Studying the Microbiome of Cyanobacterial Biocrusts From Drylands and Its Functional Influence on Biogeochemical Cycles

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Abstract

Background: Drylands are areas under continuous degradation and desertification largely covered by cyanobacterial biocrusts. Several studies have already shown that soil microorganisms play a fundamental role in the correct soil functioning. Nevertheless, little is known about the relationship taxonomy-function in arid soils and, in particular, in cyanobacterial biocrusts. An in-depth study of the taxonomic composition and the functions carried out by soil microorganisms in biogeochemical cycles was here carried out by using a shotgun metagenomic approach.

Results: Metagenomic analysis carried out in this study showed a high taxonomic and functional similarity in both incipient and mature cyanobacterial biocrusts types with a dominance of *Proteobacteria*, *Actinobacteria* and *Cyanobacteria*. The predominant functional categories related to soil biogeochemical cycles were “carbon metabolism” followed by “phosphorus, nitrogen, sulfur, potassium and iron metabolism”. Reads involved in the metabolism of carbohydrates and respiration were the most abundant functional classes. In the N cycle dominated “ammonia assimilation” and “Nitrate and nitrite ammonification”. The major taxonomic groups also seemed to drive phosphorus and potassium cycling by the production of organic acids and the presence of extracellular enzymes and specialised transporters. Sulfur assimilation was also predominantly led by actinobacteria via the acquisition of sulfur from organosulfonated compounds. The main strategy followed for iron uptake seemed to be the synthesis and release of siderophores, mostly derived from representatives of the genus *Pseudomonas.*

Conclusions: The absence of significant differences between both type of biocrusts was suggested to be due to the identical habitat-specific characteristics, the inherent variability associated with metagenomic sampling and experimental design limitations. There is metabolic diversity of respiration processes over the photosynthesis, but a diverse group of microorganisms, predominantly *Actinobacteria* and *Proteobacteria* were also involved in CO_2 fixation metabolism. A preferential uptake of ammonium over nitrate as an economic strategy to avoid the high consumption of ATP was confirmed. Moreover, the functional redundancy presented by the microbial community was interpreted as a strategy to maintain the correct functioning of the soil biogeochemical cycles and therefore of the ecosystem in general. Evidence of sythrophic growth was nevertheless observed. Biotechnological potential as plant growth promoters was also identified.

Background

Drylands occupy a vast area on the planet (approximately 41%), and much of this area is under continuous threat of degradation and desertification due to the important growth of the human population and the global change [1]. A large part of the drylands (up to 70% of the surface) are covered by biocrusts, which are associations of soil particle, bacteria, microalgae, microfungi, cyanobacteria, lichens, and bryophytes colonizing the first millimetres of soils in the interspaces between plants [2]. Cyanobacteria dominating the biocrusts are extremophiles primary colonizers organisms which improve soil conditions and fertility in dryland regions [3]. They are also able to fix CO_2 [4] and N_2 [5], and release

to the soils a wide variety of substances such as carbohydrates, polyphenols, sucrose, glucose [6], exopolysaccharides [7], phosphorus, phytormones, vitamins [8], antimicrobial compounds [9], and other biochemical metabolites including scytonemin, mycosporine-like amino acid, among other [10, 11]. The leaching of these compounds to the underlying soils provides a favourable and selective microhabitat for a wide diversity of soil bacterial communities [12]. These biocrusts have a high abundance of very active organisms enriching the geological substrate with hydrolase-type enzymes that enable the organic remains mineralization and the establishment of later successional biocrusts [13, 14].

Several studies have shown that soil microbial communities are driven by physical and chemical soil properties, such as pH, soil organic carbon and types of labile or resilient organic compounds, carbonates or amount of salts in the soils [15, 16, 17, 18, 19, 20, 21, 22]. Therefore, the effect of the cyanobacterial biocrusts on the physico-chemical soil properties, influences in turn the diversity and composition of the soil bacterial communities [12]. The soil microbial communities control the correct functioning of soil biogeochemical cycles [23, 24], which is essential for the correct functioning of arid and semi-arid ecosystems. Nevertheless, the system of interacting biogeochemical reactions in soils is highly complex, poorly characterized, and mediated by a wide diversity of soil microorganisms [25]. Specifically, soil biodiversity contributes to a multitude of soil services including the regulation of greenhouse gas emissions, nutrient cycling [26, 27], and the uptake and acquisition by the plant of minerals, organic substances, and many other metabolites such as amino acids, phytohormones, among other [28, 29]. The soil organic matter degradation is also controlled by the soil microbial communities along to environmental conditions driving the biogeochemical activities of the soil microorganisms such as temperature, pH, soil water capacity, etc [30, 31]. Moreover, soil microorganisms develop important roles in the nitrogen (i.e. nitrogen fixers; [32, 9]), phosphorus (i.e. P-solubilisers organisms; [33]), iron (i.e. siderophore production for iron sequestration in plants; [34]), sulfur (i.e. sulfur-oxidasing bacteria), and potassium cycles (i.e. potassiumlisers and mobilisers; [35]).

Nevertheless, despite a growing number of studies have shown that soil microbial communities develop a key role in biogeochemical cycles [23], surprisingly little is known about the biogeochemical processes in soils [36] and specifically about the link between the functions performed by specific soil bacterial communities in the biogeochemical cycles. Improve our knowledge about the relationship taxonomy-function is one important challenge for advancing our understanding of biogeochemical processes. In general, a good estimate of the diversity and richness of the soil microbial communities and an efficient taxonomic identification of soil bacterial communities has been carried out recently through methods based on 16S rRNA analysis [20, 12, 21, 22], but fail in the identification of functional attributes [36]. Shotgun metagenome methods are increasingly being employed to describe soil microbial communities [37, 38] and comparative taxonomic and functional profiling of the microbial communities in different environment [39, 36]. Thus, metagenomic studies are a very efficient tool toward the understanding of microbial diversity allowing for the identification of soil microbial taxa (through 16s ribosomal amplicon sequencing [MG-RAST, QIIME]) and functional profiles of entire microbial communities (through whole genome shotgun sequencing [MGRAST]) [40] as the abundance of determinate genes have been used as an indicator of biogeochemical processes [41, 42].

Taking into account the considerations mentioned above, the objective of this work is to carry out an in-depth study of the taxonomic composition and the functions carried out by soil bacteria in biogeochemical cycles, specifically the cycles of carbon, nitrogen, phosphorus, iron, sulfur and potassium in soil samples from the Tabernas desert colonized by cyanobacteria, as well as establishing the taxonomy-function relationships in those soils. Tabernas Desert (southeast Spain), is an unique Badlands landscape in Europe due to its geomorphological processes, semiarid climate, hight biodiversity, and endemic species, where cyanobacterial and lichens biocrusts are highly represented [43, 10]. Soil microbial community composition and their functions have been shown markedly differ in desert biomes compared to other biomes [44], therefore Tabernas desert is an ideal scenario to carry out this study.

Methods

Experimental area and sampling

The study area, the Tabernas Desert in southeaster Spain (Province of Almería), is one of the largest Badlands in the European continent with an approximate extension of 150km^2. It is located in the Sorbas–Tabernas catchment, mainly filled by soft Miocene rock, mostly marls and calcareous sandstone, and delimited by the Betic Chain, the Gador, Nevada, Filabres and Alhamilla Mountains. The Tabernas Desert climate is semiarid Thermo-Mediterranean characterized by a long summer and low annual rainfall (around 230 mm) due to the most rainfalls are generally intercepted by the Betic, Gador and Nevada ranges in the study area. The average annual temperature is 17.9°C, with an absolute maximum of 45°C, an absolute minimum of − 4.5°C, and high interannual and intra-annual rainfall variability [45]. The geomorphology of the landscape combines bare, eroded and poorly developed soils (Epileptic Regosol or Lithic Torriorthent) in steeper Southwest-facing slopes (gradients up to 70°). In contrast, Northwest-facing slopes with gradients less than 30° with more developed soils (Endoleptic Regosols or Lithic–xeric Torriorthent) covered by biocrusts, including many species of terricolous lichens such as *Diploschistes diacapsis* (Ach.) Lumbsch, *Squamarina lentigera* (Web.) Poelt., *Lepraria isidiata* (Llimona) Llimona & Crespo, among other and patches dominated by cyanobacterial communities. Separating some catchments are old residual hanging pediments, on gentle slope gradients exposed to the sun, where the soils are thicker (Haplic Calcisol or Xeric Haplocalcid) and covered by biocrusts in open areas with patches of annual plants (dominated mainly by *Stipa capensis* Thunb.) and scattered perennial plants (with predominance of *Helianthemun almeriense* Pau, *Hammada articulata* (Moq.) O. Bolós & Vigo, *Artemisia barrelieri* Besser, *Salsola genistoides* Poiret, among other) [46, 14].

The sampling consisted of collecting three spatial replicates from the upper soil surface to a depth of three centimetres in two cyanobacterial biocrust communities at Tabernas desert according to previous work [14, 12] using a sterile spatula and immediately transferred to sterile tubes. The cyanobacterial biocrusts types sampled were: i) Incipient cyanobacterial biocrust (IC), with a light brown colour in clearings devoid of vegetation in areas often compacted by trampling and with high solar radiation; and,

ii) Mature cyanobacterial biocrusts (MC) with brown to dark brown colour dominating in areas mainly exposed to the sun with gentle slopes where the vegetation cover was very low (see Supplementary Table S1 for metadata details). They were also rich in diverse small and pioneering lichens including *Collema* spp., *Fulgensia* spp., and *Endocarpon pusillum* Hedw.

The samples were transported to the laboratory in isothermal bags with ice and sieved through 2 mm sterile sieves. After that, a part of these samples was directly used for subsequent molecular analysis and the other part for chemical analysis.

Chemical soil properties

Soil Organic Carbon (SOC) was determined by the Walkey and Black method [47] (modified by Mingorance et al. [48]). Total nitrogen (TN) was analysed using a Variomax CN analyzer (Elementar Analysensysteme GmbH, Hanau, Germany) and assimilable phosphorus (AP) was studied by the Watanabe and Olsen method [49]. Soil pH was determined in an aqueous solution 1/2 (w/v) using a micropH 2002 Crison pHmeter (Crison, Barcelona, Spain) and the electrical conductivity (EC) was determined in aqueous extract 1/5 (w/v) with a digital conductivity meter (Basic 30, Crison, Carpi, Italy).

DNA extraction, Metagenomes (Shotgun), Next-Generation Sequencing and Bioinformatic treatment

Total soil DNA was extracted from 0.5 g of soil by using the DNeasy PowerSoil kit (QIAGEN-MoBio Laboratories, Carlsbad CA) following the manufacturer's instructions. DNA concentration and purity were estimated spectroscopically using a NanoDrop™ 2000 spectrophotometer. DNA libraries were prepared using the Illumina Nextera Flex kit as per the manufacturer's instructions and sequenced on Illumina NextSeq 550 (150 + 150 bp paired-end) by Integrated Microbiome Resource (IMR)-Dalhousie University, Canada (an overview of the entire IMR wet-lab pipeline can be found in Comeau et al. [50]). Paired (F + R) raw sequences (FASTQ files) were quality-controlled using Kneaddata (https://bitbucket.org/biobakery/kneaddata) and then concatenated for subsequent downstream analysis. FASTQ files were afterwards processed and annotated by MG-RAST V.4.0.3 server at http://www.mg-rast.org/ [51] and further quality controls involving trimming of low-quality regions by SolexaQA [52], removal of artificial duplicate reads (ADRs) [53], and near-exact match to the genome of model organisms by DRISEE [40] and Bowtie [54], respectively, by using default parameters, were performed. Through the workbench tool from MG-RAST server, SEED subsystems [55], Cluster of Orthologous Groups (COG; [56]) and Kyoto Encyclopedia of Genes and Genomes (KEGG; [57]) data sources were used to generate subsets of reads annotated for taxonomic and functional purposes.

Statistical analysis

The significant differences in physico-chemical soil properties between the IC and MC samples were studied by T-Test analysis using Statgraphics Centurion 18.1.13. For metagenomic statistical analysis, relative abundances of reads were downloaded from MG-RAST server and filtered using a minimum count of 4, prevalence in samples of 20%, and a low variance filter at 10% based on inter-quartile range.

To improve downstream statistical analysis, the low count filter was adjusted at 2 for functional analysis. Data normalization was carried following the Relative Log Expression (RLE) procedure [58] according to the recommendations suggested by Pereira et al [59]. Rarefaction curves, α- (ACE, Chao1, Fisher, Observed, Shannon, Simpson) and β-diversity (3D ordination plot based on principal coordinate analysis (PCoA)), Permutational Multivariate Analysis of Variance (PERMANOVA), and Random Forest analysis were performed by using the web-based tool microbiomeAnalyst at https://www.microbiomeanalyst.ca/ [60, 61]. Additional statistics tests were carried out by IMB® SPSS® Statistics v 25.0.0.1.

Results

Chemical soil properties

All biocrusts samples showed low values of SOC, NT, and AP being higher the SOC (0.9 ± 0.2 g kg^{-1}), TN (0.1 ± 0.01 g kg^{-1}) and AP (1.6 ± 0.4 ppm) content in MC biocrusts than in IC biocrusts with SOC values of 0.5 ± 0.2 g kg^{-1}, NT of 0.1 ± 0.02 g kg^{-1} and AP of 1.5 ± 0.3 ppm, although only SOC was significantly higher in MC than in IC (Supplementary Table S2). Soil pH was basic in all samples ranging between 7.75 and 8.85, although in the MC-biocrusts it was slightly lower than in IC-biocrusts, but instead the electrical conductivity was comparatively higher in IC biocrusts than in MC biocrusts (See Supplementary Table S2 for chemical soil properties associated with each sample). No significant differences were found between IC and MC samples.

Sequence processing details

The number of uploaded reads into MG-RAST server ranged from 1,686,580 to 2,976,285 sequences for the IC samples and from 2,407,121 to 2,931,615 for the MC samples. After quality control, the number of retained sequences for the IC samples ranged from 1,472,483 to 2,671,326 with an average length of ~ 153.3 bp, whereas 2,223,048 – 2,655,285 sequences with an average length of ~ 165.3 bp were retained for the MC samples. Regarding predicted features, from 390,736 to 706,790 and from 627,819 to 698,001 proteins were identified in IC and MC samples, respectively, observing an average of 2,517 ± 304.5 identified as rRNA features for all the samples except sample IC2 that just aligned 832 rRNA features. For additional sequence processing details and raw annotated data, see Supplementary Table S3.

Rarefaction curves calculated after statistical analysis filtering plateaued for all taxa levels (Supplementary Fig. S1), suggesting that saturation in sequencing was achieved and, therefore, an appropriate taxonomic depth was reached to describe a reliable species richness in these metagenomic samples [62].

Bacterial diversity and comparative taxonomic profiles

Observed species richness (Sobs), Chao1, ACE, Shannon and Simpson indices, at genus level, revealed an α-diversity slightly higher in MC biocrusts samples. Despite, the inherent variability observed within the IC samples may have covered up potential significant differences in diversity between the two biocrust

developments (Supplementary Fig. S2). Certain differentiation between the two biocrusts types was also observed by β-diversity index although no statistically significant pattern was identified at any taxonomic level ([PERMANOVA] F-value: 2.8161; R-squared: 0.41315; p-value < 0.2) (Supplementary Fig. S3).

Microbial communities of both IC and MC biocrusts samples showed a dominance of the domain *Bacteria* (93.9 ± 3.3 % and 90.5 ± 2.7 %, respectively), followed by *Eukarya* (5.0 ± 3.1 % and 8.6 ± 2.8%, respectively), and *Archaea* (1.0 ± 0.09% and 0.9 ± 0.1%, respectively). At phylum level, the predominant taxonomic groups were the bacterial phyla *Actinobacteria* (24.6%-45.6%) and *Proteobacteria* (26.9%-34.9%), in both IC and MC metagenomes, followed by (all ≤ 10%) *Ascomycota (Eukarya), Cyanobacteria* (4.7%-5.6%), *Firmicutes, Bacteroidetes, Chloroflexi, Plantomycetes, Verrucomicrobia*, and *Acidobacteria* (Fig. 1a). Composition of IC and MC microbial populations were also similar at genus level being the most frequent genera: *Rubrobacter* (5.2 ± 0.4%), *Conexibacter* (4.0 ± 0.3%), and *Streptomyces* (3.7 ± 0.4%), all of them belonging to *Actinobacteria*, followed by *Frankia, Mycobacterium, Geodermatophilus (Actinobacteria), Methylobacterium, Sphingomonas (Proteobacteria), Salinispora (Actinobacteria), Caulobacter (Proteobacteria), Nocardioides, Rhodococcus (Actinobacteria), Roseiflexus (Chloroflexi), Saccharopolyspora (Actinobacteria)*, and the cyanobacteria *Nostoc* and *Cyanothece* (Fig. 1a), whose abundances ranged from 0.8 ± 0.3% for IC samples to 1.74 ± 0.5% for MC samples and from 0.4 ± 0.2% to 1.3 ± 0.3%, respectively.

Considering the minor domains, it is worth mentioning that the most frequent archaeal phylum was *Euryarcheaota* comprising 99.6 ± 0.0% of the reads assigned to *Archaea*, and within this phylum, the genus *Methanosarcina* (11.3 ± 0.2%). Regarding *Eukarya, Ascomycota* encompassed 74.2 ± 9.0% of the total reads assigned to eukaryotes being the dominant genera *Neosartorya, Aspergillus, Coccidioides*, among others (See Supplementary Table S4 for raw taxonomic data).

When comparing IC and MC samples, significant differences were found in the actinobacterial families *Nocardioidaceae, Intrasporangiaceae, Dermabacteraceae* and *Micromonoporaceae* (DESq2 Pvalue < 0.05, FDR < 0.05). Within *Nocardioidaceae*, differences on the abundance of representatives belonging to the genera *Nocardioides* and *Kribella* were also determined as statistically significant. In the same way, significant differences were observed in *Intrasporangium* and *Janibacter* representatives, both affiliated to *Intrasporangiaceae*, and in the genera *Brachybacterium (Dermatobacteraceae)* and *Salinispora (Micromonosporaceae).* Finally, statistical differences between the two biocrust types were also identified for the ascomycotal genus *Ramichloridium.*

Comparative functional profiles

According to COGs database, functional categories encompassing the major abundance of reads were “General function prediction only” (R), “Amino acid transport and metabolism” (E), and “Energy production and conversion” (C) showing percentages ranging from 0.09 to 0.14 (Fig. 1b). Other categories comprising genes related to “Carbohydrate transport and metabolism” (G), “Translation ribosomal structure and biogenesis” (J), “Replication, recombination and repair” (L), and “Cell wall membrane envelope biogenesis” (M) also displayed relative abundance (0.08 – 0.05%). At gene level, a total of 2,925

orthologues were obtained, of which 2,855 (97.6 %; ~16% of processed reads) were assigned to functional categories in COG. 2,824 genes were shared between IC and MC metagenomes (core metagenome), 31 (1.1%) were unique to IC samples and 73 (2.5%) were only observed in MC samples. Both IC and MC samples presented high percentages of sequences coding for several types of dehydrogenases observing also a significant abundance of permeases (See Supplementary Table S5 TabA for raw functional data). Although no significant differences were observed between IC and MC biocrust types, when applied DESeq2 statistical test, MC samples were functionally more similar than those analysed as IC samples (Supplementary Fig. S4).

Taxonomic groups and functional genes involved in the biogeochemical cycles

Conforming to SEED subsystems functional annotation (~ 17% of processed reads), in all samples, the most predominant functional categories related to soil biogeochemical cycles were “carbon metabolism” followed by “phosphorus, nitrogen, sulfur, potassium and iron metabolism”, showing the lowest gene abundance “iron acquisition and metabolism” (See Supplementary Table S5 TabB for raw functional data). No significant differences were observed between IC and MC samples apart from some particular genes that will be later mentioned. Hence, for practical terms, joined statements are generally provided.

Carbon cycling

According to SEED subsystems, carbon cycle processes (77,260.8 ± 5576.8 reads) identified in biocrust samples were classified in carbohydrate metabolism (53,323.2 ± 4,050.2 reads), respiration (16,825.2 ± 990.0 reads), fatty acids, lipids, and isoprenoids metabolism (6,533.7 ± 500.0 reads), CO_2 fixation (3.423.0 ± 525.4 reads) and photosynthesis (760.7 ± 222.8 reads), observing a clear dominance of respiration over carbon fixation in microbial communities associated with both type of samples. Consequently, in absolute terms, it was observed a predominance of oligotrophic heterotrophs such as *Caulobacter* (Proteobacteria) and belonging to the phylum *Actinobacteria* (*Rubrobacter, Conexibacter, Frankia* and *Streptomyces*, among others) (Fig. 2a). Nevertheless, and in spite of the low number of reads assigned to “photosynthesis” category, significant relative abundances were showed for *Roseiflexus* (Chloroflexi).

Carbohydrate metabolism

Regarding the presence of extracellular enzymes involved in the degradation of complex carbohydrates, samples were rich in *α*-glucosidases (289.5 ± 27.0 reads), *β*-glucosidases (332.8 ± 40.8 reads), *β*-galactosidase (602.3 ± 87.0 reads), α-amylases (264 ± 13.7 reads), and glucoamylases (392.3 ± 25.6 reads). *Rubrobacter* and *Nostoc* resulted to be the major contributors of *α*-glucosidases while *β*-glucosidases were mainly derived from *Caulobacter. β*-galactosides and α-amylases were, nevertheless, predominant in *Deinococcus* and *Spirosoma* and *Rhodopseudomonas* and *Rhodospirillum* representatives, respectively. Contrarily, the major number of glucoamylases was found in *Caulobacter*

and other actinobacterial representatives. In addition, bacteria from the group of *Opitutus* (*Verrucomicrobia*) were postulated as the major producers of chitinases in samples (91.2 ± 14.0 reads) and minor amounts of xylanases (29.7 ± 3.9 reads), were found in reads affiliated to *Xanthomonas* and *Caulobacter* representatives.

Fatty acids, lipids, and isoprenoids metabolism

Extracellular esterases and lipases were, in general, absent or a minor component in samples. The well-known triacylglycerol acylhydrolase was absent just observing a minimal rise for extracellular lysophospholipases (40.7 ± 6.4) in the genera *Caulobacter* and *Salinispora*. Nevertheless, 1,234 ± 99.2 reads mainly derived from *Rubrobacter, Roseiflexus*, and *Thermobaculum* representatives, among others, were assigned to “glycerol and glycerol-3-phospate uptake and utilisation” category. Finally, 491.5 ± 57.4 reads identified as polyhydroxyalkanoic acid (PHA) synthases were affiliated to the taxonomic groups of *Bacillus* and *Acidiphilium* but also *Caulobacter* which showed a different behaviour between biocrust type samples according to Random Forest analysis (Supplementary Fig. S5a).

Respiration

In general terms, and as expected, respiration processes in cyanobacterial biocrusts seemed to be driven by actinobacteria (37.0 %) followed by proteobacteria (30.8 %). In particular, the actinobacterial genera *Conexibacter* and *Rubrobacter* were the predominants accounting 8.0 % and 5.9 % of recovered reads, respectively. Although no differential abundance of genes were identified statistically for any process related to respiration, Random Forest analysis indicated differences in the abundance pattern of representatives of the genus *Cytophaga* and, in a lesser extent, *Herpetosiphon*, along with others (Supplementary Fig. S5b).

At functional level, as expected under aerobic conditions, samples showed microbial respiration mainly led by glycolysis (2,754.7 ± 158.6 reads), the tricarboxylic acid (TCA) cycle (1,935.3 ± 263.8 reads), and oxidative phosphorylation (15,103.5 ± 878.7 reads) being the glucose the main substrate. Entner-Doudoroff pathway (1,230.8 ± 131.1 reads) was besides observed in *Conexibacter* representatives but also in *Nostoc, Rubrobacter, Caulobacter* and *Salinispora*, among others. In addition, 659.2 ± 61.6 reads associated with glyoxylate cycle were also identified in the genera *Conexibacter, Rubrobacter*, and *Nocardioides*, among others. The ethylmalonyl-CoA pathway of C2 assimilation showed 737.7 ± 30.8 reads mainly corresponding to actinobacterial groups such as *Conexibacter, Frankia* and *Streptomyces*, among others.

Finally, a taxonomically varied group of microorganisms exhibited a significant number of reads assigned to enzymes involved in methanotrophic metabolism. That is, methane monooxygenase (141.5 ± 16.0 reads), mainly affiliated to actinomycetes such as *Frankia* and *Gordonia;* methanol dehydrogenase (80.7 ± 9.4 reads), derived from *Bradyrhizobium*, *Candidatus Solibacter*, and *Granulibater*, and formate dehydrogenase (505.8 ± 43.5 reads), coming a significant proportion from *Thermobacullum, Sphaerobacter*, and *Geodermatophilus* representatives. No reads were, nevertheless, assigned to formaldehyde dehydrogenase. Additionally, carbon monoxide dehydrogenases (965.2 ± 63.4 reads) were

also found supporting the carbon metabolism of *Rubrobacter* but also *Frankia* and *Roseiflexus*, among others.

CO_2 fixation

Actinobacteria (31.2 %), *Proteobacteria* (27.7 %) and *Cyanobacteria* (15.8%) were the predominant phyla, followed of *Firmicutes* with 7% of OTUS recovered. At genus level, reads assigned to *Rubrobacter* (8.2%) showed the highest abundance, followed by *Conexibacter* (4.8%), *Caulobacter* (3.0%), and the cyanobacterial *Nostoc* (4.6%), *Cyanothece* (2.5%), and *Anabaena* (2.1%). Relative high abundances of the diazotrophophs *Bradyrhizobium* (3.7%) and *Frankia* (3.0%) were also observed. Random Forest analysis, besides, indicated differences in the abundance pattern of some minor bacterial components such as *Thermomonopora* and *Flavobacterium*, in a lesser extent (Supplementary Fig. S5c).

Among the alternative functional pathways, the Calvin-Benson-Bassam (CBB) cycle showed the highest number of reads (2,205.5 ± 150.4 reads) followed by the photorespiration via oxidative C2 cycle (902 ± 55.6 reads) and carboxysome synthesis (863.7 ± 97.3 reads).

Photosynthesis and phototrophy

Photosynthetic metabolism, was, as expected, mainly identified in representatives of the phylum *Cyanobacteria* (40.4 %). However, significant proportions were also found in *Proteobacteria* (12.2 %), *Actinobacteria* (10.4 %), and *Chloroflexi* (4.6 %). At genus level, the most abundant OTUS were affiliated to *Nostoc* (10.8%) followed by *Chrorella* (5.8%), *Cyanothece* (5.5%), *Synechococcus* (5.5%), *Chlamydomonas* (4.4%), *Thermosynechococcus* (4.0%), *Anabaena* (3.8%), *Trichodesmium* (2.2%), and *Nephroselmis* (1.7%). Once more, Random Forest analysis indicated differences in the abundance pattern of some minor bacterial components such as *Candidatus, Koribacter* and *Caulobacter and Geodermatophilus*, in a lesser extent (Supplementary Fig. S5d).

At functional level, the major number of reads were assigned to photosystems I and II (260.2 ± 81.5 and 322 ± 99.8 reads, respectively). On the other hand, photosystem II-type photosynthetic reaction center reads (38.3 ± 9.7 reads) were identified coming from proteobacteria, mainly from the orders *Rhizobiales, Rhodospirillales*, and *Rhodobacterales*. Apart from chlorophyll-based phototrophs, samples showed similar amount of reads involved in the synthesis of retinal component of proteorhodopsins (446.3 ± 43.2 reads). In these samples, those were mainly derived from *Bradyrhizobium, Streptomyces, Nostoc, Rhodopirellula* and *Sphaerobacter*, among others from diverse phyla. Finally, cyanobacteria, mostly *Nostoc* representatives, showed a third alternative light-harvesting system to capture sunlight based on phycobilisome (PBS) (125 ± 40 reads).

Nitrogen cycling

Actinobacteria were the major drivers in both IC and MC samples counting with almost 50 % of reads assigned to nitrogen metabolism (3,696.3 ± 171.2 reads) followed by proteobacteria (27.0 %). Indeed, most of the top abundant genera, including *Rubrobacter* (10.3 %) and *Conexibacter* (8.5 %), belonged to

actinobacteria group (Fig. 2b). Random Forest besides indicated differences between IC and MC biocrusts in the abundance pattern of some minor bacterial components such as *Sphaerobacter* and, in a lesser extent, *Alkaliphilus*, among others (Supplementary Fig. S5e).

According to SEED subsystems categories, 56.8 % of reads were identified as part of “ammonia assimilation”, 25.8 % to the subcategory “Nitrate and nitrite ammonification”, 7.0 % as part of “Nitric oxide synthase”, and 4.1 % to “Allantoin utilisation”. Minor rates were assigned to the subcategories “Amidase” (1.6 %), “Nitrogen fixation” (1.4 %), “denitrification” (1.3 %), and “cyanate-hydrolisis” (1.3 %).

The main processes driven ammonium assimilation in the samples were “ammonium transporters (Amt)” (25.5 % of reads assigned to this category), and “Glutamine synthetase-glutamate synthase (GS-GOGAT) pathway”, assigned mainly to the genera *Conexibacter* and *Rubrobacter*, among other actinobacteria.

Processes driven the assimilatory nitrate reduction (ANR) were “ATP-dependent nitrate ABC transporter” (15.5 % reads related) mainly identified in cyanobacterial representatives such as *Anabaena*, *Nostoc* and *Cyanothece*, and other “specific nitrate/nitrite transporters” (13.4 % reads) observed in other cyanobacteria such as *Actinosynnema* and representatives of *Nostoc* and in actinobacteria such as *Frankia*. Reduction of NO_3^- to NO_2^- is driven by “cytoplasmatic ferredoxin dependent enzyme nitrate reductase (Nas) (29.7% reads), mainly derived from cyanobacteria (*Nostoc* and *Anabaena*, among others) and actinobacteria (*Mycobacterium, Streptomyces* and *Saccharomonospora*, among others), which is then further reduced to NH_4 + by “cytoplasmatic ferredoxin dependent nitrite reductase (NiR) (NAD(P)H) (25.1% reads)” from bacteroidetes such as *Cytophaga* and actinobacteria such as *Kineococcus, Mycobacterium, Frankia* and *Rhodococcus*.

Contrarily, “dissimilatory nitrate (or respiratory nitrate) reduction” was insignificant. Transmembrane respiratory nitrate reductases (Nar) and periplasmic nitrate reductases (Nap) processes derived mostly from *Methylobacterium* and *Rubrobacter*. No reads associated with dissimilatory nitrite reductases were identified, but some nitrous oxide reductases were present in *Maricaulis, Salinispora*, *Albidiferax*, and so forth.

“Nitrogenase (molybdenum-iron)-specific transcriptional regulator NifA”, and nitrogenase (molybdenum-iron) associated with nitrogen fixation registered a low number of reads (59 ± 6 reads assigned to the genera *Nostoc, Cyanothece* and *Rhodopseudomonas*, among others. Some reads of transport (cyanate ABC transporter) and hydrolysis (cyanate hydratase) of cyanate were also registered in cyanobacteria (*Synechococcus* and *Nostoc*, among others), proteobacteria (*Bradyrhizobium*) and actinobacteria (*Mycobacterium*).

Moreover, a number of reads were related to the utilisation of allatoin; specifically, “the catabolism of allantoate to NH_3” were registered by representatives of the genera *Rubrobacter, Sphaerobacter, Desulfitobacterium* and *Geodermatophilus*, among others.

Finally, a low number of reads were identified as part of the extracellular enzyme urease ("urea amidohydrolase") mainly identified in representatives of cyanobacteria such as *Anabaena* and *Cyanothece*, proteobacteria such as *Alcanivorax* and *Anaeromyxobacter*, actinobacteria such as *Frankia, Saccharopolyspora* and *Geodermatophilus* and ascomycetes such as *Neurospora*.

Phosphorus cycling

5,888.8 ± 855.2 reads were assigned to phosphorus metabolism. According to SEED subsystems categories, 58.6 % were identified as part of "phosphate metabolism", 24.5 % as the subcategory "high affinity phosphate transporter and control of PHO regulon", 10.3% as "P uptake in cyanobacteria", and 5.8% as "alkylphosphonate utilisation". Phylogenetically, predominant members involved in the phosphorus cycling were allocated into the phyla *Actinobacteria* (36.2 %), followed by *Proteobacteria* (33.7 %), and *Cyanobacteria* (10.2 %). At genus level, there was a predominance of actinobacteria such as *Conexibacter* (6.7 %), *Rubrobacter* (4.3 %), *Caulobacter* (3.1 %; *Proteobacteria*), and *Salinispora* (3.0 %) (Fig. 2c). Random Forest besides indicated differences in the abundance pattern of some minor bacterial components such as *Nitrosopumilus* and *Leifsonia* and, in a lesser extent, *Tsukamurella, Chitinophaga*, and *Rhodobacter*, among others (Supplementary Fig. S5f).

Regarding organic phosphorus mineralisation, no read was assigned as phytases. Nevertheless, a significant number of reads (4.5%) mainly derived from *Rhodoseudomonas* (*Proteobacteria*), *Synechococcus* (*Cyanobacteria*) and *Sphingopyxis* (*Proteobacteria*), among others, were identified as alkaline phosphatases. In addition, 5.8% of reads, mainly coming from the taxonomic groups of *Pseudomonas*, *Methylobacterium, Salinispora, Mesorhizobium, Burkholderia Sinorhizobium*, and so forth, were assigned to the subsystem category "Alkylphosphonate utilisation".

Furthermore, two different systems were identified for the Pi transport: the high-affinity phosphate-specific transporter (Pst) regulated by PHO regulon (24.5% of assigned reads) mainly derived from *Caulobacter* and *Roseiflexus* but being also present *Dehalococcoides*, *Nostoc*, and *Sphingomonas* in good proportions, among others; and a "probable" low-affinity phosphate inorganic transporter (Pit) just represented by 2.7 % and identified mainly in reads associated with *Conexibacter, Nostoc, Anabaena*, *Magnaporthe* (*Ascomycota*), and so forth.

Sulfur cycling

An average of 2,733 ± 179.3 reads involved in "organic sulfur assimilation" (13.4 %), "inorganic sulfur assimilation" (55.5%), "sulfur oxidation" (8.6%), and sulfur respiration (2.54%) were detected. In general terms, actinobacteria seemed to drive the cycling observing a predominance of the genera *Rubrobacter, Frankia*, and *Conexibacter* followed by the alphaproteobacterial *Bradyrhizobium* (Fig. 2d). Although no significant differences were found between samples, some deviations in abundance were identified by Random Forest test in *Rhodobacter, Cellvibrio*, and *Brucella*, all of them *Proteobacteria*, and, in a lesser extent, in *Rhodopirellula (Plantomycetes), Spirosoma (Bacteriodetes), Xylella (Proteobacteria), Dictyoglomus (Firmicutes), Streptomyces*, and *Acidothermus(Actinobacteria)* (Supplementary Fig. S5g).

In particular, significant abundance of key functional genes related to organic sulfur assimilation via bacterial alkanesulfonate monooxygenase system (SsuD) and, taurine and glutathione utilisation were identified being those mainly providing from *Bradyrhizobium* representatives and, in a lesser extent, from *Gloeobacter, Caulobacter, Nostoc, Rhodopseudomonas*, and *Pseudomonas*, among others. Some genes involved in the breakdown of dimethylsulfoniopropionate (DMSP) only derived from *Roseobacter* were also present. The utilisation of L-cystine was, nevertheless, scarce and not consistently prevalent in the samples. Furthermore, *Rubrobacter* representatives appeared driving ester sulphate mineralisation by extracellular arylsulfatases (ARS; 368.8 ± 50.0 reads) and choline-sulfatases (375.8 ± 52.3 reads), noting also a minor proportion of N-acetylgalactosamine 6-sulfate sulfatase (GALNS) (22.3 ± 4.0 reads) coming from *Anabaena* and *Beutenbergia*, among others.

Contrarily, representatives of the genera *Cyanothece, Conexibacter, Frankia, Anabaena, Caulobacter, Nostoc*, and *Streptomyces*, among others, were postulated to drive inorganic sulfur assimilation. The major uptake of inorganic sulfur (usually sulfate or thiosulfate) seemed to be performed by both ABC-type transporters (mainly complex CysAWTP involved in sulfate/thiosulfate import), already identified in *E. Coli* [63], and putative sulfate permeases (some of them Trk-type). Following uptake, the intracellular sulphate activation would be dominated by sulphate adenylyltransferase in its heteromeric form (SAT1 + 2, CysDN), already studied in *E. Coli* by Kredich [63], and, in a lesser extent, by its homomeric form (DSAT, dissimilatory-type) already described in *Bacillus subtilis* [64] for dissimilatory sulfate reduction [65]. For the reduction of the activated sulfate, both adenylylsulfate kinases and phosphoadenylyl-sulfate reductases (thioredoxin) [66] were also present in significant proportion in *Rubrobacter* and *Cyanothece*, among others, and *Symbiobacterium* and *Nostoc*, among others, respectively. Interestingly, the final conversion of sulfite to sulfide may mainly be mediated by a homomeric form of sulfite reductase (hemoprotein subunit) and ferredoxin-sulfite reductases, in a minor extend, as high abundance of ferredoxins were identified in all the samples, most of them coming from *Anabaena, Conexibacter*, and *Rubrobacter* representatives. In such cases, results suggested that electrons for sulfite reductases would be derived either from the photosystem I or, in non-photosynthetic organisms from NADPH [67]. Alternatively, a significant proportion of microorganisms belonging to taxonomic groups of *Thermus* and *Nitrosomonas* would present the typical heteromeric form of sulphite reductases (SIR Flavoprotein + Hemoprotein, CysIJ), using NADPH directly as an electron donor [63]. Additionally, 3’phosphoadenylylsulfate (PAPS) produced by the action of phosphoadenylyl-sulfate reductases may be converted to adenosine-3’,5’-bisphosphate (PAP) via 3'(2'),5'-bisphosphate nucleotidase and be incorporated into carbon backbone of sulfur-containing aminoacids [68]. Unfortunately, no read was recovered to any taxonomy group after binning.

The relative abundance of sulfite oxidase (SO) besides reported the presence of sulfur oxidation (SOX system) by *Roseiflexus, Streptomyces, Magnetospirillum*, and *Nostoc* representatives, along with others. Sulfite dehydrogenases were also identified but in low proportions in some proteobacteria belonging to orders *Rhizobiales* and *Burkholderiales*.

Sulfur-reducing bacteria such as *Anabaena, Conexibacter*, *Rubrobacter*, and others, exhibited large amounts of flavodoxin reductases (ferredoxin-NADPH reductases) family 1 and significant ones of ferredoxin reductases. Members of *Mesorhizobium* and *Acidiphilium*, among others, also presented reads assigned to dimethyl sulfoxide (DMSO) reductases, Finally, thiosulfate reductase (TR) were also identified in *Sphingopyxis, Rhizobium, Anabaena, Bradyrhizobium*, *Nostoc*, and so forth.

Iron Cycling

2,087.3 ± 415.3 reads were assigned to the category “Iron acquisition and metabolism”. Out of them, 34.7% fell in line with “*Campylobacter* iron metabolism” category followed by “siderophores” (18.6%), “Heme, hemin uptake and utilisation systems in Gram negatives” (15.4%), “Iron acquisition in *Vibrio*” (13.0%), and “Heme, hemin uptake and utilisation systems in Gram positives” (8.8%). At taxonomic level, proteobacteria (45.7%) followed by actinobacteria (28.3%) were the major groups observing, in correlation, a predominance of *Rubrobacter* (*Actinobacteria*; 10.6%), *Pseudomonas* (*Proteobacteria*; 7.4%), *Caulobacter* (*Proteobacteria*; 5.8%), and *Conexibacter* (*Actinobacteria*; 4.7%) representatives (Fig. 2e). Random Forest besides indicated differences in the abundance pattern of some minor bacterial components such as *Methylibium* and *Gloeobacter* and, in a lesser extent, *Hyphomonas*, among others (Supplementary Fig. S5h).

The main iron uptake via in these samples seemed to be the synthesis and release of siderophores (18.6 %), 35.3 % coming from representatives of the genus *Pseudomonas* followed by *Cyanothece* (7.4 %), *Phenylobacterium* (7.4 %), and *Anabaena* (6.7 %), among others. Interestingly, over 40 % of those siderophore-assigned reads were identified as pyoverdine type.

As expected, Gram-negative and Gram-postive bacteria showed a predominance of reads assigned to ferric siderophore transport systems and SA14-24 Two-component regulatory system, respectively. A significant amount of heme oxigenaseswere also observed (Supplementary Table S5). Furthermore, it was noteworthy the abundant presence of ferric iron ABC transporters allocated in the category *“Campylobacter* Iron Metabolism” in spite of the fact that no reads were assigned to *Campylobacter* in significant number and those mainly derived from non-phylogenetically related genera such as *Rubrobacter, Conexibacter, Rhodococcus* and *Nostoc*, among others.

Potassium cycling

2,181.3 ± 259.4 reads mainly derived from *Rubrobacter* (10.8 %), *Caulobacter* (5.6 %), and *Nostoc* (4.5 %), along with others, were identified as part of potassium metabolism (Fig. 2f). Random Forest besides indicated differences in the abundance pattern of some minor bacterial components such as *Thermomonospora, Deinococcus* and *Chromobacterium* and, in a lesser extent, *Methylobacterium and Catenulispora*, among others (Supplementary Fig. S5i).

Out of them, 85.6 % were assigned to the “K homeostasis” category. Minor categories displayed “hyperosmotic K uptake” (5.0 %) and “K-efflux systems” (9.3 %).

In samples, the K uptake seems to be mainly performed by the K transporters Kup (it transports K^+ with low affinity) (376.3 ± 43.0 reads) that were mainly identified in reads associated with proteobacterial groups *Caulobacter* and *Sphingopyxis* and bacteroidetes *Dyadobacter, Cytophaga* and *Flavobacterium*, among others. It was closely followed by Kdp transporters (high affinity and specificity) (KdpE 128.0 ± 17.0 reads; KdpD 208.2 ± 30.4 reads), mostly affiliated to *Rubrobacter, Nostoc, Conexibacter*, *Caulobacter*, and others, and potassium channels (176.7 ± 22.1 reads), found in reads assigned to *Rubrobacter*, *Anabaena*, and so forth (all classified in "K homeostasis" category by SEED subsystems). Reads associated with the Trk system were absent.

Discussion

Spatial distribution of the microbial communities in Tabernas desert

Metagenomic analysis carried out in this study showed a high taxonomic and functional similarity in both incipient and mature cyanobacterial biocrusts types. Possibly the identical habitat-specific characteristics such as geological material, soil type, climate, surrounding vegetation, and topography under which samples were collected as could influence their taxonomic and functional composition. Moreover, only SOC showed significant differences between IC and MC samples, but the rest soil parameters, such as pH, nitrogen content and salinity level, considered the most important factors driving the structure of soil bacteria communities [15, 16, 17, 18, 19, 20, 12, 21, 22], did not show significant differences between IC and MC samples (Supplementary Table S2). Even though uniformity in physico-chemical soil parameters may explain the absence of significant differences at the functional and taxonomic level, β-diversity index indicated a certain differentiation between the two biocrusts types (Supplementary Fig. S3) and α-diversity indices were slightly higher in MC biocrusts samples (Supplementary Fig. S2). It may be justified regarding the higher percentage of cyanobacteria representatives in MC contributing to a greater amount of organic matter, nitrogen, etc. (Supplementary Table S2) and the comparatively higher diversity observed. Nevertheless, the inherent variability associated with metagenomic sampling and experimental design limitations regarding the optimal number of replicated sample set [69, 70] should also be a strong argument to consider in this case.

At phyla level the predominant taxonomic groups detected in both IC and MC metagenomes from Tabernas desert were *Proteobacteria* and *Actinobacteria*, followed by *Cyanobacteria*. Other authors already showed that the most ubiquitous phyla in desert soils from across the world are *Actinobacteria* and *Proteobacteria* but also *Bacteroidetes* [71, 72, 73, 74, 12]. Predominance of actinomycetes such as *Conexibacter, Rubrobacter, Streptomyces, Mycobacterium, Nocardioids*, and *Geodermatophilus* in arid environments, namely Tabernas Desert, is not fortuitous. Morphological and molecular features including sporulation, pigmentation, metabolic (and degradative) versatility, secondary metabolite synthesis, and presence of multiple DNA repair mechanisms [75, 76, 77, 78, 79], among others would promote adaptative advantages to harsh conditions over other taxonomic groups [80, 81]. Despite, an extensive

proteobacterial community was present in both IC and MC samples at similar levels than actinobacterial representatives. Proteobacteria have been identified as a phyla globally distributed in the world and prominent members in desert [73, 82, 83, 84, 85, 12]. In arid and semiarid areas, the high temperature and intermediary soil moisture favour the high organic carbon mineralization leading to a rapid turnover of the organic matter [86, 87]. It was demonstrated to contribute to the growth of copiotrophic bacteria such as proteobacteria [88, 89]. Moreover, the presence of proteobacteria has been suggested as functionally important in nutrient-limited arid environments as will be discussed later. Cyanobacteria was the third most abundant phylum in the samples, being comparatively greater in MC than in IC biocrusts (Fig, 1). In Tabernas desert, cyanobacteria are spatially distributed in areas with the highest global and direct radiation levels [43, 10], developing numerous adaptation strategies against UV irradiation, drying and excess salts such as production of saccharides, amino acis, MAAs [10], carotenes, scytonemin and oxalates [11] and accumulation of compatible solutes as sucrose [6] along with an effective chemotaxis [90] that would enable them a significant adaptive advantage over others phyla.

Regardless of *Bacteroidetes* is also considered a common phylum in desert soils since they often show optimum growth at basic pH which is consistent with alkaline character of desert soils [15, 85], and specifically in Tabernas desert (Supplementary Table S2), curiously, the abundance of this phylum in both metagenome biocrusts was much lower than that detected by [12] in cyanobacterial biocrusts from Tabernas desert studied by amplicon sequencing. Temporal and spatial heterogeneity influencing community composition [91] along to limitations and biases common to all molecular techniques, differences in taxonomic specificity between amplicon and shotgun sequencing approaches [92] and others associated with the use of different assembly and binning tools [93] and databases may nevertheless explain our contradictory results when compared with those previously reported. Due to the copiotrophic character of members of this phylum [94] was hypothesized that their presence may be due to carbohydrate and polyphenol soil enrichment produced by cyanobacteria [95, 96, 6]. On the other hand, the fact that the phylum *Cyanobacteria* detected is also much lower (Fig. 1a) would produce a lower secretion of labile organic matter usable by representatives of the phylum *Bacteroidetes*, reducing the proliferation of bacteria belonging to this phylum.

Functionally, microbial communities associated with biocrust types presented similar composition showing, according to COGs classification, as main functional categories those related to “General functions”, “Amino acid transport and metabolism” and “Energy production and conversion”, mainly due the high number of permeases and metalloenzymes such as dehydrogenases involved in the amino acids and other solute active transport [97] and the oxidation of hydrogen gas coupled to the energy-conserving reduction of electron acceptors and maintenance of intracellular pH and redox potential [98, 99], respectively. Predominance in permeases and dehydrogenases was already observed by other authors in metagenomic studies carried out on Namib Desert [100].

Taxonomic groups and functional genes involved in the carbon cycling

The metagenome of the biocrusts showed several bacterial and cyanobacterial communities developing an essential role in the carbon cycle. An extensive bibliography shows that biocrusts are one of the main sources of soil organic carbon in drylands [101, 95, 96, 102, 6, 5, 103]. Our results showed that the dominant processes of carbon cycle in the cyanobacteria-dominated biocrusts was the carbohydrates metabolism, fatty acids, lipids and isoprenoids metabolism and soil respiration. The three carbon cycle processes are closely related since the production in the biocrusts of carbohydrates, fatty acids, lipids and isoprenoids would stimulate the growth of mineralizing soil bacteria and, therefore, the soil respiration processes. In this sense, cyanobacteria from Tabernas desert synthesize carbohydrate, specifically saccharides as sucrose, maltose and glucose, amino acids (L-phenylalanine, L-tyrosine, and L-triptophane) and mycosporine-like amino acid [6, 10]. The washing of these compounds to the underlying layers of the biocrusts favor the proliferation of diverse soil bacterial communities [21] involved in the degradation of carbohydrates by releasing extracellular enzymes [104]. The metagenomic results corroborated that the biocrusts were enriched in a hydrolytic enzymes pool such us *α*-glucosidases, *β*-glucosidases, *β*-galactosidase, which are involved in catalysing the hydrolysis and biodegradation of *α*-D-glucopyranosides, maltose and cellobiose and *β*-galactosidases, respectively, into monosaccharides [105] and α-amylases and glucoamylases which carry out the conversion of starch to low-molecular-weight products as maltose, glucose and maltotriose [106, 107, 108]. Chitinases and xylanases enzymes, observed in much less proportion in the samples, are involved in the degradation of hemicelluloses [109]. Curiously, cellulases and invertases enzymes were very scarce in the samples, despite [14] observed cellulase activity and an especially high invertase activity in cyanobacterial biocrusts at Tabernas desert. These differences could be due to changes in the microbial populations of the soils and, therefore, changes in their metabolism as consequence of the enormous spatial heterogeneity in the soils from arid deserts. Nevertheless, the abundance of genes encoding for hydrolytic enzymes would suggest that these bacterial communities may have significant biotechnological potential [110, 111].

The main function of the fatty acids, lipids and isoprenoids metabolism was “glycerol and glycerol-3-phospate uptake and utilization” suggesting the direct utilization of those compounds by soil microbial communities as lipid precursors or their dual use as carbon sources. Moreover, *sn*-Glycerol 3-phosphate (G3P), which is an essential intermediate in the biosynthesis of phospholipids, it is also used as a carbon source via the NADH-dependent reduction of glyceraldehyde 3-phosphate in *Escherichia coli* [112]. Other important function associated to this metabolic process was polyhydroxyalkanoic acid (PHA) synthases. PHA synthase is the key enzyme in the biosynthesis of PHAs a class of aliphatic polyesters which are generally regarded as a carbon and energy reserve material in bacteria and archaea [113]. The synthesis of extracellular lysophospholipases was minimal in the biocrusts at Tabernas desert in accordance to organic matter content observed in soil samples.

Another important role of the biocrusts in drylands is their strong influence on CO_2 fluxes [114, 115, 116, 117, 118, 4]. In fact, they are considered one of the most important photosynthetic biomass pools in the dryland regions, due to their high photosynthetic potential achieving net photosynthesis rates of 11.5

μmol CO_2 m^{-2} s^{-1} under optimal conditions [119]. Nevertheless, curiously our results showed a clear dominance of respiration over carbon fixation in microbial communities associated with both biocrusts types. Specifically, the microbial respiration in biocrusts mainly encompassed glycolysis, the tricarboxylic acid (TCA) cycle and oxidative phosphorylation, being the glucose the main substrate used. Entner-Doudoroff pathway was, besides, observed as alternative to glycolisis [120]. In addition, the glyoxylate cycle, a modification of the citric acid cycle bypassing the two decarboxylation steps serving for the assimilation of acetyl-CoA [121] was also identified. An alternative pathway for C2 compounds assimilation observed in C2-assimilating bacteria lacked isocitrate lyase activity [122], the called ethylmalonyl-CoA pathway of C2 assimilation [123], was also present in the metagenome of the samples. Biocrusts also had an important pool of enzymes associated to methanotrophic metabolism such as methane monooxygenase, methanol dehydrogenase, and formate dehydrogenase, characterized by its ability to utilize methane as a sole carbon and energy source [124]. Methane is the most stable carbon compound in anaerobic environments and is a very important intermediate in the reactions that eventually lead to the mineralisation of organic matter [125], and consequently CO_2 emission into the atmosphere. No reads assigned to formaldehyde dehydrogenase, which catalyses previous step to formate dehydrogenase [126], was present suggesting that microorganisms may be uptaking formate from environment or using different mechanisms to metabolise formaldehyde. Moreover, the carbon monoxide dehydrogenase, a bidirectional enzyme playing a key role in the carbon cycle as allows organisms to both make use of CO as a source of energy (CO oxidation) and utilise CO_2 as a source of carbon (CO_2 fixation) [127], were also found.

The predominance of the respiration over the photosynthesis processes shown in the metagenome of the biocrusts are parallel to the results found by [4] in which cyanobacterial biocrusts acted as CO_2 emission sources most of the year at Tabernas desert and Cabo de Gata (Southeast Spain). Respiration overshadowed photosynthesis in cyanobacteria-dominated biocrusts especially following rainfalls after the long dry summer period. The authors attributed these findings to the washing of organic matter (i.e., carbohydrates), from the biocrust to underlying soil microbial communities. This increase of easily mineralizable labile organic matter in underlying soils favoured the growth of heterotrophs communities with high oxidative capacity in cyanobacterial biocrusts and high abundance of very active organisms with high microbial and respiratory coefficients [13], which release enzymes enabling faster degradation of organic remains [14] and in turn favouring the soil respiration processes.

The metagenomic data from cyanobacteria-dominated biocrusts corroborated a predominance of bacterial taxa releasing hydrolytic enzymes which play a key role in the “carbohydrates metabolism” (i.e. *Rubrobacter, Nostoc*, *Caulobacter, Deinococcus*, *Spirosoma, Rhodopseudomonas, Rhodospirillum*, *Opitutus* and *Xanthomonas*), as well as some bacterial taxa involved in several functions in the “fatty acids, lipids, and isoprenoids metabolism” (i.e. *Caulobacter*, *Salinispora, Rubrobacter, Roseiflexus, Thermobaculum, Bacillus* and *Acidiphilium*) and “respiration process” (i.e. *Caulobacter*, *Rubrobacter, Conexibacter, Frankia* and *Streptomyces*, among others). Specifically, *Rubrobacter, Conexibacter* and *Frankia* intervened in various functions related to the respiration processes (i.e. Entner-Doudoroff

pathway, glyoxylate cycle, ethylmalonyl-CoA pathway, and production of carbon monoxide dehydrogenases). Therefore, it is hypothezised that these soil bacterial communities would develop a vital role in the decay of organic residues, transformation of native soil organic matter, mineralisation of nutrients available for plants and soil aggregation [128]. In turn, the mineralization of the organic matter and respiration of soil heterotrophic communities are indirectly favoured by changes in the local conditions of the soils due to the biocrusts which contribute to increase the content of nutrients (organic matter, nitrogen ...) [116, 102], and the availability and duration of water content in the first centimetres of their underlying soil [129].

Nevertheless, despite the metagenome of the samples have shown that the soil bacteria pool in cyanobacterial biocrusts at Tabernas desert is performing an essential function in the mineralization of soil organic matter and respiration processes, causing CO_2 emissions to the atmosphere; the soil bacteria play also an essential role in CO_2 fixation and, therefore, in the potential role of soils as CO_2 sinks. Miralles et al. [4], showed that CO_2 fixation overshadowed C lost in different biocrusts types from different semiarid zones, being the Tabernas desert one of them. The C gain from different biocrusts types only happened in winter, after long hydration periods with high available soil moisture and mild temperatures. The capacity of CO_2 fixation was attributed mainly to cyanobacteria and lichens dominating the biocrusts by through photosynthesis. Nevertheless, our results have shown an important diversity of bacterial communities, more abundant in soils than cyanobacterial communities, whose role in CO_2 fixation has been unknown or hardly studied. Carbon fixation or assimilation, consider as the conversion of carbon dioxide to organic compounds by autotrophic organisms, is one of the most important drivers in soil carbon cycle [130]. Among the alternative pathways in CO_2 fixation dominating in all biocrusts samples, the most important process was the Calvin-Benson-Bassam (CBB) cycle, where carboxylation is performed by ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) [131]. Nevertheless, other two crucial processes in these soils for CO_2 fixation were the photorespiration via oxidative C2 cycle, which is a crucial process for the survival of oxygenic phototrophs in environments favouring the oxygenation reaction of Rubisco [132] and carboxysome synthesis, the protein macrostructures developed by cyanobacteria to contain Rubisco at high CO_2 concentrations at cell in order to enhance the efficiency of carbon fixation [133]. Autotrophic metabolism was recovered from a wide and taxonomically diverse group of microorganisms in the biocrusts highlighting *Rubrobacter, Conexibacter*, *Caulobacter*, *Sphingomonas*, *Geodermatophilus, Streptomyces* and *Bradyrhizobium* and the cyanobacterial *Nostoc*, *Cyanothece*, and *Anabaena*.

Another critical process in carbon cycle in the soils from Tabernas desert was the photosynthetic metabolism (photosynthesis and phototrophy), process essential to convert light energy into chemical energy. The principal molecular mechanisms in bacteria and archaea for harvesting light energy are: chlorophyll-based photosynthesis and microbial rhodopsins [134]. Miralles et al. [4] showed that the photosynthesis exceeded the loss of C by respiration in the biocrusts at Tabernas desert at sporadic times throughout the year depending on the interplay of several environmental variables (such as rainfall events, frequency of rainfall, soil moisture, air humidity, temperature, PAR and soil type) and their

successional stages. Moreover, the cyanobacteria are able to maintain its photosynthetic activity along the day [101], because they have developed efficient mechanisms to survive under limited water availability [135]. Among these mechanisms are included the synthesis of compounds such as saccharides (maltose, glucose, or both), carotenes and scytonemin in cyanobacteria-dominated biocrusts from Tabernas desert [10, 11], which could have an important role protecting them against desiccation and UV-irradiation [136, 137, 138, 139]. Therefore, cyanobacteria are poorly sensitive to photo-inhibition [140]. In addition, they are also able to migrate through the soil profile to protect themselves from severe desiccation on the soil surface and find water in the lower soil layers [90]. Our results have shown that the photosynthetic metabolism was, as expected, mainly identified in representatives of the phylum Cyanobacteria (i.e., *Nostoc*, *Cyanothece*, *Synechococcus*, *Thermosynechococcus*, *Anabaena*, *Trichodesmium*), although significant proportions were also found in *Proteobacteria* and *Actinobacteria*. The previous mentioned taxonomic groups of cyanobacteria presented the major number of reads assigned to photosystems I and II, as it is known that only cyanobacteria within the domain *Bacteria* contain two photosystems operating in sequence and are able to perform oxygenic photosynthesis [141]. On the other hand, photosystem II-type photosynthetic reaction center were identified coming from *Proteobacteria* (i.e., *Rhizobiales, Rhodospirillales*, and *Rhodobacterales*).

Apart from chlorophyll-based phototrophs, the biocrusts at Tabernas desert use other much simpler mechanism for the conversion of light into chemical energy, such us the synthesis of retinal component of proteorhodopsins. Retinal-binding membrane proteins perform the function of light-gated ion channels or light sensors in photodetection processes as well as the function of ion pumps in light energy conversion processes [142]. In the samples, those were mainly derived from *Bradyrhizobium, Streptomyces, Nostoc, Rhodopirellula* and *Sphaerobacter*, among others from diverse phyla. Although mostly proteorhodopsins were identified in yet uncultured marine bacteria [143], some studies already reported their presence in phyllospheres of terrestrial plants [144], in soil crust [145] and in Antarctic Dry Valley edaphic systems [146], and, recently, from cultivate *Geodermatophilaceae* representatives, bacteria isolated from extremely hot and arid environments [147]. Nevertheless, the almost absence of genes involved in the synthesis of the opsin apoprotein suggests that those may probably be involved in the synthesis of bacterial pigments such β-carotene [148] or in synthesis of the side chain of isoprenoid quinones involved in respiratory electron transport chains [149] instead. Finally, Cyanobacteria, mostly *Nostoc* representatives showed another alternative light-harvesting system to capture sunlight based on phycobilisome (PBS), integral pigmented membrane polypeptides (phycobiliproteins) that were already reported as the main-harvesting antenna in cyanobacteria [150].

Taxonomic groups and functional genes involved in the nitrogen cycling

Nitrogen (N) is a basic element for life and a crucial factor limiting terrestrial ecosystems productivity [151]. Its availability in soils largely depends on the rates of the microorganisms driving ammonification/mineralisation, nitrification, denitrification, atmospheric nitrogen fixation and ammonium update [152]. The metagenome of IC and MC biocrusts showed that the highest number of reads

assigned in the N cycle was “ammonia assimilation”, follow by “Nitrate and nitrite ammonification”, suggesting that the microbial communities associated to biocrusts presented a preferential uptake of ammonium over nitrate. It is in line with what was found by Azam et al. [153] as, energetically, NH_4^+ is a more favourable N source as it does not require to be reduced to be incorporated into organic compounds. These results corroborate the findings of Miralles et al. [13] who showed a predominance of ammonification in the biocrusts from Tabernas desert and nitrification in the deeper soils underlying. Specifically, the percentage of NH_4^+-N in the biocrust was higher than 40% of the initial inorganic nitrogen and, in turn, the net mineralization was higher in cyanobacterial biocrusts than in other lichen biocrusts types. Our metagenome results therefore suggest that there is a greater abundance of ammonifying organisms concentrated in the biocrusts than nitrifying ones as has already been suggested by other authors [90, 154, 13]. In fact, Pacchioni et al. [36] found nitrate and nitrite ammonification and ammonia assimilation as the most represent functions of the nitrogen cycle in soils subjected to an important environmental stress (strong insolation, prolonged drought, etc.) suggesting that these processes were possibly related to organic matter decomposition and mineralization [155]. In the metagenome of IC and MC biocrusts at Tabernas desert, actinobacteria, and specifically, the genera *Rubrobacter* and *Conexibacter*, among others, followed by proteobacteria may therefore be dominating the microbial uptake of ammonium and it would be facilitated by ammonium transporters (Amt), a class of membrane-integral transport proteins [156]. The predominance of ammonification in the samples could also be interpreted as an economic strategy to avoid the high consumption of ATP during nitrification or during the biosynthesis of proteins throughout the intracellular reduction of nitrate to ammonium [157, 13]. The metagenome of the samples showed that Glutamine synthetase-glutamate synthase (GS-GOGAT) pathway was the main via driving ammonium assimilation. The GS-GOGAT pathway requires ATP but has higher affinity towards ammonium being prevalent under nitrogen limitant conditions [158]. The loss of nitrate via denitrification processes [159] could also explain the predominance of ammonium in the biocrusts from Tabernas desert. The metagenome of the samples showed that denitrification (also called “nitrate reduction” or “nitrate and nitrite ammonification” by SEED subsystems database) was another important subcategory in nitrogen metabolism of these cyanobacterial biocrust communities. Both assimilatory and dissimilatory nitrate reduction were identified in these samples although with significant differences in abundance.

Assimilatory nitrate reduction (ANR) is one of the main processes in the nitrogen cycle in which nitrate (NO_3^-) is used as a nitrogen source for the growth of new cells [152]. In soil bacterial communities at Tabernas desert, NO_3^- is incorporated into the cells by a high affinity ATP-dependent nitrate ABC transporter and also by specific nitrate/nitrite transporters, being responsible of these processes mainly cyanobacterial representatives such as *Anabaena*, *Nostoc, Actinosynnema* and *Cyanothece* and some actinobacteria such as *Frankia*. Moreover, the NO_3^- is reduced to nitrite (NO_2^-) in the samples by the cytoplasmatic ferredoxin dependent enzyme nitrate reductase (Nas), which is then further reduced to ammonium (NH_4^+) by cytoplasmatic ferredoxin dependent nitrite reductase (NiR) (NAD(P)H) and incorporated into the carbon skeletons via GD-GOGAT. Different sets of bacterial communities carried out

each of the processes reducing nitrates to nitrites (i.e., *Nostoc, Anabaena*, *Mycobacterium, Streptomyces*, *Saccharomonospora*, among others), and after that ammonium (i.e., *Cytophaga*, *Kineococcus, Mycobacterium, Frankia* and *Rhodococcus*).

On the other hand, a minimum number of reads derived from *Methylobacterium* and *Rubrobacter*, mostly, and misplaced by SEED subsystem in the category nitrate and nitrite ammonification were just identified as transmembrane “respiratory nitrate reductases” (Nar) and “periplasmic nitrate reductases” (Nap), the other two prokaryotic nitrate reductases belonging to the dimethyl sulfoxide (DMSO) reductase family of molybdoenzymes, which are more associated with denitrification and anaerobic nitrate respiration, although the nitrite generated could alternatively be used as a nitrogen source depending on the organism [151]. The presence of some reads identified as nitrous oxide reductase, next step in the conversion of N_2O_2 to N_2O, would be suggested that N_2O_2 could be available in the environment for representatives of the genera *Maricaulis, Salinispora* and *Albidiferax*, among others.

Fixation of atmospheric nitrogen by microorganisms is the most important natural process for increasing the nitrogen content of soils [152]. Nevertheless, despite cyanobacteria are considered an important source of nitrogen fixation [160, 161, 9], the metagenome of the biocrusts samples showed a low number of reads associated with nitrogen fixation. The processes driving nitrogen fixation in the samples were “nitrogenase (molybdenum-iron)-specific transcriptional regulator NifA”, and insignificant the number of reads annotated as part of “nitrogenase (molybdenum-iron)”, the most common nitrogenase isoenzyme presented by all diazotrophs [162], distributed among the genera *Nostoc, Cyanothece* and *Rhodopseudomonas*, among others. Unexpectedly, no read was assigned to *Frankia* or *Bradyrhizobium*, previously presented as prevalent groups in carbon metabolism. It could be due to the effective absence of the target gene, something already observed by Nouioui et al. [163] in the type strains of Frankia inefficax or to limitations and biases associated with the experimental design as previously commented.

Minor components of cyanobacteria (*Synechococcus* and *Nostoc*, among others), proteobacteria (*Bradyrhizobium*) and actinobacteria (*Mycobacterium*) were also involved in “transport (cyanate ABC transporter) and hydrolysis (cyanate hydratase) of cyanate”, which is a small molecule containing carbon, nitrogen and oxygen atoms derived from urea and carbamoylphosphate [164]. Kamennaya et al. [165] already reported the use of cyanate as a nitrogen source for the growth of certain marine cyanobacteria under nitrogen limitation. In other bacteria and archaea the presence of cyanases, apart from the role in nitrogen assimilation, was also associated with detoxification as cyanate chemically modifies proteins via carbamylation [166] and, recently, with the use of this compound as sources of energy and reductant for growth by nitrifiers [167].

The metagenome of cyanobacterial biocrusts showed some bacterial taxa implicated in the “utilisation of allatoin”, which is a compound possibly sustained in this environment by the decomposition of surrounding plant tissues, the excretion of ureide-enriched root exudates [168], and animal urinary excretions. In these samples, the “catabolism of allantoate to NH_3” looks to proceed following the conversion of (S)-allantoate to (S)-ureidoglycolate by the Mn_2^+-dependent enzyme allantoicase by

representatives of the genera *Rubrobacter, Sphaerobacter, Desulfitobacterium* and *Geodermatophilus*, among others, to be finally hydrolyzed to glyoxylate and NH_4^+ in a reaction catalyzed by the enzyme ureidoglycolate hydrolase in or to glyoxylate and $2NH_3$ via the enzymatic reaction catalyzed by the ureidoglycolate amidohydrolase [169]. Werner al. [170] already indicated that the presence of the enzyme NAD(P)+-dependent ureidoglycolate dehydrogenase may oxidise ureidoglycolate to oxalurate, allowing urease-negative microorganisms to preserve N and energy resources more efficiently. Finally, the NH_4^+ produced may be broken down to NH_3 in either one-step reaction via urease as described in some rhizobiales bacteria [171] or in a two-step reaction catalysed by the biotindependent enzyme urea amidolyase (UAL) complex comprised by the enzymes urea carboxylase and the upregulated gene msmeg_2189 encoded allophanate [172].

Unexpectedly, some soil microbial communities including *Anabaena, Cyanothece, Alcanivorax, Anaeromyxobacter, Frankia, Saccharopolyspora, Geodermatophilus* and *Neurospora* presented extracellular enzyme urease (“urea amidohydrolase”), involved in the hydrolysis of urea into carbon dioxide and ammonia [173] and frequently used as an indicators of the health of microbial communities [174].

Taxonomic groups and functional genes involved in the phosphorus cycling

In dryland regions, phosphorus inputs into soils are mainly coming from the deposition of atmospheric dust and, in a lesser extent, from weathering of parent material [175] being biocrusts one of the major factors controlling P retention at surface and bioavailability [176]. Biocrust microorganisms, therefore, play a key role in the solubilisation of insoluble inorganic (mineral) phosphorus [177] and in mineralisation of insoluble organic phosphorus. Phylogenetically, predominant members involved in the phosphorus cycling in biocrusts samples from Tabernas desert were allocated into the phyla *Actinobacteria*, followed by *Proteobacteria*, and *Cyanobacteria*. At genus level, there was a predominance of actinobacteria such as *Conexibacter*, *Rubrobacter*, *Caulobacter* (*Proteobacteria*), and *Salinispora*. The major mechanism of soil P solubilisation is lowering of soil pH by microbial production of organic acids or the release of protons [33, 177]. It was widely reported that these organic acids are the product of microbial central metabolism, mostly by oxidative respiration [178], and type, amount and, therefore, efficiency of solubilisation differ from one organism to other [179] and is dependent of soil characteristics and environmental conditions. The data obtained by Miralles et al. [13] supported this classical view of the P cycle in which the hydrolysis of primary calcium phosphates (usually present in the geological substrate) could be favoured by acidification at the surface, induced by the organisms forming the biocrusts from Tabernas desert. It, therefore, makes sense, phylogenetic correlations observed between carbon and phosphorus cycling regarding major microbial groups (*Rubrobacter*, *Conexibacter* and *Caulobacter*, among others) in biocrusts samples from Tabernas desert. As drivers in the carbon metabolism, those may presumably have a principal role in P cycles by the production of organics acids naturally obtained in the form of intermediates, primarily citric, gluconic, 2-ketogluconic,

malic, fumaric, and, succinic acids, among others, derived from microbial metabolism (i.e. TCA, ...). Chelating substances and inorganic acids such as sulphideric, nitric, and carbonic acid are considered to be like other mechanisms for phosphate solubilisation [180].

Regarding organic phosphorus mineralisation, as expected for these samples and in correlation with soil analysis in organic carbon, no read was assigned as phytases, extracellular acid phosphatases involved in the release of phosphorus from organic materials (phytates or inositol phosphate) in soil [181]. Nevertheless, a significant number of reads mainly derived from *Rhodoseudomonas* (Proteobacteria), *Synechococcus* (Cyanobacteria) and *Sphingopyxis* (Proteobacteria), among others, were identified as extracellular alkaline phosphatases, enzymes liberating P by hydrolysis [182]. Miralles et al. [10] showed low enrichment ratio values for the phosphomonoesterase activity in biocrusts at Tabernas desert, suggesting a protective mechanism in that ecosystem, as the scarce hydrolysis of the phosphate esters present in the organic remains could contribute to an increasingly P-rich soil organic matter. On the other hand, the taxonomic groups of *Pseudomonas*, *Methylobacterium, Salinispora, Mesorhizobium, Burkholderia*, and *Sinorhizobium*, among others, were assigned to the subsystem category "Alkylphosphonate utilisation". Organophosphonates encompass a group of compounds of biogenic and xenobiotic origins that are characterised by possession of a direct carbon-phosphorus bond and are usually used by microbes as the sole phosphorus source under conditions of phosphorus limitation [183].

The transport of phosphorus sources is essential for the growth of all living organisms. Organic compounds such as the mentioned phosphonates can enter intact cell while others are required to be hydrolysed before being transported [184]. For the transport of inorganic phosphate (Pi), the preferred source of phosphorus in soil microorganisms [185], specialised transport system has been developed. In the samples from Tabernas desert, two different systems were identified for the Pi transport: The high-affinity phosphate-specific transporter (Pst) regulated by PHO regulon mainly derived from *Caulobacter* and *Roseiflexus* but being also present *Dehalococcoides*, *Nostoc*, and *Sphingomonas* in good proportions, among others, a system induced by phosphate-starvation [186]; and a "probable" low-affinity phosphate inorganic transporter (Pit), identified mainly in reads associated with *Conexibacter, Nostoc, Anabaena*, and *Magnaporthe* (Ascomycota), among others.

Taxonomic groups and functional genes involved in the sulfur cycling

Sulfur, in its available form SO_4^{-2}, is an essential element for the growth of organisms but a limiting nutrient in low organic matter and eroded soil areas [187], such as drylands, because of the mobile nature of sulphate ions and the limited S that can be mineralised in such soils [188]. The sulfur cycle includes oxidation and reduction reactions that are principally performed by soil microorganisms [189]. Like carbon, nitrogen and phosphorus, our results showed that a diverse group of microbes, predominantly actinobacteria, may lead sulfur assimilation in either mineral and organic form in cyanobacterial biocrusts at the Tabernas Desert. In particular, over 50% of reads were designated to the category "inorganic sulfur assimilation" in spite of the fact that inorganic species of sulfur were already postulated

to be less prevalent in terrestrial habitats [190, 191] suggesting that reads numbers obtained possibly due to the complexity of metabolic pathways involved.

In soil environments most of the available organic sulfur occurs as organosulphonates or organosulphate esters, being particularly abundant taurine (2-aminoethanesulfonate) that can be used by many bacteria as a source of cell carbon, sulfur or nitrogen[192].Therefore, it is not surprising that the main assimilation via showed in our samples was “alkanesulfonate monooxygenase system (SsuD) and, taurine and glutathione utilisation”, all three involved in the acquisition of sulfur from organosulfonated compounds during limiting sulfur conditions [193, 194, 195]; and the major taxonomic group *Bradyrhizobium* as sulfur molecules are critical components of nitrogenase and genes involved in organic sulfur assimilation are highly expressed during plant nodulation (symbiotic nitrogen fixation) [196]. On the other hand, *Rubrobacter* representatives may be driving ester sulfate mineralization by the presence of extracellular arylsulfatases (ARS). ARS catalyzes a key step for soil S availability [197], and therefore, has potential to be used to assess the turnover and cycling of S in soil [198] and predicts changes in response to global environmental changes such as land use change or global warming as its activity is affected by soil organic matter, soil pH, soil texture, microbial biomass carbon and nitrogen [199].

Regardless many soil bacteria are not only able to utilize these organically bound forms of sulfur for gaining cell biomass, but can use the carbon skeleton of these compounds as growth substrate plant [200]. Relative amounts of reads identified as sulfite oxidising enzymes (sulfite oxidases (SOX system) and sulfite dehydrogenases), which play a key role in cell detoxification by sulfite and sulfur dioxide [201] and are one of the most energy-yielding reactions in sulfur-oxidising bacteria [202], were identified in phototrophic and lithoautotrophic bacteria such as *Roseiflexu*s and *Nostoc* and *Magnetospirillum*, respectively. Besides, some reads were also assigned to orders (*Rhizobiales and Burkholderiales*) typically characterized as N-fixing bacteria suggesting that their ability to grow using inorganic sulfur may be related to their survival in biocrusts in the absence of suitable host plants as previously proposed for *Sinohrizobium melitoti* by Wilson & Kappler [200].

At the same time, in cyanobacterial biocrusts at the Tabernas Desert, assimilation may compete with dissimilation (use of sulfate or sulfur as the terminal electron acceptor of the respiratory chain) by the presence of a physiologically and phylogenetically diverse group of anaerobic bacteria (Sulfate-reducing microorganisms) including *Anabaena, Conexibacter* and *Rubrobacter* that exhibited large amounts of flavodoxin reductases (ferredoxin-NADPH reductases) family 1 and significant ones of ferredoxin reductases allowing the use of flavodoxin and ferrefoxin as terminal electron acceptors.

In the same way, *Mesorhizobium* and *Acidiphilium representatives* may also be able to anaerobically respire using dimethyl sulfoxide (DMSO) reductases, a molybdoenzyme using DMSO as the terminal electron acceptor already described in *E. coli* [203]. Finally, thiosulfate reductase (TR), a type of unusual enzymes containing a molybdopterin active-site cofactor able to catalise sulfur-sulfur bond cleavage in *Salmonella enterica* LT2 [204] were also identified in *Sphingopyxis, Rhizobium, Anabaena,*

Bradyrhizobium, and *Nostoc*, among others, allowing the utilisation of elemental sulfur as an electron acceptor.

Taxonomic groups and functional genes involved in the iron cycling

Iron is a major component of Earth's crust and substantial quantities are present in most soils [205]. In spite, due to geochemistry, its availability is limited under aerobic conditions and neutral pH [206]. Soil microbial communities have, therefore, developed different systems for iron uptake and homeostasis that, consequently, influence iron solubility and, in turn, the chemical equilibrium between the different Fe forms and, hence, the weathering of Fe-bearing mineral in soil [207]. The main strategy followed by microbes inhabiting cyanobacterial biocrusts in the Tabernas Desert seemed to be the synthesis and release of siderophores, low molecular-weight compounds able to bind Fe III at high affinity [208], mostly derived from representatives of the genus *Pseudomonas*, a well-known taxonomic group characterised by their capabilities in plant growth-promotion and biocontrol [209]. In correlation, over 40 % were assigned to pyoverdine type, the major siderophore produced by fluorescent pseudomonads [210] and found to be important for bacterial colonisation (biofilm development) and as a signal molecule associated with virulence factors and quorum sensing [211]. It would confer to *Pseudomonas* representatives not only of certain microbial specialisation within the cyanobacterial biocrust microbiome but also advantages on competitive dynamics in soil as drivers of ecological and evolutionary dynamics as well as an essential role in cooperation interactions in natural communities [212] as pyoverdine was already reported as a public good that can be shared among cells, and be exploited by siderophore non-producers being cheats [213]. The presence of an potential active microbial group in Fe uptake would therefore support what was previously suggested by other studies regarding the positive correlation between the presence of biocrusts in arid ecosystems and improvements in the bioavailability of soil metallic micronutrients such as Fe [214] as well as the potential role of biocrusts as modulators of the responses of soil metallic nutrients to altered temperature and rainfall regimes caused by climate change and, consequently, as preserving and protecting agents of metallic nutrients in dryland soils [215].

Furthermore, the predominance of reads assigned to ferric siderophore transport systems in Gram-negative bacteria suggests that ferri-siderophore complexes are taken up via outer membrane receptor proteins, mostly involved in Fe transport, by the energy-transducing TonB-ExbB-ExbD system [207]. Contrarily, in Gram-positives, ferri-siderophores seemed to be transported via SA14-24. Two-component regulatory system that may be implicated in iron-responses in a similar way that BasS-BasR is involved in *Escherichia coli* [216]. A significant amount of heme oxigenases, an enzyme cleaving haem group into $Fe^{2+,}$ biliverdin and CO [217] and involved in strong resistance to oxidative stress [218], were also observed suggesting the presence of very specialized mechanisms of adaptation to harsh conditions in microorganisms inhabiting cyanobacterial biocrusts.

Taxonomic groups and functional genes involved in the potassium cycling

Most of reads involved in the potassium cycling were assigned to “K homeostasis” category and derived from major taxonomic groups. It is in correlation with potassium being the most abundant intracellular cation because of its key role in maintaining turgid pressure of microbial cells [219] and, therefore, in bacterial osmoadaptation and pH regulation. Nevertheless, the significant amount of reads identified as part of K uptake systems also supported the central role in the natural K cycles solubilising potassium from insoluble mineral that soil microorganisms hold [220]. Just like the phosphorus cycling, the production of organic acids by saprophytic bacteria is considered to be the principal mechanism for solubilisation of mineral potassium in soil [180, 221]. Acidolysis presumably produced by representatives of *Caulobacter*, *Sphingopyxis*, *Dyadobacter, Cytophaga* and *Flavobacterium*, among others, would lead the conversion of insoluble K contained in phyllosilicates present in the collection site [222] in soluble form (K ions) increasing the availability of K to microorganisms and plants [223]. Then K ions would directly be transported via K^+ transporters Kup (formerly encoded by the single *trkD* gene [224]), a system having an affinity for K + similar to that of the Trk system (the major low-affinity K^+ uptake system at neutral and slightly alkaline pH [224]), but more efficient under acidic conditions [225]. To date none representative of *Caulobacter*, *Sphingopyxis*, *Dyadobacter, Cytophaga* and *Flavobacterium* genera has not been described as a silicate solubilising microorganism. Nevertheless, their unambiguously role in K + uptake in our samples as well as their saprophytic nature and the ability to work as potential plant growth-promoting bacteria of some of their representatives under harsh conditions [226, 227, 228] could suggest that those taxa may effectively and specifically be driving potassium availability in cyanobacterial biocrusts of the Tabernas Desert as potassium solubilising bacteria (KSB). They would therefore support the growth of both microbial and plant communities and, consequently, contribute to enhancing of the fertility status of soils.

However, the acidification does not seem to be the only mechanism able to improve mineral K uptake in these samples identifying others based on enhancing chelation of the cations bound to K [219] such as the presence of the high-affinity and specificity K^+ uptake system Kdp. In fact, the rates of Kup transporters over Kdp ones observed in our samples could suggest an ability in certain taxonomic groups (*Rubrobacter, Nostoc, Conexibacter* and *Caulobacter*, among others) characterised by their tolerance to oxidative stress and their capability to colonise deteriorated rocks [229, 230, 231] and frequently associated with plants [232, 233] to increase their cellular K^+ uptake at low concentrations [234]. It would contribute to a major survival under harsh conditions since potassium availability in soil may largely differ depending on soil nutritional status, mineral type, amount, and size, and environmental factors [219] and reveal a biotechnological potential as well adapted and efficient KSB in arid ecosystems.

Conclusions

The metagenome of the bacterial communities in both types of cyanobacterial biocrusts did not show significant differences at the taxonomic and functional level, possibly due to the identical environmental conditions of soil formation (lithology, vegetation, topography, time, and climate) colonised by the

biocrusts. The shotgun-based approach as well as experimental limitations could nevertheless also haved influenced the absence of significant differences between both types of biocrust.

Biocrust bacterial communities were mainly represented by the phyla *Actinobacteria, Proteobacteria* and *Cyanobacteria.* Their members, particularly *Rubrobacter, Conexibacter, Streptomyces, Frankia, Caulobacter, Mycobacterium* and cyanobacteria *Nostoc* and *Anabaena* resulted to be the predominant taxa inhabiting cyanobacterial biocrusts. Those showed a rich metabolic potential able to drive the C, N, P, S, Fe and K cycles in drylands. Such functional redundancy was here interpreted as a strategy to maintain the correct functioning of the soil biogeochemical cycles and, therefore, of the ecosystem, in general. However, the functional specialisation showed in some processes such as photosynthesis (i.e. cyanobacteria), siderophore production (i.e. pseudomonads), and those observed in metanogenic and sufate-reducing bacteria, among others, denoted a type of trophic coordination or synthrophic growth (public goods). It would allow the survival of the microbial community under a range of environmental conditions.

The C cycle seemed to be the most important in the metagenome of biocrusts, showing a high diversity in metabolic pathways. The most important processes in the carbon cycle were carbohydrate metabolism, favouring the degradation of carbohydrates by the release of enzymes such as *α*-glucosidases, *β*-glucosidases, *β*-galactosidase, α-amylases glucoamylases, chitinases and xylanases. The following processes according to the abundance of reads assigned were respiration, fatty acids, lipids, and isoprenoids metabolism, CO_2 fixation and photosynthesis, observing a clear dominance of respiration over carbon fixation processes. Despite, it was not possible to hypothesise from these metagenomic data the potential role of microbial communities as a CO_2 sink or source. Additional studies should be carried out to identify the metabolic pathways being expressed in desert soils colonised by cyanobacterial biocrusts. Biocruts showed also a diverse group of microorganisms hardly studied as the responsible of CO_2 fixation processes in the ecosystem.

In the N cycle the key process was “ammonia assimilation” followed by the subcategory “Nitrate and nitrite ammonification”. It suggested that microbial communities associated with cyanobacterial biocrusts presented a preferential uptake of ammonium over nitrate as a strategy to avoid the high consumption of ATP. Nevertheless, the unexpected low number of reads assigned to "Nitrogen fixation" revealed further limitations and biases associated with the experimental design. A potential absence of target genes in diazotrophs was also hypothesised.

Biocrust microorganisms, in particular actinobacterial members, also demonstrated to play a key role in the solubilisation of insoluble inorganic phosphorus, potassium and iron. The major mechanism of solubilisation for P and K seemed to be the production of organic acids derived from the central metabolism. Phylogenetic correlations between carbon and phosphorus and potassium cyclings supported this hypothesis. Alternative P solubilising mechanisms such as alkaline phosphatases and organophosphonate utilisation-related genes and the presence of high-affinity and specificity K^+ uptake systems were, nevertheless, also present indicating their utilisation under phosphorus and potassium

limitating conditions, respectively. Differently, the main strategy for iron uptake followed by microbes inhabiting cyanobacterial biocrusts in the Tabernas Desert seemed to be the synthesis and release of siderophores, mainly derived from pseudomonads.

Similarly, data showed that a diverse group of microbes, predominantly actinobacteria, may be leading sulfur assimilation in either mineral and organic form in cyanobacterial biocrusts at the Tabernas Desert. As expected in drylands, organosulfonated compounds seemed to be the main assimilation products. High abundance of ARS presented by major group *Rubrobacter* besides suggested that it may be driving the ester sulphate mineralisation in the microbial community. In addition, the unexpected presence of sulfite oxidising enzymes in the orders *Rhizobiales* and *Burkholderiales* was related to their successful survival and ability to grow using inorganic sulfur in biocrusts. Atypical TRs identified in *Sphingopyxis, Rhizobium, Anabaena, Bradyrhizobium*, and *Nostoc* besides supported the utilisation of elemental sulfur as an electron acceptor by these microorganisms under anaerobic conditions.

Finally, members of microbial communities associated with cynobacterial bioctrusts showed a high potential to be used in biotechnology. On one hand, the abundance of extracellular hydrolytic enzymes involved in the degradation of carbohydrates may be exploited as an industrial pool for novel *α*-glucosidases, *β*-glucosidases, *β*-galactosidase, α-amylases and glucoamylases well adapted to harsh conditions. On the other hand, the identification of novel taxonomic groups (i.e. representatives of *Caulobacter*, *Sphingopyxis*, *Dyadobacter, Cytophaga* and *Flavobacterium)* as silicate and phosphorus solubilising microorganisms and those able to synthesise siderophores (i.e. pseudomonads), highlighted the role that microbial communities associated with cyanobacterial biocrusts may perfom as modulators of soil metallic nutrients to environmental changes in dryland soils and, consequently, as plant growth promoters.

Declarations

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable

CONSENT FOR PUBLICATION

Not applicable

AVAILABILITY OF DATA AND MATERIALS

The datasets generated and/or analysed during the current study are available in the MG-RAST database within the study BIOSOC-RYC2016-21191 (See in https://www.mg-rast.org/linkin.cgi?project=mgp84274).

COMPETING INTERESTS

None declared.

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AUTHORS CONTRIBUTIONS

IM conceived and funded the study. IM and MdCM-C designed the study. IM and RO collected soil samples, extracted DNA and performed soil analysis. MdCM-C performed bioinformatic and statistical analysis. MdCM-C and IM interpreted results and wrote the manuscript. All authors read and approved the manuscript.

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Figures

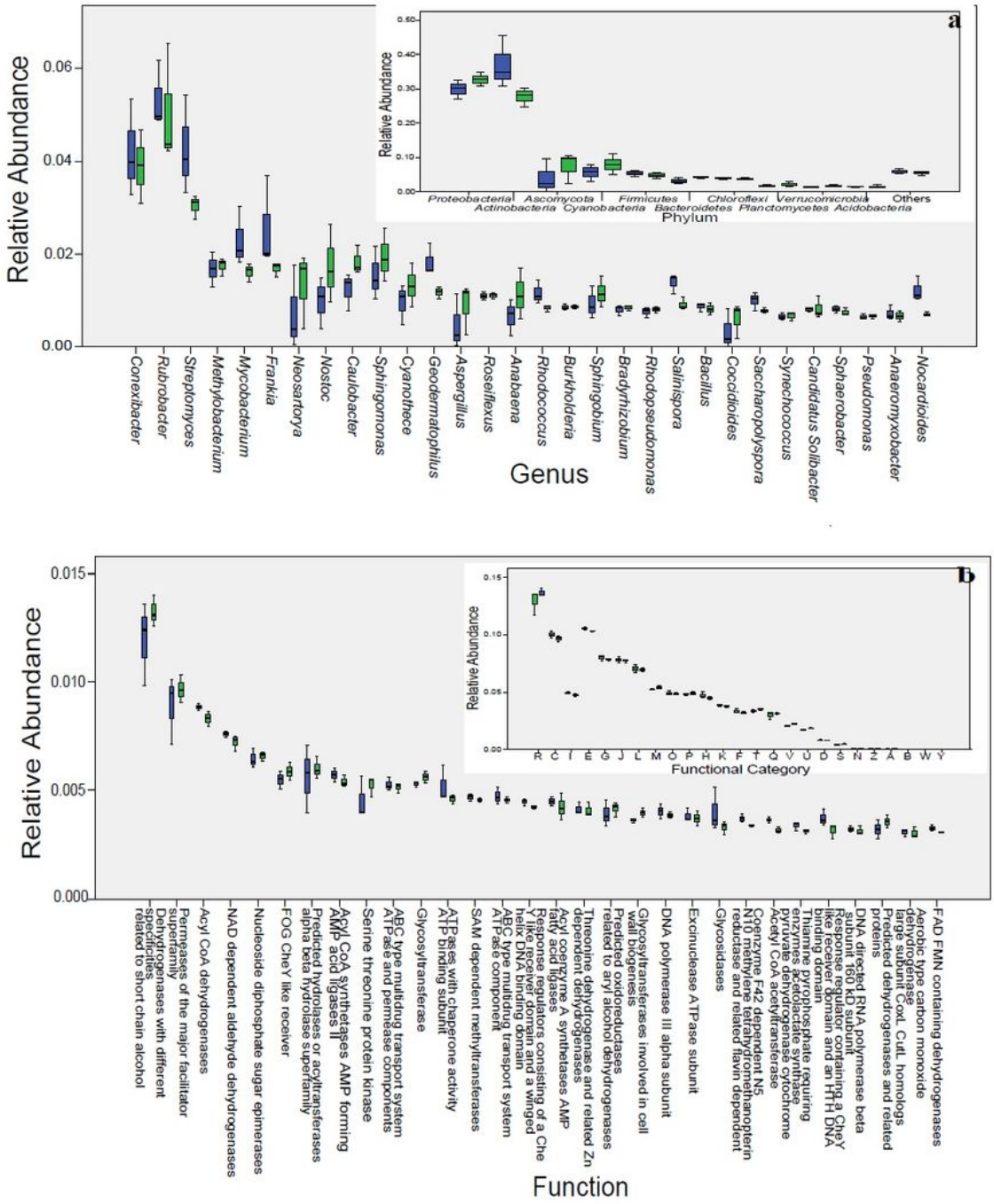

Figure 1

Phylogenetic and functional profiles of cyanobacterial biocrust microbiome. a, Genus abundance variation box plot for the 30 most abundant genera as determined by read abundance. Inset shows phylum abundance box plot; b, Orthologous group abundance variation box plot for the 30 most

abundant orthologous groups as determined by COG database. Inset shows abundance box plot of 17 COG functional categories. Box colours indicates incipient cyanobacterial biocrust (blue) and mature cyanobacterial biocrusts (green). Classification of the COG functional categories: J, translation, including ribosome structure and biogenesis; L, replication, recombination and repair; K, transcription; O, molecular chaperones and related functions; M, cell wall structure and biogenesis and outer membrane; N, secretion, motility and chemotaxis; T, signal transduction; P, inorganic ion transport and metabolism; C, energy production and conversion; G, carbohydrate metabolism and transport; E, amino acid metabolism and transport; F, nucleotide metabolism and transport; H, coenzyme metabolism; I, lipid metabolism; D, cell division and chromosome partitioning; R, general functional prediction only; S, no functional prediction

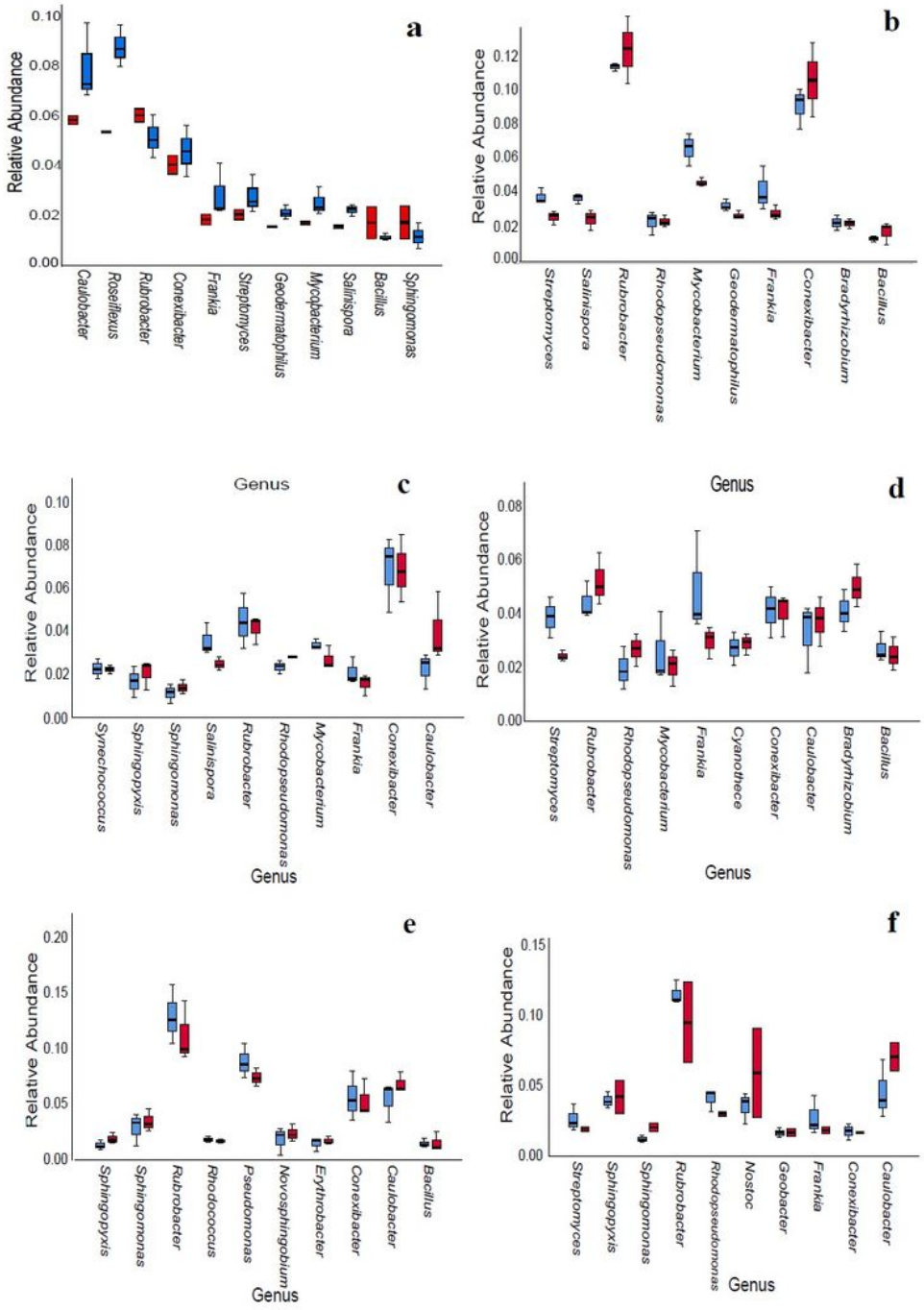

Figure 2

Genus abundance variation box plot for the 10 most abundant genera observed in phylogenetic profiles of cyanobacterial biocrust microbiome as determined by read abundance according to biogeochemical cycles. a, Carbon cycle; b, Nitrogen cycle; c, Phosphorus cycle; d, Sulfur cycle; e; Iron cycle; f, Potassium cycle. Box colours indicates incipient cyanobacterial biocrust (blue) and mature cyanobacterial biocrusts (green).

Supplementary Files

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