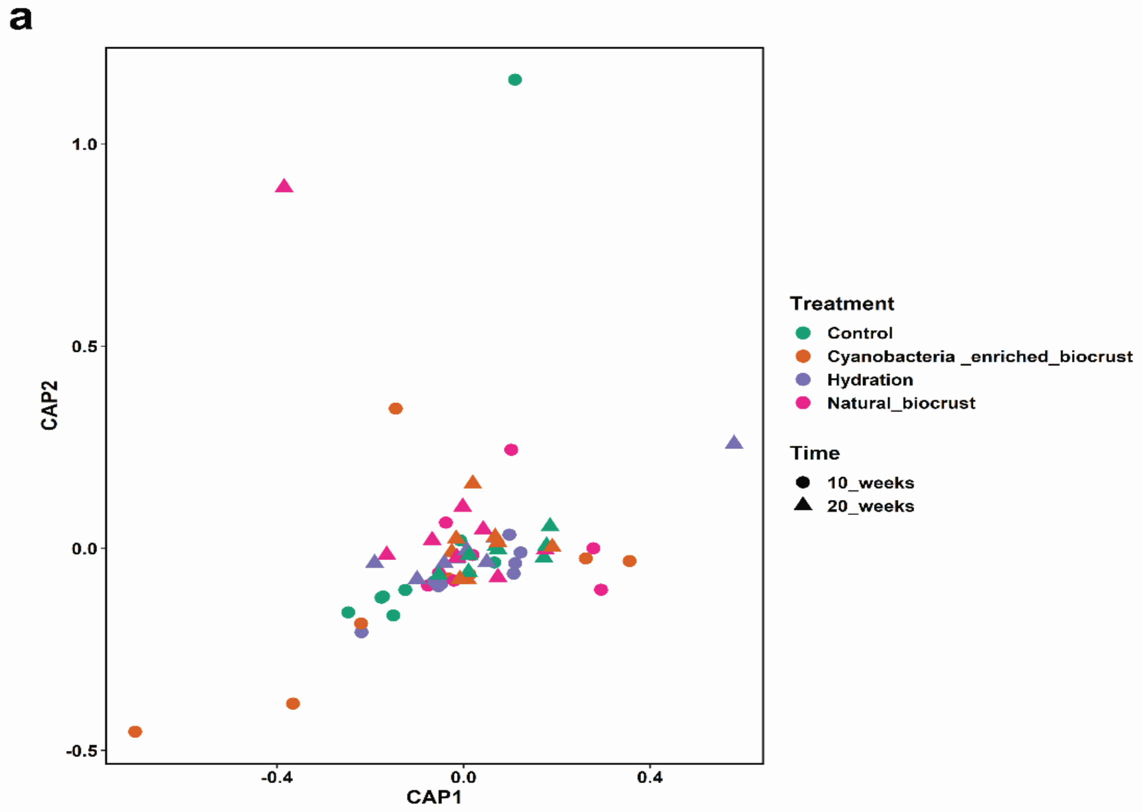
4.3.2.4.1. Biocrust layer

The CAP ordination suggests that there is no clustering according to time or treatments (Fig. 17a). Adonis test (Morisita-Horn index ~ Treatment × Time) revealed no significant differences between treatments ($F = 0.07$, $R^2 = 0.003$, $p = 0.67$) or time ($F = 1.64$, $R^2 = 0.02$, $p = 0.26$). Likewise, the interaction between the factors was not significant ($F = 1.31$, $R^2 = 0.06$, $p = 0.37$).

4.3.2.4.2. Topsoil layer

The CAP ordination suggests that the topsoil community after 10-week incubation cluster separately from the communities after 20-weeks incubation (Fig. 17b). Adonis test (Morisita-Horn index ~ Treatment × Time) revealed no significant differences between treatments ($F = 0.95$, $R^2 = 0.03$, $p = 0.45$). However, community composition differed significantly between

samples incubated for 10- and 20- weeks ($F = 29.9$, $R^2 = 0.33$, $p < 0.01$), regardless of treatment, as the interaction between factors was not significant ($F = 0.90$, $R^2 = 0.02$, $p = 0.47$).



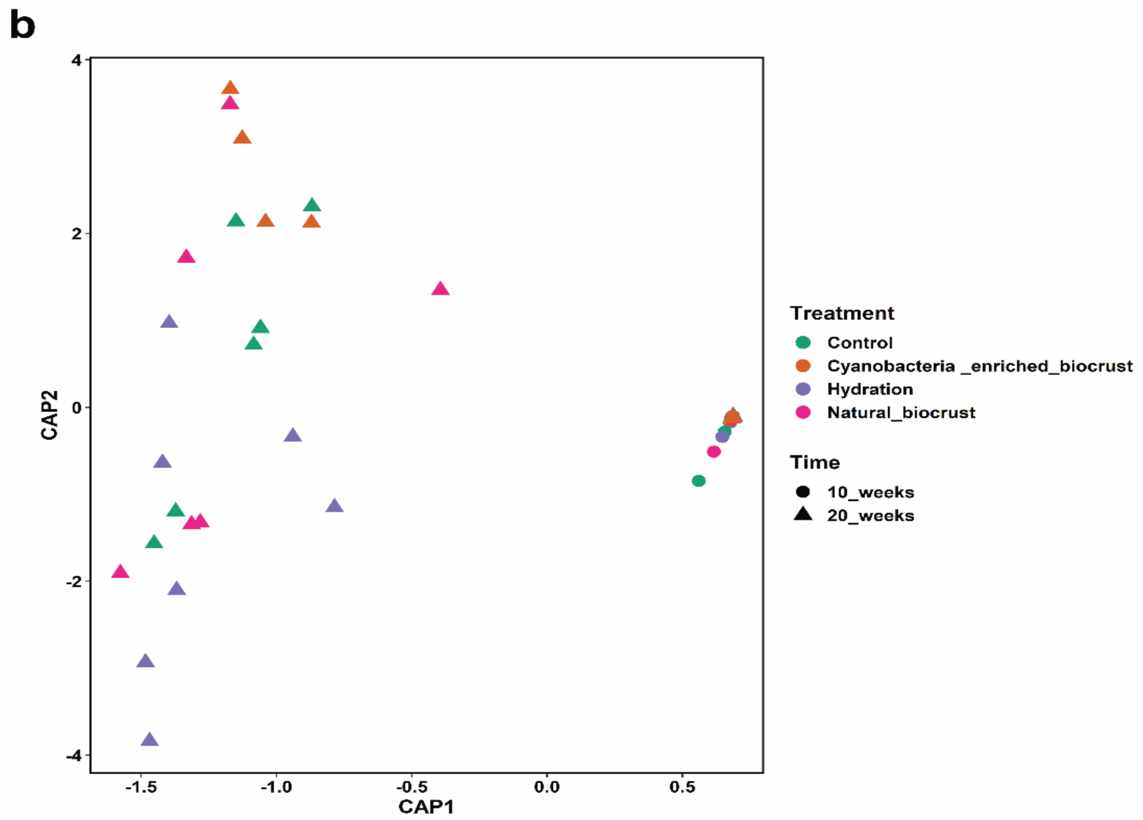


Figure 17. CAP ordination of community composition for biocrust (a) and topsoil (b) experiment samples. Each color is a different treatment and shapes represent the time.

5. **Discussion**

5.1. Biocrust bacterial communities – spatial scale

In accordance with our hypothesis, we observed distinct bacterial communities in the study sites (Fig. 6). The dominant phyla in the sites included Cyanobacteria, Proteobacteria, Actinobacteriota and Chloroflexi (Table 2), corresponding to previously reported bacterial community composition in biocrusts (Nagy et al., 2005, Angel et al., 2013, Maier et al., 2018). Reference biocrusts were dominated by Cyanobacteria and Chloroflexi, and post-mining biocrusts were dominated by Actinobacteria and Proteobacteria (Fig. 6; Tables 2,3).

To our knowledge, there are very few studies on the effects of mining on hyper-arid biocrust communities. However, other mechanical disturbances such as fire, trampling and agricultural land-use were shown to decrease the abundance and diversity of cyanobacteria (Williams et al., 2008, Blay et al., 2017, Katra et al., 2017). Similarly, mining disturbance could explain the low cyanobacterial abundances across all post-mining sites (Fig. 5). Given their dominance in the Negev biocrusts, and their role as a key primary producers in biocrusts, low cyanobacterial abundance could affect recovery times of the entire biocrust community (Zhao et al., 2016). It is likely that the availability of cyanobacterial propagules has decreased in post-mining sites, hindering biocrust recovery (Pointing & Belnap, 2012).

5.2. Biocrust bacterial communities – temporal scale

On the temporal scale, we did not observe significant differences in diversity indices between the post-mining sites (restoration years - 2007, 2010, 2012 and 2015, for Gov, Hagor, Afik and Saif, respectively; Table A5). Community composition differed between Afik and two other sites (Fig. A2), but the abundances of the dominant groups were similar across all sites (Table A6). Also, the abundances of cyanobacteria and Chl *a* concentration did not differ significantly between sites (Fig. 5b, Table A6).

5.3. Topsoil properties of Zin mines

Changes in soil physico-chemical characteristics often occur in soil excavated during mining (Sengupta, 2021, Ward, 2000, Lei et al., 2016), which could in turn contribute to changes in soil microbial communities (Harris et al., 1989). The soils in Zin mines are highly saline, with an average EC of 24 dS/m in reference biocrusts (Table A1). Post-mining soils are also saline, and salinity levels do not differ significantly between plot types (Table A1). Furthermore, Levi et al. (2021) concomitantly analyzed topsoil samples from three sites

(Afik, Hagor and Gov) and found that most physico-chemical properties did not differ between post-mining and reference plots. However, they found significant differences in soil biological indicators including soil organic matter, proteins, and polysaccharides (Levi et al., 2021). Moreover, in the topsoil collected from stockpiles, salinity levels were between 3-4 times higher than in topsoils collected from post-mining and reference plots (Table 5). It is possible that the process of excavating the soil from the ground changed the soil properties. Also, the stockpile was exposed for at least 2 years, which likely changed the soil characteristics and a reduced soil quality and biodiversity (Cooke & Johnson, 2002).

5.4. Photosynthetic potential of post-mining and reference biocrusts

Cyanobacterial abundances and Chl *a* concentrations were consistently lower in post-mining compared to reference biocrusts (Figs. 2, 4; Tables 2, 3), indicating lower primary productivity in post-mining biocrusts. The differences were most significant in the oldest (Gov, Fig. 1) and newest (Saif, Fig. 1) restoration sites. However, when we collected the soil from another site in Gov, incubated in microcosms and tested Chl *a* concentrations, the measures were slightly higher in post-mining compared to reference biocrusts, both in dry and hydrated soils (Fig. 9). The 96-hr incubation with water resulted in most biocrust samples displaying some degree of greening, yet with more greening in reference biocrusts (Fig. 8). Despite this, Chl *a* concentrations and the abundance of genes related to photosynthesis did not differ significantly between reference and post-mining biocrusts (Fig. 9, 10). The discrepancy between the photosynthetic measures could result from sampling at different locations of the Gov post-mining site. These differences strengthen the importance of microenvironments in determining the functionality of biocrusts (Garcia-Pichel & Belnap, 1996). Alternatively, the similarity in active communities and photosynthetic potential may be due to differences in the development of biocrusts between the sampling plots.

5.5. Proliferation of bacteria in biocrusts following hydration

After a 96-hr incubation with $H_2^{18}O$, we observed very little bacterial proliferation (3.9% active ASVs for reference biocrusts and 7.7% active ASVs for post-mining biocrusts). Post-mining biocrusts had a higher amount of active ASVs compared to reference biocrusts, however, the differences were not significant. Also, composition and taxonomic identity of the active communities did not differ between reference and post-mining biocrusts.

Biocrust organisms are known to activate quickly following hydration, initiating functions such as damage repair, germination, nutrient cycling, and growth (Harel et al., 2004, Rajeev

et al., 2013, Green & Proctor, 2016, Thomas et al., 2022). Photosynthetic activity is usually observed in biocrust organisms, particularly cyanobacteria, within minutes to hours following hydration (Lange, 2003). Previous $H_2^{18}O$ SIP assays showed a quick response of soil bacteria to hydration, within 72-96 hrs of incubation (Aanderud & Lennon, 2011, Aanderud et al., 2015). Thus, our assumption was that after 96-hrs of incubation with water under favorable laboratory conditions, we will observe significant bacterial proliferation. However, the growth patterns of biocrust organisms are affected by local environmental conditions (Kim & Or, 2017). Zin mining fields are in a hyperarid region, where extreme heat events are frequent in the summer, and rains are scarce and unpredicted. Moreover, in recent years there were only two or three rain events during each rainy season (Zin factory meteorological data). Hydration is the most important factor affecting biocrust organisms' growth rate, while long desiccation periods negatively affect growth (Zaady et al., 2016). Also, salinity levels in Zin valley soils are high (Levi et al., 2021). We suggest that due to these conditions, the hyperarid biocrust communities prioritize activation and preparation for desiccation over growth. It is known that in high stress environments, biocrust microorganisms resume carbon and nitrogen fixation upon hydration. The resulting organic carbon and nitrogen compounds can be metabolized during the long desiccation periods (Belnap, 2003a; Colesie et al., 2014)

5.6. SIP as a method to assess activity in biocrusts

Given that bacteria in Zin biocrusts do not proliferate, RNA-SIP could have been a better approach to estimate biocrust functionality in Zin biocrust, as nutrient cycling and repair activities could be assessed. However, this is challenging since previous attempts of extracting RNA from Zin soils have been unsuccessful. Alternatively, 96-hr incubation may not be sufficient for estimating bacterial growth in these biocrusts. In fact, another extreme environment, the dry arctic soils, showed increase in activity after a 30-day incubation with $H_2^{18}O$ (Schwartz et al., 2014). Thus, it is possible that longer incubation times are required to see significant activation of the biocrust bacteria. Further SIP assays with longer incubation time or a time series of incubations could provide further insight regarding bacterial response to hydration. It is important to note, that while longer incubation times in the laboratory will provide an understanding on the time and amount of hydration needed to observe significant bacterial growth, it is not applicable in field conditions, as water evaporates quickly from the soil, and repeated hydration would be technically and logistically difficult to apply. Thus, in the field it is unlikely to mimic conditions that allow significant growth.

5.7. Natural recovery of biocrust bacterial communities in Zin mines

Natural recovery times and trajectories of biocrusts succession have been long debated, especially since they depend on many factors such as precipitation, site stability and frequency of disturbances. Thus, it is difficult to reach a consensus on biocrust recovery times, necessitating a case-by-case consideration (Belnap & Eldridge, 2003, Weber et al., 2016). Recovery times in extreme environments, such as hyper-arid ecosystems, are expected to be longer due to low precipitation, high radiation, long droughts and high temperatures (Weber, Büdel, et al., 2016). Studies examining the recovery of lichen biocrusts in the hyper-arid Namib Desert, estimated recovery times of hundreds of years following severe mechanical disturbances, while recovery times after mild disturbances were estimated at 5 to 28 years (Lalley & Viles, 2008). Also, it has been shown that during early stages of biocrust establishment, heterotrophic bacteria dominate the biocrust before being outcompeted by filamentous cyanobacteria (Zhang, 2005). Given these patterns, we suggest that biocrusts from our post-mining sites are still at the early stages of succession.

Low proliferation rates, particularly in post-mining biocrusts, further support the estimated long establishment and recovery of biocrusts. The topsoil from a stockpile is used to cover the mining pits. This soil does not contain a rich biocrust seed bank that was destroyed and buried during the mining processes. Further increase in bacterial biomass might highly depend on the dispersal of biocrust propagules to the site from distant natural areas through wind or water. Also, these sites experience very short activity times for biocrust development due to the infrequent hydration events as described above. The establishment and restoration of biocrusts in post-mining sites was reported to be greatly affected by the proximity, availability, and dispersal timing of biocrust propagules (Bowker, 2007, Walker et al., 2007)

5.8. Current restoration practices in Zin mines

Restoration of mining sites is often passive (i.e., allowing a natural recovery of the ecosystem), resulting in very slow recovery times and in different communities from the original or surrounding sites (Cooke & Johnson, 2002). One restoration-oriented practice is the preservation of the topsoil layer (top 10 cm), which is later reapplied to mined pits (Burke, 2003). In some cases, the topsoil is mixed with nutrient sources such as fertilizer (Chambers et al., 1994) or even mine tailings (Kumaresan et al., 2017). Moreover, it was shown that if the topsoil is excavated and exposed for a short period of time, plants and microbial seedbanks could be partially preserved (van Etten et al., 2012, Merino-Martín et al.,

2017, Ngugi et al., 2018). Rotem ICL mining company excavates the top 50-80 cm of soil and considers it topsoil, which means that the topsoil seedbanks are mixed with deeper soil layers, that were shown to support different microbial communities (Wang et al., 2021). Also, this topsoil is exposed for a few years, which probably leads to considerable biodiversity loss (Harris et al., 1989, Ngugi et al., 2018). Therefore, the current restoration practices in Zin mining sites do not suffice for restoring ecosystem functions and more active restoration measures are required. Such active restoration practices include soil inoculation with local cyanobacterial propagules (Acea, 2003, Wang et al., 2009, Zhao et al., 2016, Velasco Ayuso et al., 2017), increased hydration (Morillas & Gallardo, 2015, Zhang et al., 2018), or a combination of various treatments (Maestre et al., 2006, Antoninka et al., 2018).

5.9. Natural and cyanobacteria enriched biocrust communities used as inoculates

To test the effectiveness of restoration treatments in establishing biocrusts on bare soils, we used two inoculation treatments and increased hydration of the soil. The communities of natural and cyanobacteria enriched biocrusts that were used as inoculates, differed in their composition. The taxonomic composition of natural biocrusts was consistent with our pervious characterization of natural biocrusts in Zin (Fig. 7) and was dominated by Actinobacteria, Cyanobacteria and Proteobacteria. The cyanobacteria enriched biocrusts had a higher abundance of cyanobacteria but were also dominated by Firmicutes and Proteobacteria compared to natural biocrusts (Fig. 14). This was expected, since the topsoil used to grow the biocrust was not autoclaved, thus bacteria that occur naturally in the soil were enriched, despite the media targeting non-nitrogen fixing cyanobacteria. Previously reported restoration experiments inoculated cyanobacterial strains directly onto the target soil (Lan et al., 2014, Giraldo-Silva et al., 2019). We tried the same approach here in preliminary experiments but failed to establish cyanobacterial biocrusts with direct inoculation of culture. Instead, we incubated the soil with liquid media, which allowed the cyanobacteria to attach to the soil particles and grow.

5.10. Performance of restoration treatments on biocrust establishment in Zin soils

After 20 weekly hydration events of stockpile samples inoculated with either natural cyanobacteria enriched biocrusts, we could not detect biological crust formation on the surface of the incubated mesocosms, but rather a physical crust. It appears that the soil particles aggregate due to the large amount of salt in the soil rather than a biological process (Fig. 15). Moreover, a similar trend was observed in the mesocosms that underwent only

hydration treatment without inoculation. Chl *a* concentrations were significantly higher in topsoil samples at the middle of the experiment compared to the end (after 10 weeks compared to 20 weeks of hydration). It is possible that the photosynthetic activity of cyanobacteria decreased in the duration of the experiment due to the high salinity levels in the soil (Table 5) that seem to be concentrated on the soil surface. High salinity can limit biocrust development and affect the availability of nutrients in the soil (Ullmann & Büdel, 2003, Zhao et al., 2016). A previous study showed that biocrusts in highly saline costal soils had lower abundances of cyanobacteria, and other known biocrust phyla, and were dominated by halotolerant bacteria (Abed et al., 2019). Another study on biocrusts in highly saline mining tailing piles found only salt-tolerant bacteria in biocrusts near the piles (Sommer et al., 2020). Given that biocrust activity affects soil properties such as soil organic matter (Chamizo et al., 2012), the low organic matter measurements in bare soils and after 20 hydration events further indicate low activity.

Another factor that could have contributed to the reported results is the high initial concentration of NO_3 and NH_4 in the soil. It was previously shown that inorganic nitrogen enrichment in biocrust disrupt nitrogen fixation processes (Bu et al., 2014). After 20 weeks of hydration, there was a significant decrease in both compounds, possibly through gaseous losses or leaching (Barger et al., 2016).

Community composition in the biocrust layer did not vary between treatments or weeks of hydration events. However, in the topsoil layer, samples clustered according to time, indicating that there was a change in the community between 10 and 20 weeks of hydration (Fig. 17). It appears that some of the salt in the soil rose to the surface via capillary flow, thus explaining the decrease in salinity in the topsoil for all treatments (Table 5).

It is important to test the feasibility of biocrust restoration in degraded soils since they provide key ecosystem services in arid environments (Bowker et al., 2018). It seems that despite increased hydration and inoculation, the high salinity levels in the soil did not allow the propagation of biocrust organisms on the surface. Pre-conditioning of the inoculants to withstand high saline conditions could provide a solution to the challenge of establishing biocrust on saline soils. The pre-conditioning of inoculants is well known in biocrust restoration (Antoninka et al., 2018, Giraldo-Silva et al., 2020). However, the efficiency of this approach is debated, and it is largely dependent on habitat characteristics (Bowker et al., 2020).

Our experiment was done in mesocosms placed in a climate-controlled incubator under optimal climatic conditions. The effect of water and inoculation will likely be different under field conditions, where leaching through a much deeper soil column may prevent the capillary flow of the salt. Salt accumulation might not occur also because of different soil properties in different stockpiles, shorter exposure times or different handling. On the other hand, the extreme climate conditions in Zin, with strong UV radiation and high temperatures most of the year, might negatively affect biocrust formation in disturbed soil. Biocrust organisms, particularly cyanobacteria, developed adaptations to survive in such extreme conditions (Belnap et al., 2003). However, increased hydration events under the prevailing conditions might impair the bacterial adaptations. Previous field experiments showed a decrease in photosynthetic activity of cyanobacterial biocrusts even under frequent hydration events, due to their inability to produce protective pigments in response to UV stress (Belnap et al., 2004, Belnap et al., 2008). Shading could be a potential treatment to alleviate the UV and temperature stresses in the field, that has been identified as an effective treatment in previous research (Chock et al., 2019, Antoninka et al., 2020, Bowker et al., 2020). Another possible treatment to address the issue of UV stress is using cyanobacteria strains that reside slightly below the surface, where radiation is lower. A previous study conducted on Negev Desert biocrusts showed that filamentous cyanobacteria are active below the surface, where radiation is diminished (Raanan et al., 2016)

Conducting a similar experiment in the mining fields of Zin valley is necessary to fully understand whether alternative restoration measures are feasible for the Zin mining sites.

6. Conclusions

Following mining activities in the Negev Deserts, biocrust bacterial communities differ in diversity and composition compared to reference (undisturbed) biocrusts and suffer a loss of primary producers. There is no clear trajectory of succession in these hyper arid biocrust, which is not surprising given harsh environmental conditions. Low proliferation of biocrust bacteria after incubation with water suggests very slow recovery times of the biocrusts. Furthermore, the potential recovery may largely depend on site conditions and the ability of biocrust propagules to disperse to post-mining sites. The establishment and recovery of biocrusts largely depends on the microenvironments in post-mining sites. Extreme soil parameters hinder the establishment of biocrusts, even under optimal climatic conditions.

Current restoration-oriented mining practice in Zin mines are thus insufficient to achieve the recovery of the biocrust community, particularly the primary producers (i.e., cyanobacteria). Therefore, further study of active restoration measures offers the best potential to accelerate the recovery process.

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8. Appendices

8.1. Appendix A

8.1.1. Figures

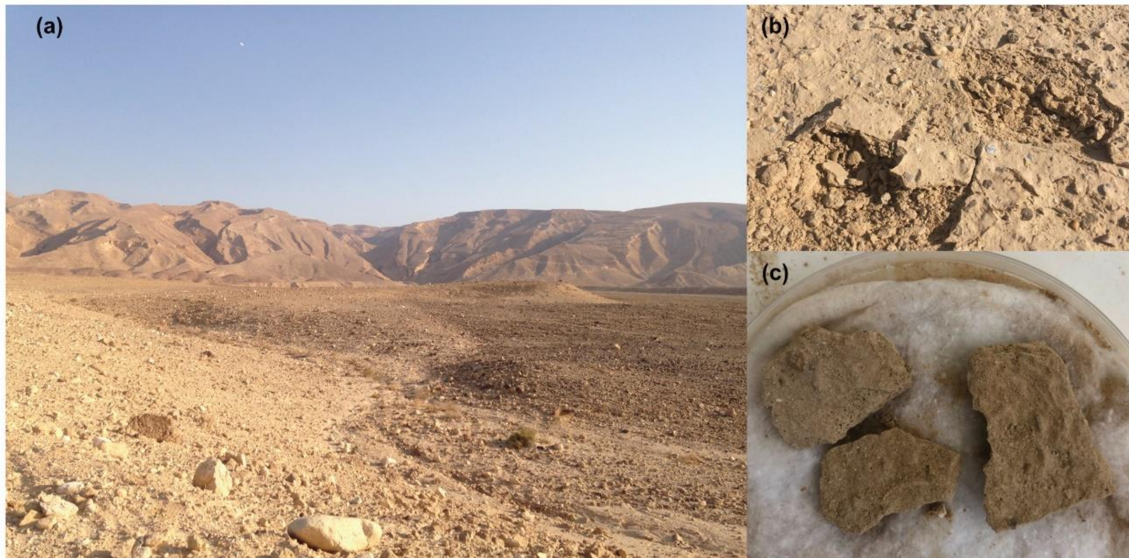


Figure A1. Examples of one of the research sites and biocrust samples: (a) Gov research site. The right side (dark soil) is the reference plot, and the left side (light soil) is the post-mining plot; (b) close-up of biocrusts in Gov mining site; (c) close-up of Gov biocrusts after collection.

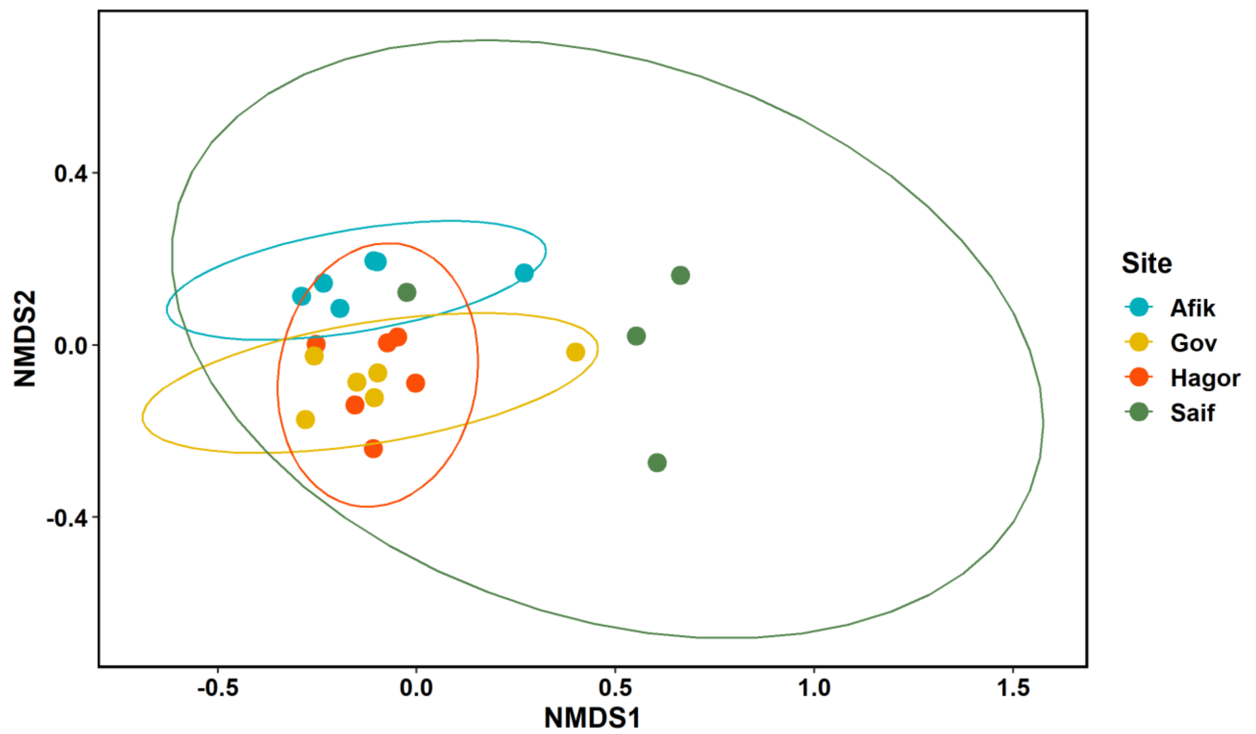


Figure A2. NMDS plots of samples post-mining plots. Each color represents a different mining site (different restoration time). Ellipses are 95% confidence interval ellipsoids. Stress value = 0.13.

8.1.2. Tables

Table A1. Texture and features of the soil for three of the sites used in this study (Saif –restored in 2015, Afik – restored in 2012, Hagor – restored in 2010 and Gov – restored in 2007). Values are means and standard errors.

Soil characteristics	Afik		Hagor		Gov	
	Reference	Post-mining	Reference	Post-mining	Reference	Post-mining
pH	7.91±0.18	8.13±0.10	7.98±0.20	7.61±0.24	8.01±0.25	7.95±0.10
EC (dS/m)	24.26±14.05	3.8±0.97	37.63±10.69	44.37±13.52	13.04±7.74	17.86±5.66
NH4 (mg/kg)	1.33±0.24	6.083±1.17	2.21±0.35	3.3±0.79	1.46±0.39	6.61±2.17
NO3 (mg/kg)	381.43±224.33	33.14±17.90	289.31±97.95	310.71±109.04	146.34±93.89	47.91±10.30
Sand (%)	70.5±2.88	73.9±1.41	60.5±4.26	69.41±3.70	68.18±5.25	70.26±2.17
Silt (%)	16.36±4.44	8.55±2.52	23.63±4.78	17.68±3.71	19.75±3.89	18.33±2.65
Clay (%)	13.13±2.06	17.55±1.40	15.86±2.95	12.9±2.27	12.06±2.51	11.4±2.35

Table A2. list of 16S rRNA encoding genes primers used in this study

Organism	Primer	Sequence (5' to 3')	Reference
Bacteria	341F	CCTACGGGAGGCAGCAG	Takahashi et al. 2014
	806R	GGACTACGGGTWTCTAAT	
Bacteria	341F	CCTACGGGAGGCAGCAG	Klindworth et al. 2013
	515R	TTACCGCGGCTGCTGGCAC	
Cyanobacteria	CYA359F	GGGGAATTTTCCGCAATGGG	Nubel et al. 1997
	CYA781R(a)	GACTACTGGGGTATCTAATCCCATT	
	CYA781R(b)	GACTACAGGGGTATCTAATCCCTTT	

Table A3. Sequencing and ASV information

Features	No. of reads
Min. counts/sample	5,604
Max. counts/sample	76,103
Mean counts/sample	557,373
Total counts	2,563,908
Number of ASVs (not filtered)	6,733

Features	No. of reads
Min. counts/sample	5,604
Max. counts/sample	76,103
Mean counts/sample	557,373
Total counts	2,563,908
Number of ASVs (not filtered)	6,733

Table A4. Comparisons of alpha diversity indices between reference and post-mining plots (Saif – restored in 2015, Afik – restored in 2012, Hagor – restored in 2010 and Gov – restored in 2007). Statistically significant results are highlighted.

Site	**Saif**		**Afik**		**Hagor**		**Gov**	
Diversity index	Reference	Post-mining	Reference	Post-mining	Reference	Post-mining	Reference	Post-mining
Observed ASVs	271.66	266.75	297.66	253.50	**352.16**	**196.66**	391.50	260.00
Chao1	288.28	295.61	319.14	274.88	**372.14**	**206.14**	415.29	275.55
Fisher's alpha	41.80	48.99	47.56	38.96	**58.31**	**29.81**	63.81	41.31

Table A5. Comparisons of alpha diversity indices between post-mining plots in the four mining sites (Saif – restored in 2015, Afik – restored in 2012, Hagor – restored in 2010 and Gov – restored in 2007).

Diversity index/Site	**Saif**	**Afik**	**Hagor**	**Gov**	***χ2***	***p*-value**
Observed ASVs	266.75	253.50	196.66	260.00	1.67	0.62
Chao1	295.61	274.88	206.14	275.55	1.90	0.59
Fisher's alpha	35.87	38.95	29.81	41.30	1.91	0.59

Table A6. Comparisons of relative abundance at the phylum level between post-mining plots in the four mining sites (Saif – restored in 2015, Afik – restored in 2012, Hagor – restored in 2010 and Gov – restored in 2007). Statistically significant results are highlighted.

Phylum/Site	**Saif**	**Afik**	**Hagor**	**Gov**	***χ2***	***p*-value**
Cyanobacteria	2.44	10.53	8.32	12.18	0.87	0.83
Chloroflexi	1.64	<1%	1.04	1.97	0.87	0.83
Actinobacteriota	28.93	43.21	46.80	29.79	4.69	0.19
Proteobacteria	51.68	30.31	23.96	38.53	6.53	0.06
Bacteroidota	2.52	7.14	6.92	4.03	**8.80**	**0.03**
Deinococcota	**<1%**	3.49	**5.69**	4.22	**9.11**	**0.02**
Patescibacteria	**1.25**	1.84	1.88	**3.22**	**8.51**	**0.03**
Gemmatimonadota	<1%	<1%	**3.21**	**3.42**	**13.66**	**0.003**
Firmicutes	1.45	<1%	1.40	<1%	4.24	0.23
Desulfobacterota	**2.13**	<1%	<1%	<1%	**10.87**	**0.01**
Myxococcota	<1%	<1%	<1%	<1%	1.29	0.73
Verrucomicrobiota	4.50	<1%	<1%	<1%	5.84	0.11

8.2. Appendix B

8.2.1. Figures

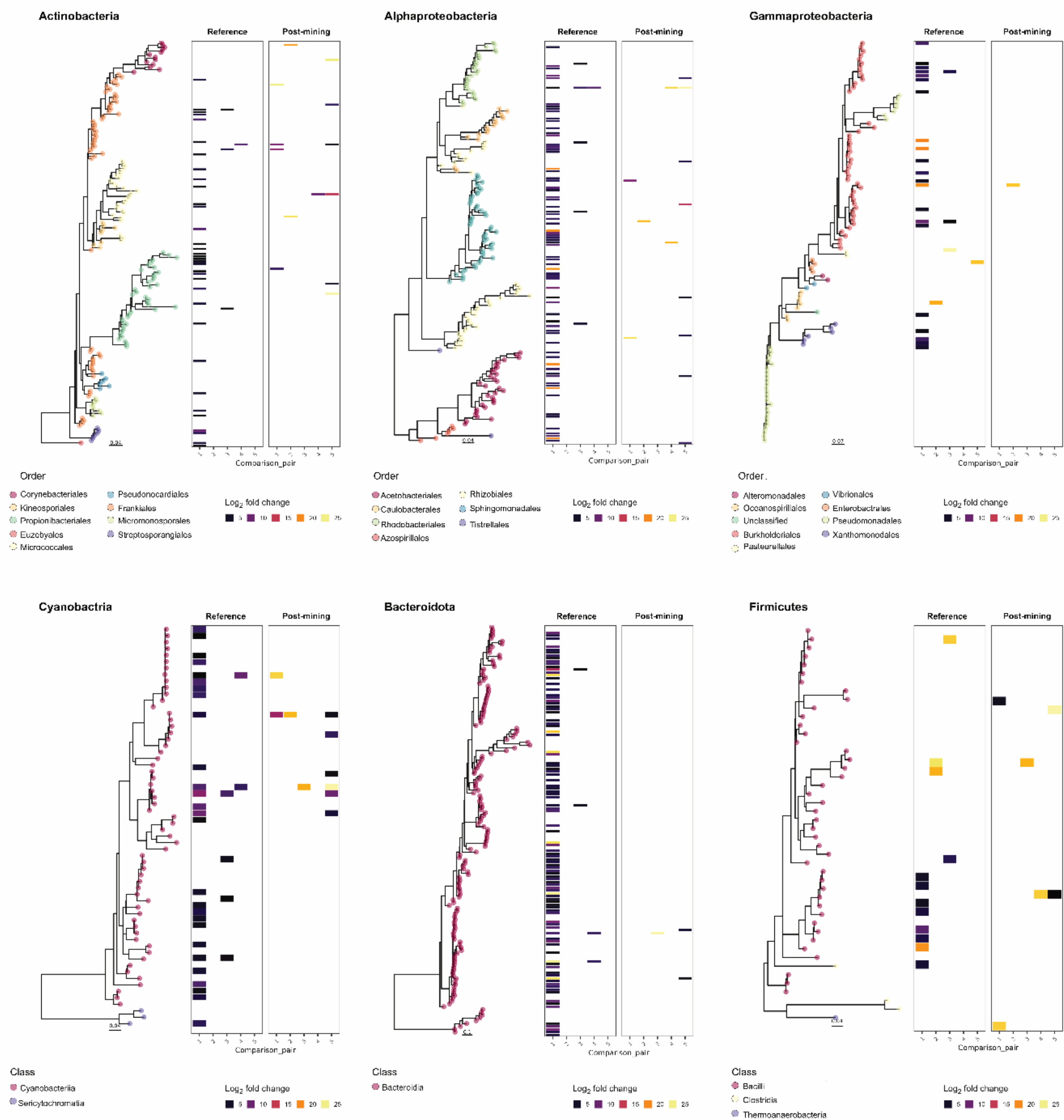


Figure B1. Heatmaps and phylogenetic trees of active bacterial groups. Each figure represents a different phylum or class, the different colors in the tips of the phylogenetic trees represent different orders/classes. The squares in the heatmaps represent active sequences assigned to a specific taxa and the color of each square is the number of Log_2 fold change. Every column represents a natural or post-mining sample.

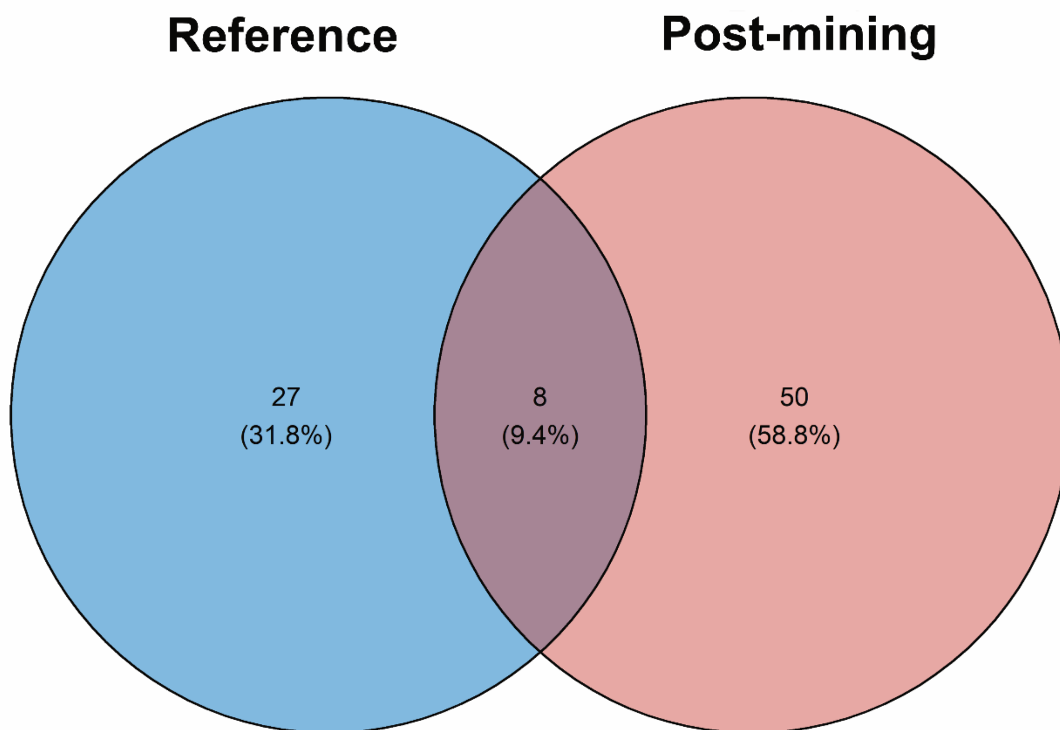


Figure B2. Venn diagram representing unique sequences in natural (blue) and post-mining (pink) samples. Overlap represents sequences that appear in both groups.

8.2.2. Tables

Table B1. Read information for samples.

Min.	602
Max.	291129
Median	23739
Mean	47311

Table B2. Numbers of ASVs throughout the pipeline.

Non-chimera ASVs	10275
Number of ASVs after decontamination	10100
Number of ASVs after prevalence filtering	1404
Labelled ASVs (used for differential abundance modelling)	1266

Table B3. Means of functional gene abundances of the 11 function categories. Significant differences are highlighted.

Function category	Reference mean abundance	Post-mining mean abundance
Autotrophy	713.20	483.83
DNA conservation	8248.72	2959.93
DNA repair and degradation	8012.15	1938.69
Inorganic and gaseous energy sources	7301.73	1541.39
Light energy or sensing	71.80	**475.22**
Nitrogen	7249.59	2055.00
Organic energy source	145174.15	21677.39
Phototrophy	2805.44	11180.22
ROS-damage prevention	12654.13	2394.11
Sensing and motility	33402.52	5759.34
Sporulation capsule and C-storage	7220.42	2789.87

8.3. Appendix C

8.3.1. Figures

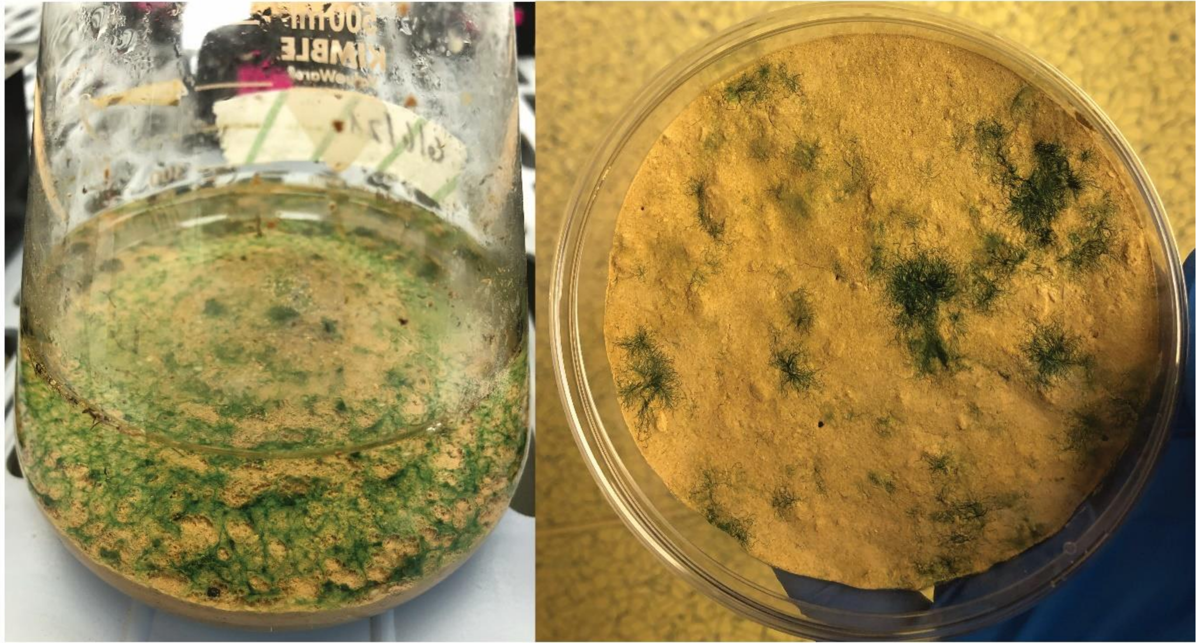
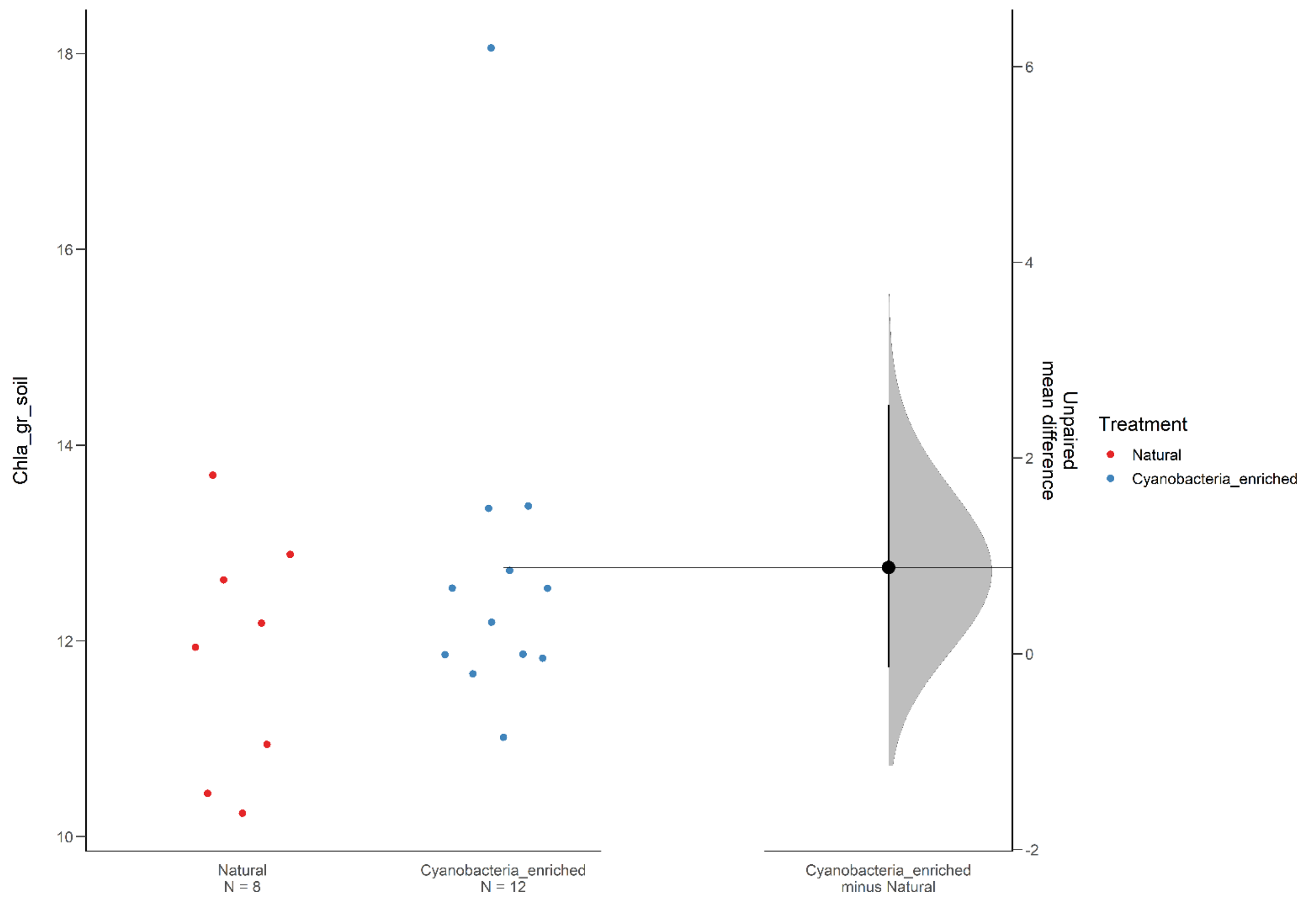


Figure C1. examples of cyanobacteria enriched biocrust in Erlynmeyer flask after the 10-14 days incubation period (left) and in a petri dish after transferring and drying in room temperatue under a 12-hr photoperiod (right).



Figur C2. estimation plot of Chl *a* concentrations for natural and cyanobacteria enriched biocrusts.

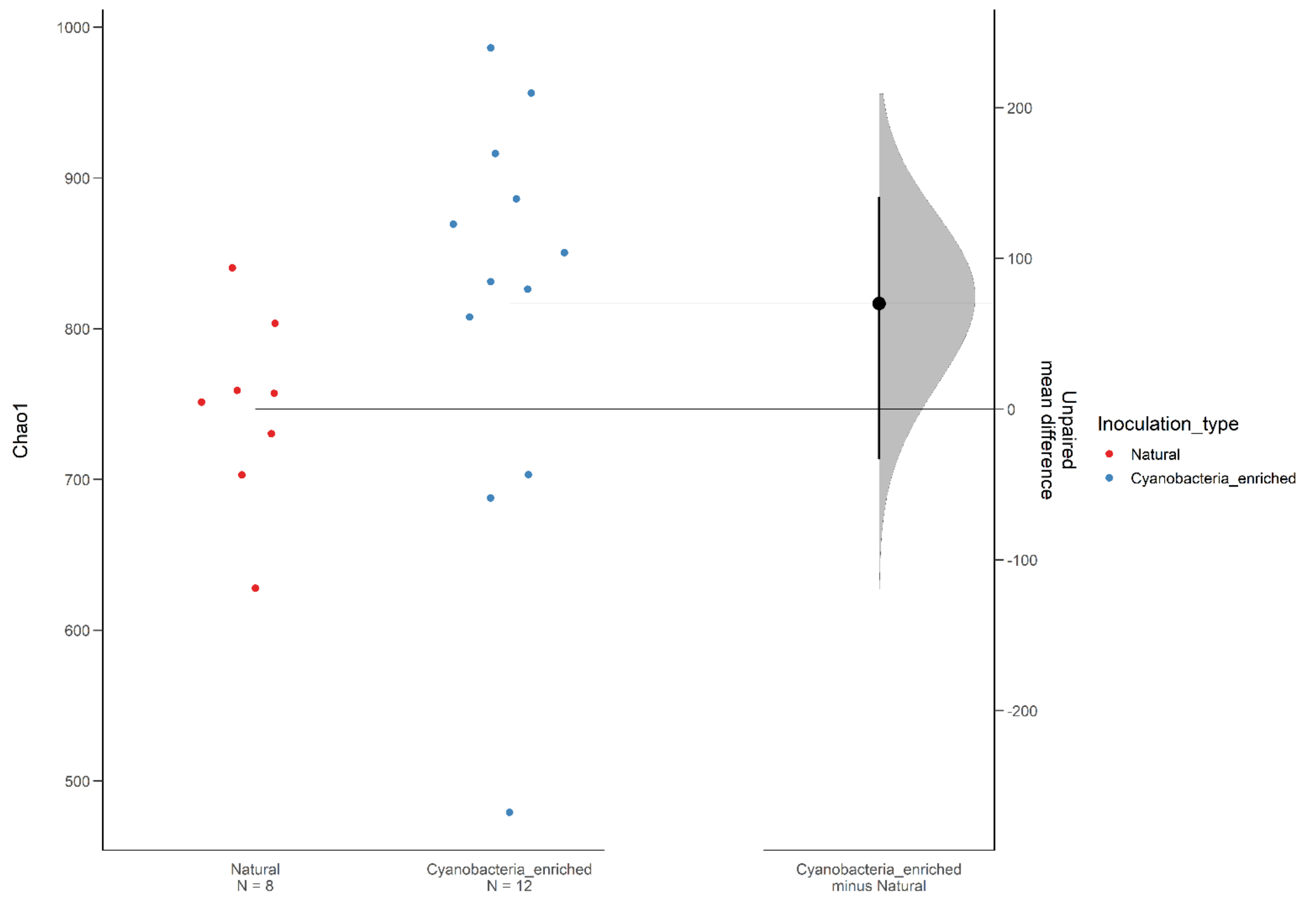


Figure C3. estimation plot of Chao1 diversity index for natural and cyanobacteria enriched biocrusts.

8.3.2. Tables

Table C1. relative abundance of bacterial phyla in natural and cyanobacteria enriched biocrusts. Significant differences are highlighted.

Phylum	Natural	Cyanobacteria enriched
Actinobacteriota	**38.25**	9.82
Cyanobacteria	**24.28**	**29.01**
Proteobacteria	**18.07**	**31.01**
Chloroflexi	6.74	4.23
Bacteroidota	4.49	4.58
Gemmatimonadota	2.64	1.38
Firmicutes	1.90	**10.48**
Deinococcota	1.52	less than 1%
Myxococcota	1.35	2.91
Rare	0.77	1.02
Acidobacteriota	less than 1%	2.90
Planctomycetota	less than 1%	1.64
Bdellovibrionota	less than 1%	1.03

Table C2. Results of Tukey's post-hoc test, comparing Chl *a* results between treatments and time.

10 weeks	20 weeks	P value
Control	Control	0.0082928
Control	Hydration	0.0386585
Control	Natural biocrusts	0.0007346
Control	Cyanobacteria enriched biocrusts	0.0099446
Hydration	Cyanobacteria enriched biocrusts	0.0084843
Natural biocrusts	Control	0.0464736
Natural biocrusts	Natural biocrusts	0.0053593
Natural biocrusts	Cyanobacteria enriched biocrusts	0.0542873
Cyanobacteria enriched biocrusts	Control	0.0000101
Cyanobacteria enriched biocrusts	Hydration	0.0000724
Cyanobacteria enriched biocrusts	Natural biocrusts	0.0000006
Cyanobacteria enriched biocrusts	Cyanobacteria enriched biocrusts	0.0000126

תקציר

הפרעות אנתרופוגניות מובילות להרס וקיטוע של בתי גידול טבעיים בשטחים גדולים, מה שגורם לאובדן תפקוד תקין של המערכת האקולוגית וירידה במגוון ביולוגי. דוגמה להפרעה אנתרופוגנית היא כרייה, המערבת הרס הקרקע בשטחים נרחבים, יחד עם כל האורגניזמים החיים בה. באזור היפר יובשני של הנגב, מתבצעת כריית פוספטים מזה 60 שנה. בשנים האחרונות, חברת הכרייה החלה ליישם פרקטיקה של שיקום תוך כדי כרייה, אך ההשפעות של פרקטיקה זו על המערכת האקולוגית ככלל, וקרומי הקרקע הביולוגיים בפרט, לא נבחנו. קרום הקרקע הביולוגי הוא השכבה העליונה של קרקעות בבתי גידול יובשניים, המכיל קבוצות מגוונות של אורגניזמים. קרומי קרקע ביולוגים נותנים שירותי מערכת חשובים כגון קיבוע פחמן וחנקן, ייצוב הקרקע והשפעה על משטרי זרימת מים בקרקע. לאור מיעוט הצמחייה במערכות מדבריות והחסינות של קרומים ביולוגיים לתנאי סביבה קיצוניים, הם מהווים איי פוריות, ולכן חשוב לשמור על שכבת קרום המתפקדת בצורה מלאה. במחקר הדוקטורט היו לי שלוש מטרות: (1) אפיון חברות החיידקים בקרומי קרקע משוקמים וטבעיים (שלא עברו כרייה) על מנת להבין את השפעת הכרייה על החברה; (2) בחינת החברות החיידקיות הפעילות בקרומי קרקע משוקמים וטבעיים, באמצעות שימוש באיזוטופ יציב של מים; (3) ביצוע ניסוי מעבדה הכולל טיפולי הדבקת קרקע עם קרומים ביולוגיים והשקיה על מנת לבחון את יעילות הטיפולים בהתבססות של קרומים על קרקע חשופה (ללא קרומים).

במחקר הראשון אספנו דוגמאות מארבעה אתרי כרייה (כל אתר שוקם בשנה אחרת) ובשטחים טבעיים הסמוכים להם. שיערנו כי חברות החיידקים בקרומי קרקע לאחר כרייה יהיו שונות מחברות טבעיות, לאור ההתבססות האיטית של קרומים ביולוגיים לאחר הפרעות מכאניות. בנוסף, שיערנו כי החברות מאתרי שיקום שונים יהיו שונות אחת מהשנייה, בהתבסס על הזמן שעבר מאז השיקום. על מנת לבחון השערות אלו, אפיינו את החברות בעזרת ריצוף גן ה S16 החיידקי. כמו כן, מדדנו את ריכוז הכלורופיל בקרקע, שכן זה מהווה מדד לפוטנציאל קיבוע הפחמן בקרומים. מצאנו כי המגוון החיידקי נמוך יותר בקרומים המשוקמים וכי מבנה החברה שונה. השפע של ציאנובקטריה וריכוזי הכלורופיל היו נמוכים יותר בקרומים המשוקמים, מה שמרמז על פוטנציאל פוטוסינתטי נמוך יותר. לא מצאנו הבדלים משמעותיים בחברות המשוקמות מזמני שיקום שונים. במחקר השני, בחרנו אתר כרייה אחד על מנת לבדוק אילו קבוצות חיידקיות פעילות בקרומי הקרקע המשוקמים וטבעיים בעזרת איזוטופ יציב של מים, שכן האורגניזמים בקרומי קרקע נהיים פעילים רק לאחר הרטבה. הדגרנו דוגמאות קרקע עם מים במשך 96 שעות. בנוסף, בדקנו מדדי קרקע, ריכוזי כלורופיל וכמויות של גנים הקשורים לתהליכים ביוכימיים שונים, בהתבסס על כמויות הגנים של S16 החיידקי. מצאנו כי יש מעט מאוד פעילות חיידקית בקרומים המשוקמים וטבעיים. לא מצאנו הבדלים בהרכב החברות הפעילות, מדדי קרקע, ריכוזי כלורופיל או גנים הקשורים לתהליכים ביוכימיים. במחקר השלישי אספנו טופסויל מערמת קרקע שהוצאה בתהליך הכרייה על מנת לבחון שלושה טיפולים שונים שמטרתם לזרז תהליך יצירת קרומי קרקע על קרקע חשופה – השקיה, השקיה + הדבקת קרקע עם קרומים טבעיים והשקיה + הדבקת קרקע עם קרומים שיוצרו בתנאי מעבדה. הניסוי נעשה בעציצים קטנים בחדר עם בקרת אקלים. לאחר 20 אירועי השקיה (פעם בשבוע), לא ראינו הבדלים בהרכב החברה של הקרומים בטיפולים השונים לעומת הקרומים לאחר 10 אירועי השקיה. בנוסף, ריכוז הכלורופיל ירד לאחר 20 אירועי השקיה, מה שמרמז על ירידה בפעילות קיבוע הפחמן. תוצאות התזה מראות כי חברות החיידקים בקרומי קרקע משוקמים שונות מחברות טבעיות, ללא קשר לזמן שעבר מאז השיקום. הירידה בשפע של ציאנובקטריות וכלורופיל מרמזות על ירידה בפוטנציאל קיבוע הפחמן של קרומים משוקמים. הגידול המועט בקרומים המשוקמים וטבעיים מרמזים על כך שגם בזמן פעילות, חיידקי הקרומים לא מכפילים את עצמם. הוספת טיפולים לקרקע לא הובילה ליצירה מהירה של קרומי קרקע, שכן לא נוצרו קרומים ביולוגיים לאחר 20 אירועי השקיה, כנראה עקב כמויות מלח קיצוניות בקרקעות ששמשו לניסוי. אנו מסיקים כי פעילות השיקום שנעשית כיום בשטחי הכרייה בנגב אינה מאפשרת התבססות של חברות החיידקים בקרומי הקרקע, בפרט קבוצת הציאנובקטריה. בנוסף,

השתקמות טבעית של קרומי הקרקע צפויה להיות איטית במיוחד עקב תנאי האקלים הקשים באיזור צין והגידול המועט של חיידקים בזמן פעילות. ניסויים נוספים לבחינת שיקום אקטיבי בשטחי הכרייה חשובים, שכן זה הפתרון האופטימלי לזירוז תהליך שיקום הקרומים.

<u>בחינת השפעות כריית פוספטים בנגב על חברות קרומי קרע ביולוגיים</u>

מחקר לשם מילוי חלקי של הדרישות לקבלת תואר "דוקטור לפילוסופיה"

מאת

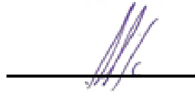
טליה גבאי

הוגש לסינאט אוניברסיטת בן גוריון בנגב

תאריך עברי	תאריך לועזי
כ"ב תשרי ה'תשפ"ג	17 באוקטובר 2022

באר שבע

אישור המנחה : **ירון זיו** 29.09.2022

אישור המנחה: אסנת גילאור  15-10-2022

אישור דיקן בית הספר ללימודי מחקר מתקדמים ע״ש קרייטמן

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כ״ב תשרי ה׳תשפ״ג 17 באוקטובר 2022

באר שבע

העבודה נעשתה בהדרכת

פרופ׳ ירון זיו

פרופ׳ אסנת גילאור

במחלקה : מדעי החיים ומכון המים

בפקולטה : מדעי הטבע והמכונים לחקר המדבר

הצהרת תלמיד המחקר עם הגשת עבודת הדוקטור לשיפוט

אני החתום מטה מצהיר/ה בזאת (אנא סמן):

✓ חיברתי את חיבורי בעצמי, להוציא עזרת ההדרכה שקיבלתי מאת מנחה/ים.

✓ החומר המדעי הנכלל בעבודה זו הינו פרי מחקרי מתקופת היותי תלמיד/ת מחקר.

תאריך 17/10/2022 שם התלמיד/ה טליה גבאי

חתימה