Title

Ecological relevance of abundant and rare taxa in a highly-diverse elastic hypersaline microbial mat, using a small-scale sampling

Authors

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Abstract

We evaluated the microbial diversity and metabolomic signatures of a hypersaline elastic microbial mat from Cuatro Ciénegas Basin (CCB) in the Chihuahuan Desert of Coahuila, México. We collected ten samples within a small scale (1.5-meters transect) and found a high microbial diversity through NGS-based ITS and 16S rRNA gene sequencing. A very low number of taxa were abundant and shared between all sites, whereas the rare biosphere was more phylogenetically diverse (FPD index) and phylogenetically disperse (PSC index) than the abundant taxa for both analyzed libraries. In regard to potential biotic interactions (Pearson analysis), there were more positive correlations than negative. We also found a distinctive metabolomic signature for each sample and were able to tentatively annotate several classes of compounds with relevant biological properties. Together, these results reveal cohesive, diverse and fluctuating microbial communities, where abundant and rare taxa appear to have different ecological roles.

Kewords: biotic interactions, Cuatro Ciénegas Coahuila, metabolomic analyses, microbial communities, phylogenetic diversity, rare biosphere.

Introduction

Microbial mats are tightly interacting communities, usually self-sustaining, that are horizontally stratified with multilayered biofilms embedded in a matrix of exopolysaccharides binding cells and inorganic substances together (Bolhuis et al., 2014; Prieto-Barajas et al., 2018; Stolz, 2000). These stratified structures, as well as stromatolites, are the oldest communities found in the fossil record, informing our understanding of ancient metabolic interactions (Gutiérrez-Preciado et al., 2018; Nutman et al., 2016).

Although modern microbial mats and stromatolites are geographically widespread (Gerdes, 2010), their distribution is restricted to extreme environmental conditions where multicellular algae and grazing organisms cannot grow (Bebout et al., 2002). One such site, with a high diversity of microbial mats and stromatolites, is the desert oasis of the Cuatro Ciénegas Basin (CCB), found in the Chihuahuan Desert of the

state of Coahuila in northern México. This small site (150,000 km^2) is extremely biodiverse – in particular for microbes (Souza, Eguiarte, et al., 2018) – and is considered of international importance by the RAMSAR convention (www.ramsar.org). CCB is characterized by an imbalanced stoichiometry (i.e., very low concentration of phosphorus) (Elser et al., 2005, 2006; Papineau, 2010; Planavsky et al., 2010), which appears to be a strong selective pressure driving microbial lineages to adapt locally and form extremely cohesive and co-adapted microbial communities (Souza, Eguiarte, et al., 2018). The uniqueness of microbial life in CCB -- characterized as an "Astrobiological Precambrian Park" (Souza et al., 2012) -- is reflected in the presence of ancient marine lineages (Rebollar et al., 2012; Souza et al., 2006; Souza, Eguiarte, et al., 2018), rich prokaryotic communities (López-Lozano et al., 2013; Pajares et al., 2012, 2013; Souza, Olmedo-Álvarez, et al., 2018) , and diverse fungal (Velez et al., 2016) and viral communities (Taboada et al., 2018), as well as a high microbial diversity within microbial mats and stromatolites (Bonilla-Rosso et al., 2012; De Anda et al., 2018; Peimbert et al., 2012).

In March 2016, we discovered an outermost elastic layer microbial mat in CCB, which enables the formation of dome-like structures under wet conditions (Fig. 1).

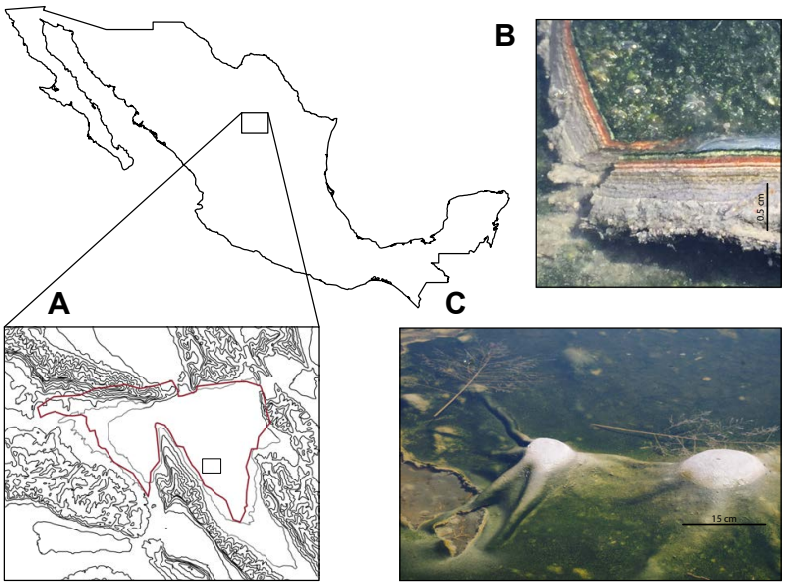


Figure 1

(A) The Cuatro Ciénegas Basin within México, and geographic location of Archaean Domes in Pozas Azules Ranch. (B) Detail of an Archaean Domes slice. (C) Domes when the sampling was conducted (note the size scale). Photos by David Jaramillo.

The internal microbial layer, insulated by an outer aerobic community, thrives under an anaerobic environment, rich in methane and hydrogen sulfide, and small volatile hydrocarbons that recreate the atmosphere of the Archaean Eon, hence we named these mats “Archaean domes” (Medina-Chávez et al., 2020). Similar types of elastic mats have previously been described in marine tidal zones, although their formation is relatively rare (Gerdes et al., 1993).

Given their functional richness, microbial mats are ideal systems for analyzing the role of microbial interactions in maintaining community structure under extreme conditions (Paerl & Yannarell, 2010; Prieto-Barajas et al., 2018). In this sense, there are three approaches that can improve our understanding of biotic interactions and processes at an evolutionary scale (Mayfield & Levine, 2010) for microbial mat communities. First, determine the phylogenetic diversity of communities under extreme environmental conditions where, for example, abiotic filtering and competitive exclusion have been reported as drivers of phylogenetic clustering (Goberna et al., 2014;). Second, investigate the ecology of microbial subcommunities, such as those of rare taxa, a less-studied topic than the whole community (Liu et al., 2019) but important to explore for their potential contribution to ecological processes (Jia et al., 2018). Third, analyze the metabolomics of communities, which is one of the functional expressions of diversity and an underexplored aspect of microbial communities that can help complete our understanding of the observed diversity. Moreover, metabolomics can also elucidate ecological aspects (Sardans et al., 2011) that help uncover the reciprocal interactions between ecology and evolution, known as eco-evolutionary feedbacks (D. M. Post & Palkovacs, 2009).

In this study, we evaluated the microbial diversity, the rare biosphere, and its metabolomic signature within this elastic hypersaline microbial mat from CCB, across ten samples collected within a 1.5-meter transect. Specifically, we aimed: 1) to explore microbial diversity analyzing amplicons of the 16S rRNA and ITS regions,

110 2) to determine whether rare and abundant taxa are phylogenetically structured; 3)
111 to evaluate microbial metabolomic signatures as an approximation to eco-
112 evolutionary feedbacks; and 4) to approximate biotic interactions.
113
114 **Results**
115 ***Microbial taxonomic composition***
116 16S rRNA gene and ITS libraries revealed the occurrence of different microbial
117 lineages, as genera composition showed (Fig. 2).

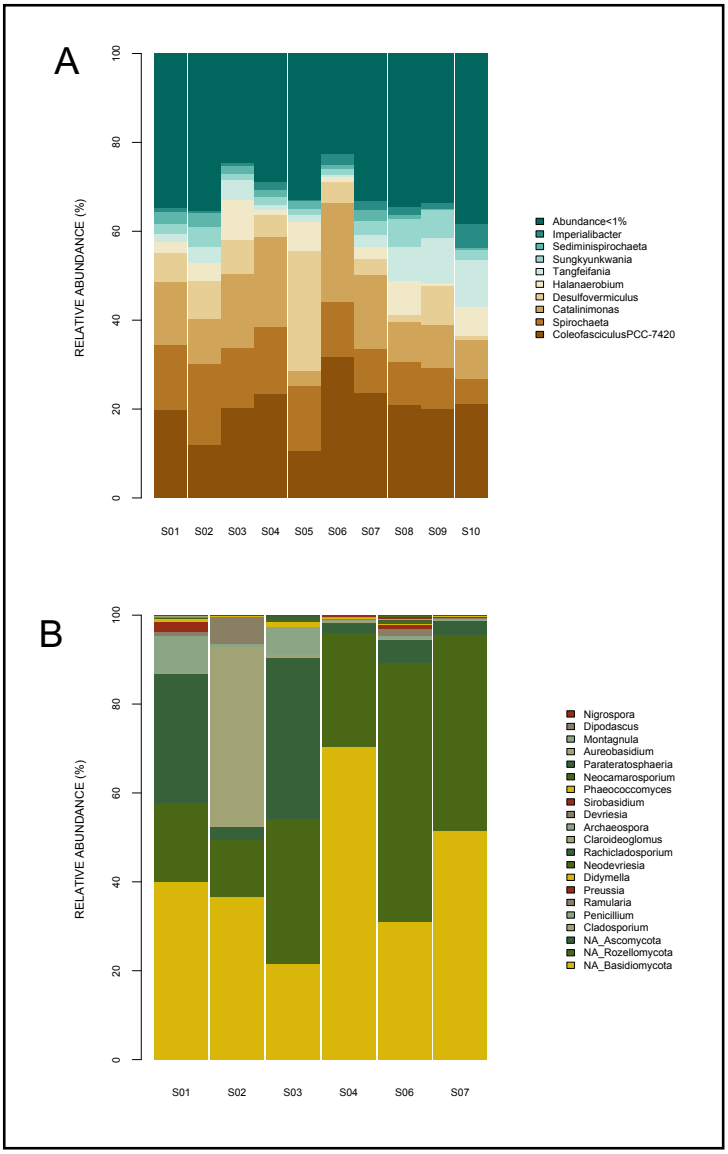
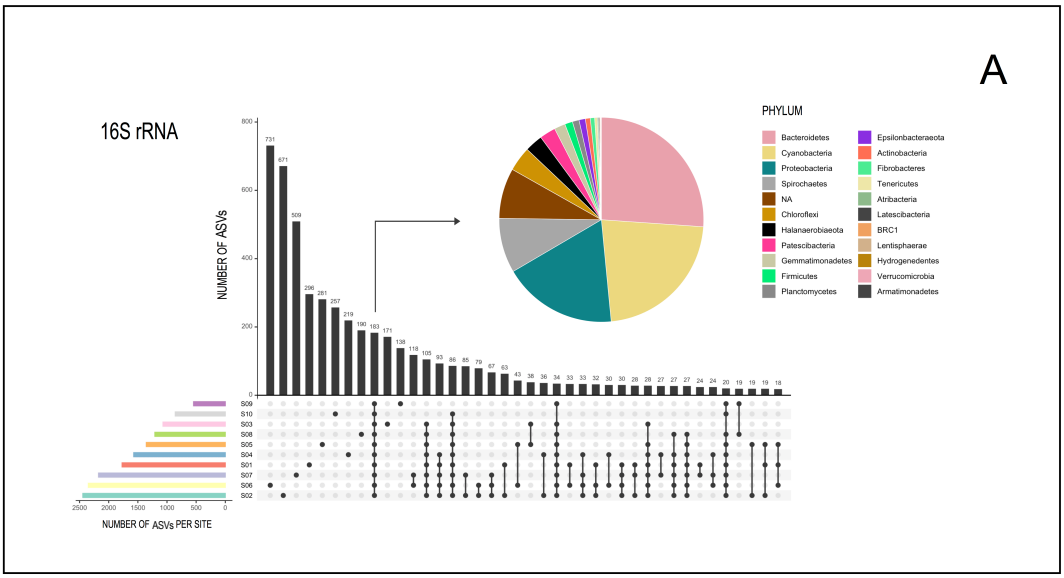


Figure 2
Genera relative abundance of taxa in Archaean Domes, Cuatro Ciénegas Basin sample. A) Bacteria (only showed the most common taxa, more than 1% of diversity) B) Fungi (using Merge database, only fungal ASVs. NA is for ASVs that were not assigned to any genera, but phylum only).

125 Overall, 40 prokaryote phyla were detected from the 16S rRNA gene libraries (Suppl.
126 Fig. 1A), and a total of 6,063 different ASVs were found, with a proportion of 11.8%
127 of unclassified reads (Suppl. Table 2). Bacteroidetes was the most abundant phylum,
128 representing around 23% of the prokaryotic diversity in the samples, followed by
129 Proteobacteria with ≈ 19% and Cyanobacteria with nearly 18%. Spirochaetes and
130 Chloroflexi represented ≈ 8% and ≈ 4% of the prokaryotic diversity, respectively.
131 Nearly 2% were Firmicutes, Patesibacteria and Halanaerobiota (Suppl. Fig. 1A).
132 A total of 410 genera of Bacteria were detected in the 10 sampled sites (S1-S10) at
133 a 1.5 m-scale. From those, only 9 genera were abundant (representing ≈ 70% of
134 total abundance, Fig. 2 A): *Coleofasciculus* (or *Microcoleus*), *Spirochaeta*,
135 *Catalinimonas*, *Desulfovermiculus*, *Halanaerobium*, *Tangfeifania*, *Sungkyunkwania*,
136 *Sediminispirochaeta* and *Imperialibacter*. The remaining 401 genera can be
137 classified as rare biosphere, with ≈ 30% of total abundance (less than 1% each, Fig.
138 2 A). Moreover, 28 ASVs of Archaea were obtained using our 16S primers,
139 comprising two phyla, Woesearchaeota and Euryarchaeota (Suppl. Fig. 1a).
140 Halobacteria class was the most abundant, and we detected 4 genera: *Halovivax*,
141 *Halorubrum, Halorussus* and *Haloasta* (Suppl. Table 2).
142 For the ITS region libraries, we successfully obtained reads for six of the sampled
143 sites (S1 to S4, S6 and S7). For the Forward-Only ITS database, we detected 923
144 different ASVs. The majority of the classified reads belong to Alveolata protists (77%,
145 Suppl. Fig. 1B), followed by Fungi (11%) and Viridiplantae (8%); Metazoa, Protista,
146 Amoebozoa, Rhodoplantae, Chromista and Stramenophila comprised the remaining
147 4%. Unassigned taxa were 50% of all reads (Suppl. Table 2). The majority of ASVs
148 within Forward ITS database could not be accurately classified at lower taxonomic
149 categories, particularly for three common ASVs (ASV0 (unclassified), ASV1
150 (Ciliophora) and ASV2 (Ciliophora)), which comprise nearly 70% of the abundance
151 in ITS reads for all sites. Using Merge ITS ASVs database for a better taxonomic
152 assignment, Fungal ASVs comprising 18 genera were detected, with phyla
153 Ascomycota, Basidiomycota and Rozellomycota present in all samples (Fig. 2B).
154
155

156 ***Alpha and beta diversity***

157 Rarefaction curves (Suppl. Fig. 2A and 2B) showed similar results for both the
158 Forward-Only ITS region libraries (S1, S2, S3, S4, S6, S7), and 16S rRNA gene
159 libraries, as all sites reached an asymptote, suggesting an adequate sampling effort.
160
161 Only 183 ASVs (3% of the diversity) were shared between all sites for 16S rRNA
162 gene libraries (Fig. 3A), forming a very small core, but comprised 72.4% of all reads
163 (composed of twenty-one phyla), while most of the ASVs were unique to a specific
164 site or shared between two or three sites. For ITS libraries, the 56 shared ASVs (6%)
165 comprised 94.7% of all reads (Fig. 3B).
166
167
168



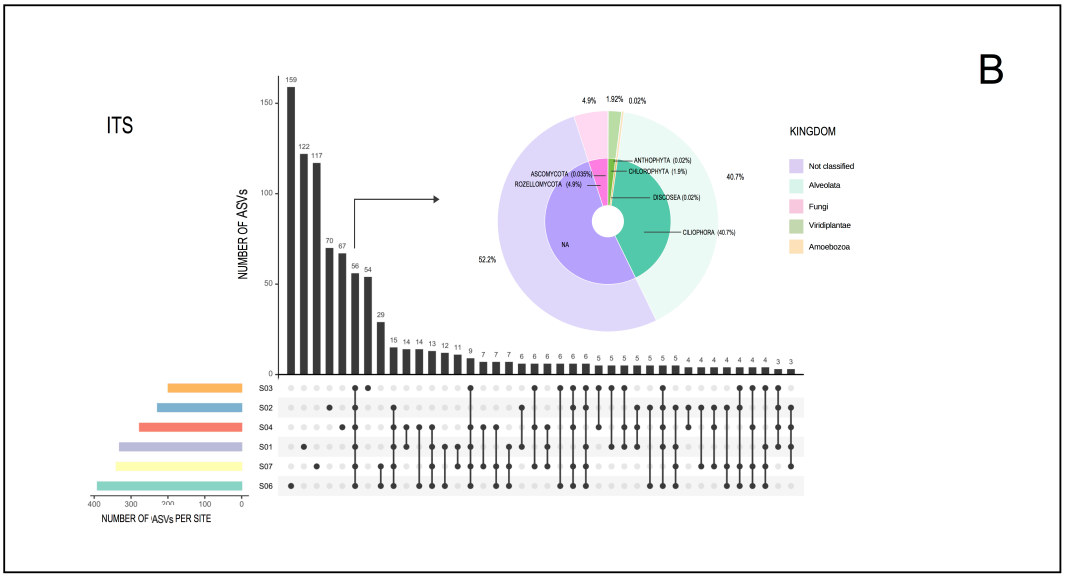


Figure 3
Graphic representation of shared ASVs within Archaean Domes mats sampling sites. Number of shared ASVs is represented by black bars, showing the exact number on the top. Dots below bars denote sites where those ASVs are shared, and lines joining dots indicate the shared sites. Colored bars at bottom left of the main figure are for ASVs number within each site. Pays show taxonomy of shared ASVs within all sites (a few but abundant ASVs core). (A) 16S Archaea and Bacteria, rRNA gene database, (B) Fungi and other Eucarya, Forward-Only ITS database.

179 16S rRNA ASVs alpha diversity (Table 1) ranged from 2470 expected ASVs
180 according to Chao1 in S2 (Shannon = 7.4, highest Shannon index value), to 544
181 expected ASVs in S9 (Shannon = 6.1). In the case of the ITS region libraries
182 (Forward-Only ITS database), the most ASV Chao1 diversity for Eukarya was S6
183 with 393 expected ASVs and a Shannon diversity of 2.5; S3 had the lowest Chao1
184 diversity, with 199 expected ASVs, but this site had the highest Shannon value of 3
185 (Table 1).

ITS region						
	α diversity			Phylogenetic diversity		
Sample	Observed	Chao	Shannon	Faith PD	Faith PD rare	Faith PD abundant
1	322	322	1.827	86.309	42.596	18.161
2	226	226	2.713	60.622	40.846	14.516
3	199	199	3.062	47.289	30.370	14.774
4	270	275	1.783	83.008	51.513	16.287
6	383	393	2.501	131.435	75.352	28.794
7	337	344	2.008	114.983	60.352	18.865

186

16S rRNA						
	α diversity			Phylogenetic diversity		
Sample	Observed	Chao	Shannon	Faith PD	Faith PD rare	Faith PD abundant
1	1761	1773.203	6.863	544.704	290.491	44.159
2	2399	2470.398	7.402	789.807	368.430	84.442
3	1068	1071.318	6.256	211.745	156.204	20.064
4	1564	1568.664	6.499	500.787	259.828	40.627
5	1344	1358.842	6.882	220.536	172.299	21.557
6	2345	2353.855	6.301	851.168	302.103	131.337
7	2156	2172.218	6.838	759.536	328.238	97.486
8	1195	1217.568	6.487	205.987	139.839	22.798
9	544	544	6.126	160.368	94.201	18.577
10	828	875.634	5.115	283.017	148.655	36.514

187
188 Table1

189 Alpha diversity indices for 16S rRNAgene and Forward-Only ITS region databases
190 from Archaean Domes CCB microbial mats.

Paired beta bacterial diversity among microbial mat samples for both the 16S rRNA gene and ITS region databases ranged from 0.2 to 0.7 for the Bray-Curtis diversity values and 0.3 to 0.7 according to the Jaccard-1 values (Suppl. Fig. 3 A and B). For both indexes, S10 is different from the rest of the 16S rRNA gene libraries, and S1, S2, S4, S6, S7 were associated in a cluster. In the ITS libraries, there are two main clusters (S1, S2 and S4; S3, S6 and S7). A significant geographic pattern in the Mantel test (p=0.0402) was found for Jaccard-1 of the 16S library, however we did not find significant values based on geography for ITS libraries for either Jaccard-1 or Bray-Curtis.

Phylogenetic Diversity

For both libraries, Faith Phylogenetic Diversity showed that rare ASVs were more diverse than abundant ASVs (Table 1), and Phylogenetic Species Clustering (PSC) measures showed that abundant ASVs were clustered within phylogeny, while rare ASVs were disperse (Fig. 4 A and B).

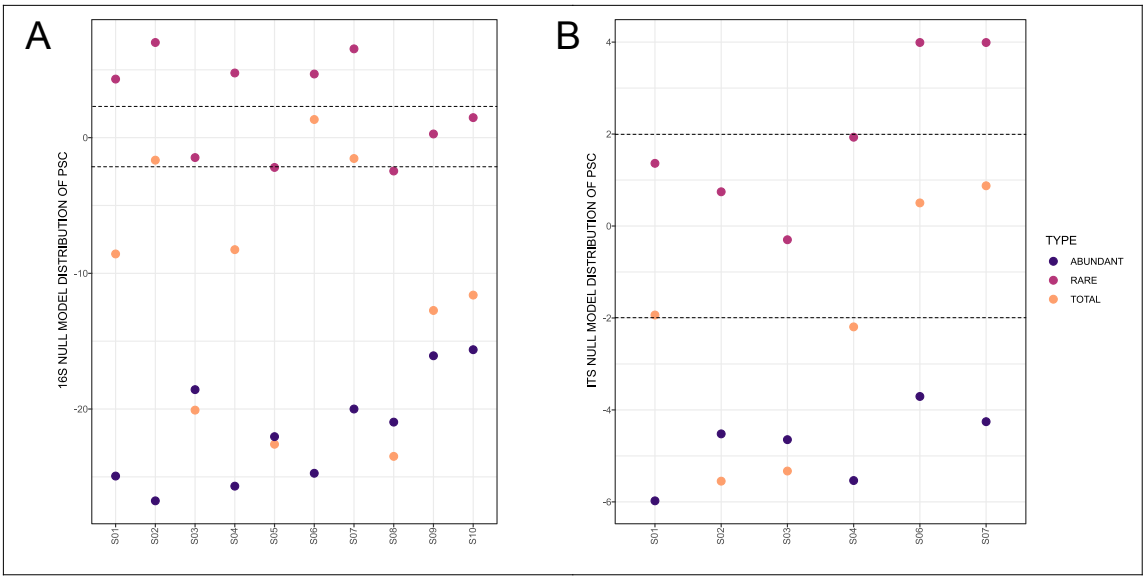


Figure 4
Graph representing null model distribution of Phylogenetic Species Clustering (nmdPSC) values for all community, abundant and rare taxa from 16S rRNA database (A) and Forward-Only ITS database (B). Dotted lines are showing nmdPSC significant values: > +2 is consistent with a sample that had more phylogenetic overdispersion than expected by chance alone, and nmdPSC value < -2 indicates more phylogenetic clustering than expected by chance alone; values in

the range +2 and -2 had a null model distribution. nmdPSC values are reported in Suppl. Table 5.

Approximation to biotic interactions: Pearson analysis

In order to identify potential biotic interactions within the dominant taxa from the three domains of life within the Archaean Domes, we calculated Pearson correlations between ASVs (Fig. 5).

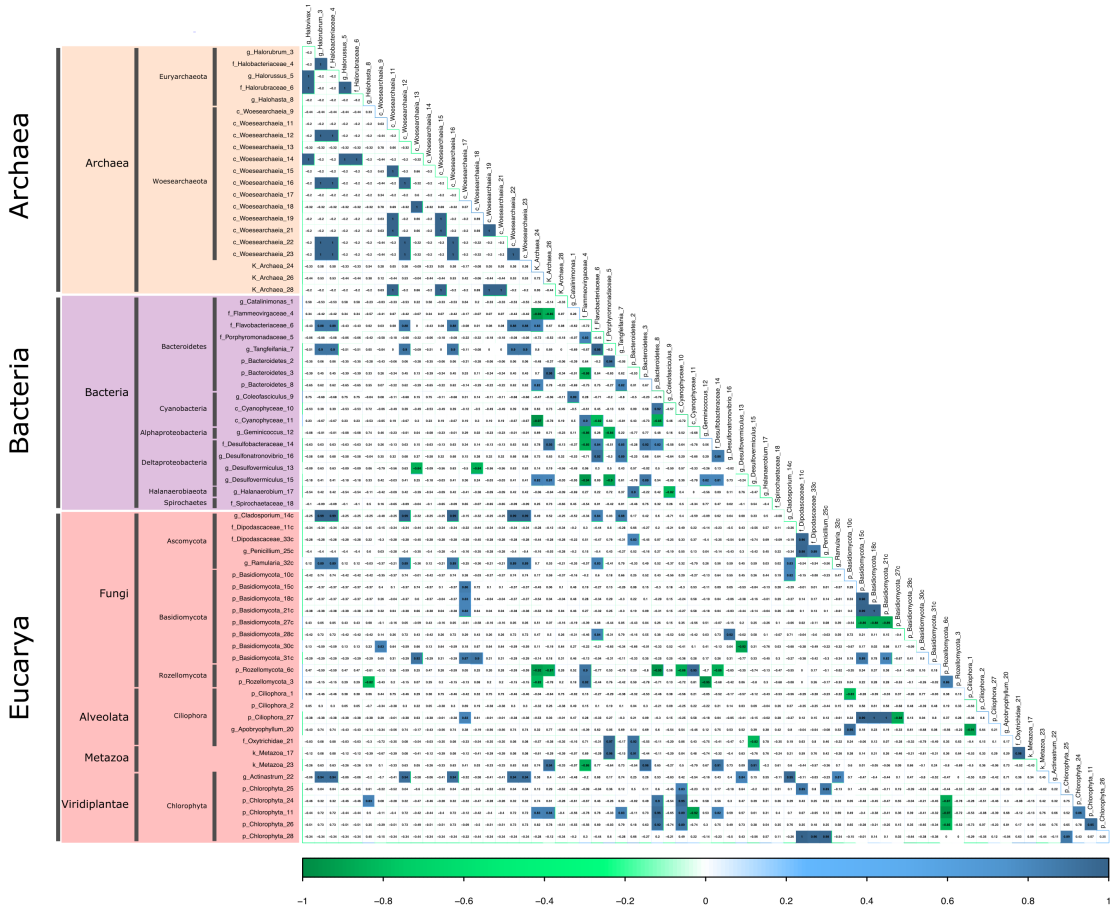


Figure 5
Pearson correlation analysis within the most common (i.e., involving more than 1% of diversity) Bacteria, and all genera of Archaea and Fungi (from Merge ITS database). Colored squares are for significant correlations (P≤0.05), blue for positive ones and red for negatives.

We analyzed the most abundant ASVs (above 1%) from the 16S rRNA gene libraries and both ITS databases, along with 22 Archaea ASVs. We observed that most of the negative co-occurrence correlations are associated with Bacteria, in particular

231 the Bacteroidetes phylum. Within the complete correlation matrix, we observed 22
232 negative co-occurrence correlations, along with 9 for Archaea,14 for Fungi, and 6 for
233 Eukarya (see Suppl. Table 3 for interaction counts). In addition, we observed that
234 Archaea-Archaea correlations (Fig. 5) were mostly positive (80), the majority of them
235 enclosed within Archaea ASVs (32), whereas Eukarya-Eukarya interactions had the
236 fewest positive correlations (4). Remarkably, in the cross-kingdom fungal
237 interactions, most of the negative fungal correlations (8 of 14) were observed in
238 Rozellomycota.
239
240 ***Metabolomic profile***
241 We found a wide range of metabolites based on heatmap and GNPS molecular
242 networking analysis (Fig. 6 and Suppl. Fig. 4 A&B).

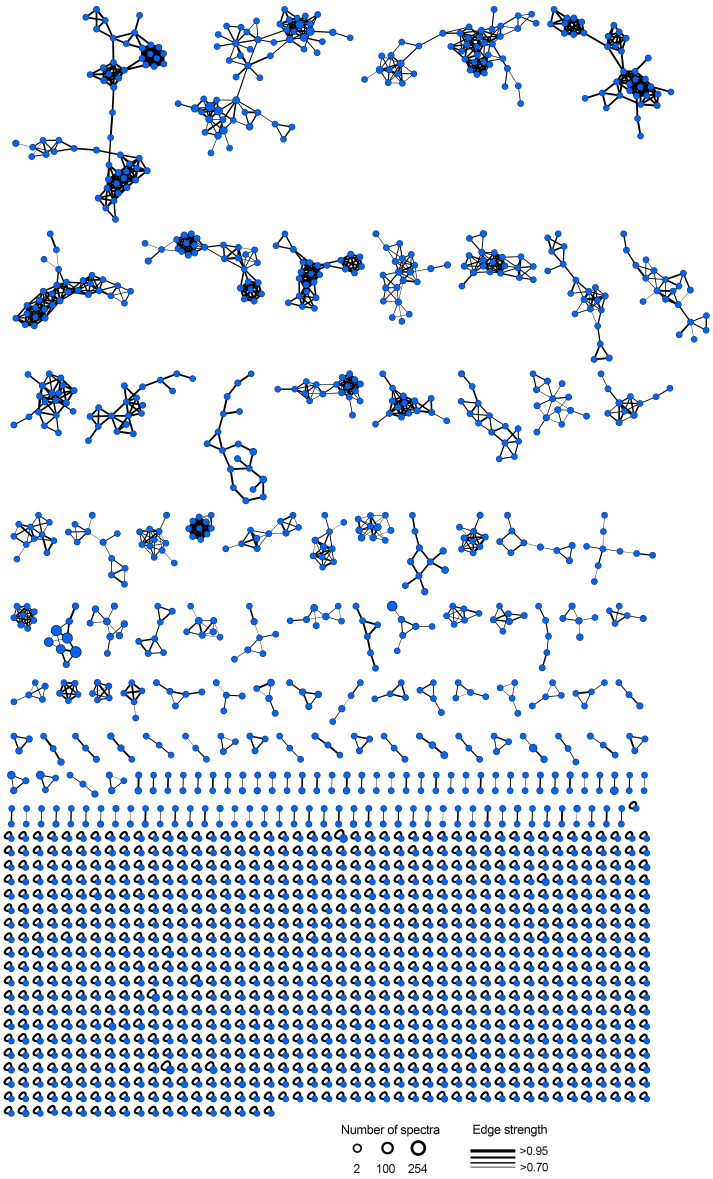


Figure 6
Molecular network of soil samples grouped metabolite features into 140 chemical families. Each node in the network represents one metabolite feature. Nodes connected to each other are structurally-related (cosine MS^2 similarity score ≥0.7).

Untargeted metabolomics analysis of microbial communities is complicated, due to the complexity and heterogeneity of this matrix and the scarcity of the secondary metabolites extracted. The 1,341 molecular features at unique retention times (Suppl. Fig. 4 A&B) shared several common regions across all samples, allowing us

253 to group them into three main clusters (cluster 1, samples 3-5 and 10; cluster 2,
254 samples 1, 2 and 6; and cluster 3, samples 7-9, Suppl. Fig. 4B). We found highly
255 heterogeneous metabolic profiles in all samples, and no significant associations
256 were observed between metabolite features and ITS or 16SrRNA abundance
257 matrices.
258 On the other hand, GNPS molecular networking analysis grouped the metabolite
259 features in 18,547 nodes distributed into 83 chemical families (sub-networks) of ≥3
260 members, 57 families of two metabolite features, and 875 singletons (Fig. 6).
261 Detected compounds in the GNPS and detailed metabolomic analysis of the main
262 ions observed in LC-MS/MS data, focused in microbial metabolites, allowed us to
263 tentatively identify several secondary metabolites that passed our quality criteria (≤
264 5 ppm mass error; Suppl. Table 4 and Fig. 7).

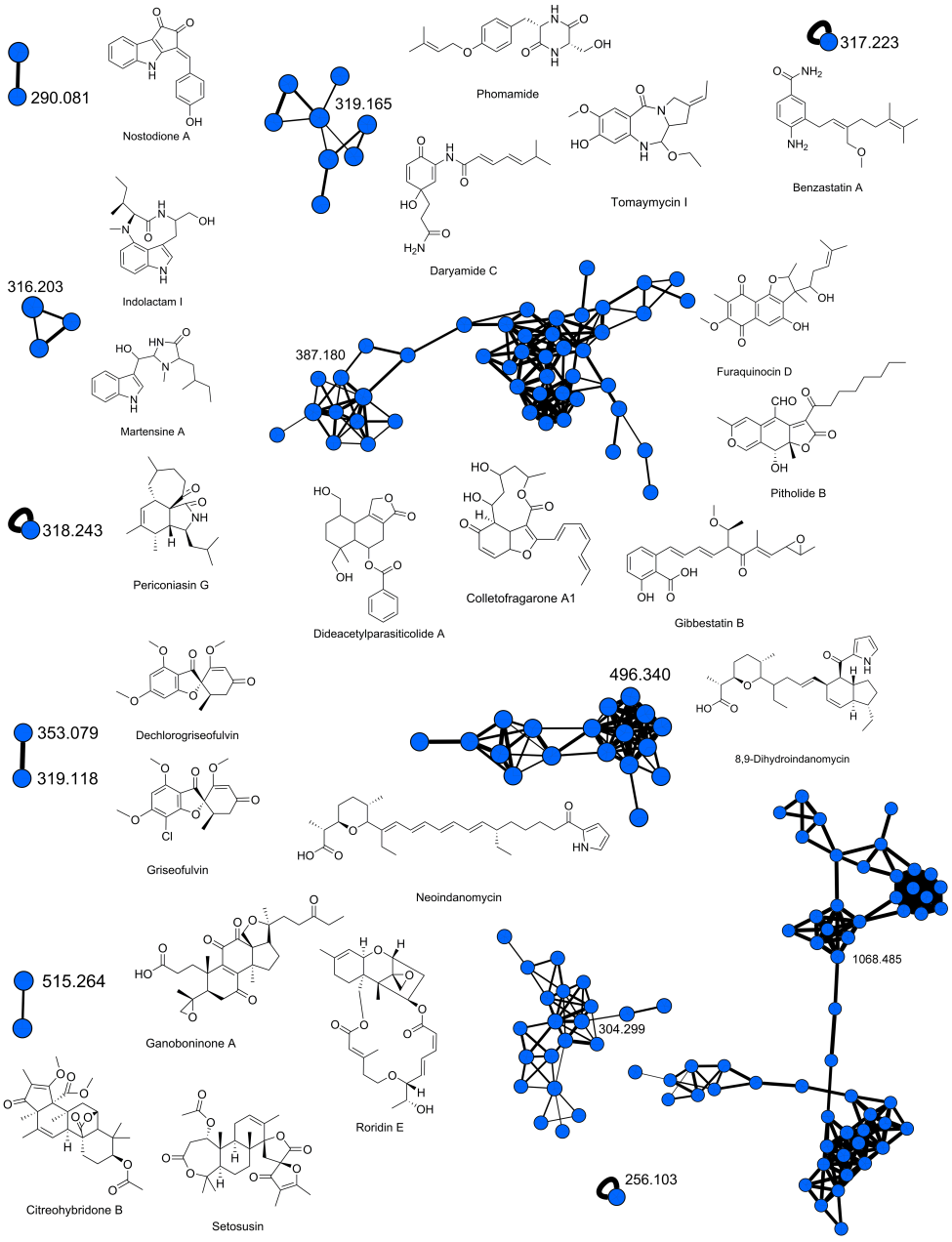


Figure 7
Structures of microbial metabolites tentatively annotated in samples S1-S10 (≤ 5 ppm mass error).

Discussion

Microbial composition at a microscale

Our study revealed a remarkably high microbial diversity within a 1.5 m scale in an elastic microbial mat located at an extreme site. Altogether, we found 40 prokaryotic phyla, including 410 genera of Bacteria and 4 of Archaea, as well as 18 Fungal genera (Fig. 2 and Suppl. Fig. 1).

Bacteria

The bacterial phyla (Suppl. Fig. 1) detected in our study site are common components of hypersaline microbial mats (Bolhuis et al., 2014; Visscher et al., 2010): Bacteroidetes, Proteobacteria, Cyanobacteria, Chloroflexi, and Firmicutes, among other abundant phyla, which are similar at this broad taxonomic level to other sites in CCB (Bonilla-Rosso et al., 2012; Breitbart et al., 2009; De Anda et al., 2018; Nitti et al., 2012). As expected from a microbial mat, a primary producer was present and abundant in all of our samples: *Coleofasciculus* (*Microcoleus)* (Fig. 2), a filamentous nitrogen-fixing cyanobacteria genus well-adapted to saline conditions (De Wit et al., 2013; Ramos et al., 2017) that has been reported in Guerrero Negro microbial mats (Des Marais, 2010; Wong et al., 2016). We also found the abundant taxon *Catalinimonas,* which is a halophilic Bacteroidetes marine genus (Choi et al., 2013) that has not been previously reported in microbial mats. Another common taxon in our samples is the *Spirochaeta* genus (Bolhuis et al., 2014; Margulis et al., 2006), an anaerobic chemo-organotrophic taxa abundant in hypersaline marine mats (Ben Hania et al., 2015). Interestingly, the *Spirochaetes* phylum has not been detected previously in any CCB mats (Bonilla-Rosso et al., 2012; de Anda et al., 2018; Peimbert et al., 2012). We also observed *Desulfovermiculus*, a sulfate-reducing Deltaproteobacteria, and *Halanaerobium*, a thiosulfate-reducing Firmicute that is halophilic and can form spores (Liang et al., 2016), both of which are common genera in marine microbial mats (Bolhuis et al., 2014).This suggests that this shallow salty pond could be more similar to marine lineages than other sites in CCB (see Rebollar et al., 2012; Souza et al., 2006, 2018).

301 Previous metagenomic analysis from a mat in Churince within CCB reported 1,431
302 OTUs (de Anda et al., 2018) – four times less diverse than the Archaean Domes
303 (6063 ASVs). Other studies within CCB, also with complete metagenomes, retrieved
304 250 expected OTUs using 31 core genes (Bonilla-Rosso et al., 2012). Therefore, it
305 is possible that using high coverage 16S tags may reveal a hidden high-diverse rare
306 biosphere in these microbial mats.
307 Regarding the high diversity within the Archaean Domes and comparing it with other
308 microbial mats (since ASVs and OTUs are reported providing similar ecological
309 results (Glassman & Martiny, 2018)), Cardoso et al. (2019) reported 977 OTUs in
310 marine microbial mats, and Wong et al. (2015) found 4800 OTUs in Shark Bay, which
311 are considered some of the most diverse microbial mats worldwide.
312
313 *Archaea*
314 In a recent metagenomic study of the Archaean Domes (Medina-Chávez et al.,
315 2020), Archaea taxa formed a constant core that is more diverse than any other site
316 within or outside of CCB. In those metagenomes, Archaea represented nearly 5% of
317 the total reads, accounting for 230 ASVs.
318 In our data, by using 16S rRNA gene amplicons, we only detected two main phyla,
319 as expected given the nature of the amplified region (Suppl. Fig. 1 and Suppl. Table
320 2). These were Woesearchaeota, a mainly halophilic phylum and potentially
321 syntrophic within methanogens (Liu et al., 2018; Ortiz-Alvarez & Casamayor, 2016),
322 and Euryarchaeota, within which we found four halophilic genera: *Halovivax*,
323 *Halorubrum, Halorussus* and *Haloasta*, all of them previously reported within saline
324 habitats and sediments (McGonigle et al., 2019; Zaitseva et al., 2018). The
325 metagenomic data (Medina-Chávez et al., 2020) and the 16S rRNA gene amplicons
326 were sampled from the same pond at different times and space, and both showed
327 lineages common to hypersaline habitats.
328
329 *Fungi*
330 Fungal members represent a diverse and common component of hypersaline water
331 columns and mats (Cantrell et al., 2013) and have been suggested to play a role in

332 the degradation of EPS (exopolymeric substances) (Cantrell & Duval-Pérez, 2013).
333 Our results suggest that unidentified uncultured members of the Ascomycota and
334 Rozellomycota comprise the core mycobiota of the analyzed mats, as they occurred
335 across all the samples (Fig. 3B). In addition, it is feasible that these abundant
336 heterotrophs occupy a unique niche within the micro-scale of microbial mats, where
337 they can degrade the abundant EPS produced in the CCB microbial mats (Breitbart
338 et al., 2010; De Anda et al., 2018; Peimbert et al., 2012). In particular, halotolerant
339 taxa such as members within the genera *Aspergillus*, *Penicillium*, and *Nigrospora*
340 have been repeatedly reported in microbial mats at other locations (Cantrell et al.,
341 2013; Cantrell & Duval-Pérez, 2013), evidencing their adaptation to extreme
342 conditions. Thus, it appears that, similar to other microbial mats, fungal members
343 occupy a unique niche within the micro-scale of the Archaean Domes (Cantrell et
344 al., 2011).
345
346 *Other eukaryotes*
347 The overall diversity of protists in the Archaean Domes is comparable to the diversity
348 observed in other microbial mats (329 ASVs, Suppl. Table 2). For example, Saghaï
349 et al. (2017), reported nearly half of the 586 18S rRNA OTUs they analyzed as
350 Protozoa in a saline shallow pond mat in Chile, while ≈ 500 eukaryotic 18S rRNA
351 OTUs were observed in mats from hydrothermal vents in Guaymas Basin (Pasulka
352 et al., 2019), where half of them were likewise classified as Protozoa taxa. More than
353 1500 eukaryotic 18S rRNA OTUs were detected in microbialites in Highborne Cay,
354 Bahamas, and Hamelin Pool (Shark Bay), Australia (Edgcomb et al., 2014), with also
355 a half proportion being Protozoa.
356 In our sample, there are three abundant ASVs of Alveolata protists that represent
357 nearly 70% of the ITS region (Suppl. Fig. 1B, Forward-Only ITS database) in all
358 sequenced sites (S1-S4, S6, S7). Two of the three ASVs are Ciliophora (i.e. ciliates),
359 which is the most diverse phylum of protists, with 3,500 described species, most of
360 which are cosmopolites (Gao et al., 2016). It will therefore be interesting to analyze
361 more molecular markers in the future to distinguish the protist lineages, as well as to
362 microscopically study the protists to reach a closer taxonomic identification. Other

363 Protozoa ASVs were observed at low numbers and belong to the Amoebozoa
364 phylum, and the ancestral algae from the kingdom Chromista were also observed at
365 a low abundance (Suppl. Fig. 1B).
366 Although research on halophilic protozoan is still young, it has made great progress
367 in recent years (Harding & Simpson, 2018). Protozoa in general are grazing
368 organisms, as is the case with *Fabrea salina* ,one of the few classified low
369 abundance ITS ASVs in our study (Merge database, Suppl. Table 2). This ciliate is
370 present in microbial mats and feeds on *Cyanotece sp.*, a Cyanobacteria associated
371 with algae mats (Carrasco & Perissinotto, 2012). Indeed, in hypersaline
372 environments, most ciliates are known to be important components of the pelagic
373 microbial food loop (Liu et al., 2016). Amoebae have also been observed as grazers
374 (Hauer et al., 2001; Hauer & Rogerson, 2005; F. Post et al., 1983) on cyanobacteria
375 in surface mud samples from a salt pond in Eilat, within the Salton sea in Southern
376 California (Hauer & Rogerson, 2005). Even though the diversity of protists is low,
377 their prevalence, along with the large amount of viruses found in metagenomic
378 samples (28% of the metagenomes from Medina-Chávez et al., 2020), strongly
379 suggests that predator-prey interactions could be very important for the dynamics of
380 the system (Carreira et al., 2020).
381
382 ***Alpha and beta diversity***
383 The studied microbial mats –which are subject to extreme and fluctuating
384 environmental conditions– have a similar Shannon diversity as soil bacterial
385 communities (Dang et al., 2019; Wang et al., 2018). The Shannon Index accounts
386 for both species evenness and abundance; hence, it could sub-represent diversity if
387 most of the diversity is part of the rare biosphere. For example, Site 3 for the Eukarya
388 database had the highest Shannon value, despite having the lowest ASV number
389 (Table 1), due to its higher evenness distribution of taxa. Meanwhile, Chao1 only
390 compares the observed diversity with the expected, given a species distribution,
391 which more accurately reflects the total diversity of the site (Table 1; Suppl. Fig. 2A
392 and 2B).

393 Given the extraordinary patchiness of the observed diversity, exemplified by genera
394 composition differences between sample sites (Fig 2A), as well as unique
395 metabolomic patterns in each sample (Suppl. Fig. 4A&B), beta diversity analysis
396 showed a large turnover between sites despite the small scale (Suppl. Fig. 3).
397 Accordingly, the different domains analyzed (i.e., the Bacteria database or the
398 Eukarya database) had a distinctive association pattern, showing a statistically
399 significant geographic association only for the bacterial database.
400 Our results suggest a small taxonomical and functional core in these hyper-diverse
401 10 samples of the Archaean Domes, formed by bacterial and Eukaya ASVs shared
402 among all sites, that make up a low fraction of the total taxa, but are highly abundant
403 (183 16S rRNA ASVs, that comprises 3% of taxa (Fig. 3A) and 72.4% abundance of
404 all reads; 56 (6%) shared ITS ASVs with 94.7% abundance of all reads) (Fig. 3B).
405
406 ***Interactions in the Archaean Domes, inferred from the co-occurrence or co***
407 ***absence between sites.***
408 In this study, we performed a Pearson correlation analysis (Fig. 5) to identify
409 potential ecological roles and interactions between the most abundant ASVs within
410 the three databases and 22 archaeal ASVs. Negative correlations (i.e. absence or
411 lower than expected co-occurrence) can be used to identify metabolic competition,
412 separated niches, antagonistic interactions, or any other relationship precluding co-
413 occurrence (Saghaï et al., 2017). Meanwhile, positive correlations (i.e. co-occurr
414 frequently or always observed) encompass a variety of relationships that include
415 mutualism, symbiosis, parasitism, predation, common abiotic niche requirements
416 (which are particularly relevant to complex microbial mat communities), associations
417 with redox gradients, and metabolic syntrophy (Saghaï et al., 2017). We found more
418 positive correlations than negative (Suppl. Tab. 3) in our analysis. However, these
419 correlations do not necessarily indicate direct interactions, since two taxa that are
420 co-present may not be in direct physical or metabolic contact, due to the spatial
421 heterogeneity intrinsic to microbial mats. Therefore, this result should be taken
422 cautiously, given the Pearson correlation assumptions, as well as the consideration
423 that only a small (but abundant) fraction of the diversity is taken into account.

424 On the other hand, some of the twenty-two archaeal ASVs analyzed (Fig. 5) formed
425 a pattern of co-presence associations, which is worth noting since the same ASVs
426 were also part of the Archaea core observed in a time series metagenomic study
427 (Medina-Chávez et al., 2020). These positive associations reflect the presence of
428 taxa within samples: for example *Halorubrum*, *Halobacteria* and Wosearchaes are
429 positively associated because they were detected (i.e. coexist) in sample 2, and
430 *Halovivax*, *Haolorusis* and *Halorubraceae* were detected in sample 6; a similar
431 situation occurs for the fungi and Eukarya ASVs associated with them. Future
432 detailed studies, including metagenomic analyses, are required to understand the
433 mechanism of this potential cooperation, since co-cultivation techniques are difficult
434 given the differential life strategies of Archaea, which grow very slowly compared
435 with the faster growth of Fungi in culture.
436 It is interesting that, while most of the known fungal species are cosmopolitan and
437 generalists, a few others are specialists, usually adapted to extreme conditions
438 (Cantrell et al., 2011). For instance, Rozellomycota is a relatively large and
439 widespread group of eukaryotes, branching at the base of the fungal tree of life
440 (Corsaro et al., 2014). This group of fungi includes a number of amoebae parasites
441 that have developed unique morphological and genomic adaptations to intracellular
442 parasitism (Vávra & Lukeš, 2013). Thus, the utmost negative correlations observed
443 in two Rozellomycota ASVs (Fig. 5) might suggest the endoparasitic lifestyle of
444 these early-diverging fungi in the analyzed Archaean Domes.
445 The majority of the negative associations found among the abundant taxa of this
446 study were bacterial interactions (Fig. 5; Suppl. Table 3). Among them, bacteria-
447 bacteria negative interactions were mainly between different phyla. These
448 interactions are likely not based on metabolic competition, as they are mainly found
449 between different phyla that could not be sharing a niche. However, antagonism has
450 been reported previously in Cuatro Ciénegas bacterial taxa (Aguirre-von-Wobeser
451 et al., 2014; Moreno Fontalba, 2017; Pérez-Gutiérrez et al., 2013) and within cross-
452 kingdom interactions (Velez et al., 2018), which may account for the negative
453 correlations observed here. Nevertheless, it is important to remember that these are

454 only negative correlations, and experimental data is needed to demonstrate
455 antagonism in this system.
456
457 ***Phylogenetic diversity***
458 *Rare taxa*
459 A third of the abundance of the bacterial genera detected in our samples belongs to
460 rare bacterial taxa (Fig. 2A). The rare biosphere was more phylogenetically diverse
461 (Faith Pylogenetic Diversity index, Table 1) and phylogenetically disperse
462 (Phylogenetic Species Clustering, Fig. 4) than the abundant taxa within these
463 samples for both the 16S rRNA and ITS libraries, suggesting that environmental
464 filtering is not occurring for the rare biosphere in this site. This evidence suggests
465 that these rare taxa could be a large reservoir of genetic diversity, as well as a matrix
466 of rich microbial interactions and diverse metabolic functions within this microbial
467 mat (Jia et al., 2018), perhaps presenting collective adaptations to the extreme
468 conditions.
469
470 *Abundant taxa*
471 The pattern of phylogenetic clustering of the abundant ASVs is supported by
472 Pearson analysis, with a higher number of positive associations in general (reflecting
473 co-presence of taxa) and negative bacterial-bacterial associations between different
474 phyla. This pattern could be similar to some soil bacterial communities that typically
475 show a coexistence of phylogenetically close relatives (Bryant et al., 2009; Horner-
476 Devine & Bohannan, 2006), which may be the product of abiotic filtering or
477 competitive exclusion (Goberna et al., 2014). Competitive exclusion seems to be a
478 likely explanation for the phylogenetic clustering observed for abundant bacterial and
479 eukaryotic ASVs, as proposed by Goberna et al. (2014), since the presence of
480 compounds with antimicrobial properties suggests high levels of competition among
481 those abundant microbes.
482
483
484

485 ***Metabolomic profile***

486 The dynamic and complex metabolomic profiles of the studied microbial mat was
487 consistent with the highly diverse microbial community observed in the metagenomic
488 analysis (Medina-Chávez et al., 2020) (Fig. 6 and Suppl. Fig. 4A&B).
489 Even though the expression of regulatory genes responsible for secondary
490 metabolites production depends on several factors (e.g. nitrogen or phosphate
491 starvation, stressors like heat, pH and damage to the cell wall, among others) (Baral
492 et al., 2018), along with the lack of soil metabolomics studies and the solubility of the
493 compounds for LCMS analysis, we were still able to tentatively annotate several
494 classes of compounds with interesting biological properties (Fig. 7). Many of these
495 compounds were found to overlap between most of the samples: the antibiotics
496 indolactam 1 (Irie et al., 1990), 8,9-dihydroindanomycin (Li et al., 2009),
497 neoindanomycin (Rommel et al., 2011) described from *Streptomyces* species, and
498 martensine A reported from the marine red alga *Martensia fragilis* (Kirkup & Moore,
499 1983); the cytotoxic daryamide C (Asolkar et al., 2006), gibbestatin B (Kinashi &
500 Sakaguchi, 1984) and furaquinocin H (Ishibashi et al., 1991), from *Streptomyces*
501 strains; the germination inhibitor colletofragarone A1, from fungi *Colletotrichum*
502 *fragariae* and *C. capsiciae* (Inoue et al., 1996); benzastatin A (Kim et al., 1996) and
503 periconiasin G (Zaghouani et al., 2016) from *S. nitrosporeus* and *Periconia* sp. F-31
504 with free radical scavenger and weak anti-HIV activities; and phomamide and
505 tomaymycin I form *Phoma lingam* (Ferezou et al., 1980) and *Nocardia* sp. (Tozuka
506 et al., 1983) with no biological activity described. Other metabolites of interest were
507 found in specific samples, for example the antifungal agents griseofulvin and
508 dechlorogriseofulvin, produced by microorganisms of the genera *Penicillium*,
509 *Aspergillus*, *Xylaria*, *Coriolus*, *Palicourea*, *Memnoniella*, *Khauskia*, *Carpenteles*,
510 *Arthrinium*, *Nigrospora*, *Stachybotrys*, and *Streptomyces* (Atta-ur-Rahmanro, 2005;
511 Kimura et al., 1992; Knowles et al., 2019; Park et al., 2006) were identified only in
512 S7; the antimitotic agent nostodione A from the blue-green alga *Nostoc commune*
513 (Kobayashi et al., 1994) commune in samples S2 and S6-S8; and the mycotoxins
514 citreohybridone B (Kosemura, 2003), setosusin (Fujimoto et al., 1996), roridin E
515 (Böhner et al., 1965) and ganoboninone A (Ma et al., 2015) in samples S8-S10.

Interestingly, several observed ions (Suppl. Table 4) do not match with any data reported and could represent potentially new secondary metabolites.

Several compounds associated with fungi and osmotrophs, as well as to other Eukarya, reflect the importance of studying the potential role of microorganisms besides bacteria, within microbial mats (Carreira et al., 2020).

Perspectives

In view of our results, the complex metabolomic data suggests local adaptive processes, following eco-evolutionary dynamics. These dynamics would create cohesive, diverse and fluctuating communities where taxa constantly and actively modify their own ecological niche and that of others (niche construction) (Laland et al., 2016; Odling-Smee et al., 2003, Van Der Hooft et al., 2020). However, this should be tested in future studies, exploring the possibility that the rare biosphere could fulfill the majority of the trophic levels and metabolic tasks, implementing metabolomics, mesocosms, and metagenomic time series approaches. Additional perspectives could evaluate the effect of environmental filtering, deepening the analysis of the biotic interactions, including the rare biosphere.

The high phylogenetic diversity and phylogenetic dispersion of the rare biosphere may suggest that there is a diverse “seed bank” in the aquifer of CCB, a deep biosphere fed by magmatic energy (Wolaver 2013) that constantly “seeds” the Archaean Domes community, along with deep water and particular minerals. Therefore, in this scenario, eco-evolutionary feedbacks could enhance community cohesion in Red Queen-like dynamics (Haafke et al., 2016), a hypothesis stating that “biotic factors provide the basis for a self-driving perpetual motion of the effective environment and so of the evolution of species affected by it” (Van Valen, 1973). In our system, there may be a constant change in biotic interactions, as well as evolutionary landscapes, while the abiotic variables (i.e., gases, nutrients, pH, salts) are also constantly changing, as a product of community feedback within the deep aquifer. In the Archaean Domes, active niche construction may have allowed the communities to co-evolve around a stable species core, given an influx of new neighbors of microbes and viruses (Taboada et al., 2018) from the deep biosphere,

547 which constantly assembles complex and new interactions. These ideas require
548 further testing, along with the community's resilience to fluctuations within these
549 extreme conditions.
550
551 **Methods**
552 *Sampling site and sample collection*
553 During the dry season (i. e. most of the year at CCB; Montiel-González et al., 2017),
554 the microbial mats of the Archaean domes remain humid and active under a salty
555 crust, which is then dissolved during the rainy season, allowing for increased
556 photosynthetic activity and the development of the dome-like structures.
557 The microbial mats of the Archaean domes were sampled during the rainy season
558 of 2018 (October), with ten evenly spaced samples (S1-S10) along a 1.5-meter
559 transect (26º 49' 41.7" N 102º 01' 28.7" W) in the 'Pozas Azules' ranch of Pronatura
560 Noroeste within CCB (Fig. 1). The salinity and pH were constant along the 1.5-meter
561 transect (salinity 53%, pH 9.8), and the average nutrient content showed a slight
562 stoichiometric imbalance (C:N:P 122:42:1), similar to the one measured during the
563 rainy season of 2016 (Medina-Chávez et al., 2020). Microbial mats and associated
564 sediments (8 cm) were collected and transferred to sterile conical tubes (50 mL),
565 stored at 4ºC, and subsequently frozen in liquid nitrogen until processing in the
566 laboratory.
567
568 *Total DNA extraction and amplicons sequencing*
569 Prior to DNA extraction, approximately 0.5 g of each sample was rinsed with sterile
570 water. Total DNA extraction was performed following the protocol reported in de
571 Anda et al. (2018), which is based on slight modifications from the Purdy et al. (1996)
572 protocol.
573 DNA was sequenced at the Laboratorio de Servicios Genómicos, LANGEBIO for the
574 V3-V4 region of the 16S rRNA gene (357F primer (Rudi et al., 1997), and the ITS
575 nDNA region (ITS1F/ITS4R for ITS; (White et al., 1990). For each sample**,** 5 mg of
576 genomic DNA (DO 260/280 1.8) was used; 16S amplicons were sequenced within
577 Illumina Next-Seq 500 platform (150 SE), and the ITS amplicons were sequenced

578 with a Mi-Seq platform (2x300 PE). All raw sequences generated are available at
579 GeneBank Bioproject PRJNA705156 (ITS accession SAMN18070760; 16S
580 accession SAMN18070761.
581
582 *Bioinformatics analyses*
583 The 16S rRNA amplicon reads were quality filtered and dereplicated using the QIIME
584 2 bioinformatics platform version 2019.7 (Bolyen et al., 2018). Sequences were
585 demultiplexed and truncated at the 20th bp from the left, and the 120th bp from the
586 right. Divisive Amplicon Denoising Algorithm 2 (dada2) was then used to filter by
587 sequence quality, remove chimeras, denoise, and create a table of amplicon
588 sequence variants (ASV) per sample. ASVs were then taxonomically classified using
589 the silva_132_99_v3v4_q2_2019-7 database. For all subsequent analyses the final
590 ASV table was rarefied to 760,000 reads. For abundant ASVs (>1%) and
591 Archaeobacteria ASVs, an additional taxonomic classification was conducted by
592 selecting those taxa with coincident classification across the results of *blastN* (both
593 type strain and 16S blast) (Altschul et al., 1990), the RDBP classifier (Q. Wang et
594 al., 2007), and Qiime2 (Silva database) (Bolyen et al., 2018; Quast et al., 2013).
595 The ITS amplicon reads were processed using the ITS-specific workflow of the
596 *dada2* v1.13.1 package (Callahan et al., 2016) in R (R Core Team, 2020). Briefly,
597 *cutadapt* was used to remove primer and adapter contamination from the raw reads
598 (Martin, 2011). Prior to determining ASVs, reads with inconsistent bases were
599 excluded, and reads were required to have less than two expected errors based on
600 their quality scores. ASVs classification was inferred from forward and reverse reads
601 using the run-specific error rates (Qiime2 and R code is available at
602 https://gitlab.com/lauasuar/archaean-domes-repository).
603 Due to the wide range of sizes of the ITS amplicons reported for fungal species in
604 CCB (485-782 bp; (Velez et al., 2016)), two different strategies were implemented
605 to define taxonomic diversity. First, the pre-denoised ASVs were merged after
606 excluding reads with an overlap of less than 12bp and reads with mismatches in the
607 overlapping region. Chimeras were removed using the consensus method of
608 "removeBimeraDenovo" implemented in dada2. The taxonomic assignment was

609 performed with the UNITE database (Nilsson et al., 2019), using the RDP naive
610 Bayesian classifier (Wang et al., 2007) available in the *dada2* package with a
611 minimum bootstrap value of 80. ASVs with a minimum of 85% identity and with E-
612 values lower than e-45 were retained (Merge ITS database, Suppl Table 1). ASVs
613 without taxonomic assignment (mostly non-fungi organisms) were classified using
614 *blastn*, where only those ASVs within identities greater than 90% were enlisted
615 (Suppl. Table 1). The remaining ASVs were considered non-classified.
616 As a great proportion of the reads failed to merge (~56%), a parallel classification
617 was implemented using the forward independently. The RDP naive Bayesian
618 classifier was used on the two sets of reads (forward and reverse) to check for
619 congruence in the classification between of ASVs. ASVs that had an assignment
620 bootstrap value lower than 50, or showed inconsistent classifications among forward
621 and reverse reads, were aligned to the NCBI database to corroborate their
622 taxonomic assignment. Even though some ASVs reached a genus level assignment,
623 the taxonomic classification of forward reads was limited to the phylum level (ITS
624 table, Suppl. Table 2); a more robust classification of ASVs was reached when using
625 forward ASVs, thus all subsequent analyses were performed using the forward-only
626 ASVs. The Eukaryotic classification followed the UNITE database. The ITS table
627 abundance was rarefied to 167,660 reads using the *vegan* package (Oksanen et al.,
628 2013) in R (R Core Team, 2020).
629 In sum, the 16S rRNA amplicon filtered database included 760,326–1,973,982 high
630 quality reads per sample, with a total of 6,063 ASVs. The ITS amplicon filtered
631 database included 167,660–352,720 high quality reads per sample, with a total of
632 923 ASVs. ITS amplicons were obtained for only six samples (S1, S2, S3, S4, S6
633 and S7) due to DNA depletion in four samples (S5, S8, S9 and S10). The ASV
634 abundance tables and the amplicons sequences are available at
635 https://gitlab.com/lauasuar/archaean-domes-repository.
636
637
638
639

640 *Alpha and beta diversity estimates*

641 To evaluate all samples from 16S (S1-S10) and 6 samples (S1-S4, S6, S7) from the
642 Forward-Only ITS database, we generated rarefied diversity plots using the *vegan*
643 package (Oksanen et al., 2013) in R (R Core Team 2020).

644 In order to visualize and count the number of overlapping ASVs within the ten
645 samples from the 16S rRNA gene and the six samples from the ITS region, we
646 constructed an UpSet plot using the *UpSetR* package (Conway et al., 2017) in R (R
647 Core Team 2020).

648 Alpha (i.e., Chao1, Shannon) and beta (i.e., Bray-Curtis, Jaccard) indices were
649 estimated using the 16S rRNA ASV abundance table, as implemented in Qiime2.
650 For the ITS ASV abundance table, alpha and beta diversity indices were estimated
651 with the the *phyloseq* package (McMurdie & Holmes, 2013) in R (R Core Team,
652 2020). The beta diversity indices were used to construct an UPGMA dendrogram of
653 samples (Carteron et al., 2012). Mantel tests (999 permutations) (Legendre &
654 Legendre, 2012) were conducted with the *vegan* package (Oksanen et al., 2013) to
655 unveil the relationships between beta diversity and geographic distance, as well as
656 between beta diversity and the metabolomic profile distance matrix (see below).

657

658 *Phylogenetic diversity*

659 We estimated phylogenetic diversity within samples using Faith's Phylogenetic
660 Diversity index (Faith, 1992) and the Phylogenetic Species Clustering index (Helmus
661 et al., 2007) using the *picante* package (Kembel et al., 2010) in R (R Core Team
662 2020). Given a phylogenetic tree, phylogenetic diversity (PD) estimates the total
663 length of branches encompassing a subset of taxa (*e.g.*, sampled communities),
664 where PD increases as the subset of taxa is more phylogenetically diverse (Faith,
665 1992). Phylogenetic Species Clustering (PSC) is a measure of the degree of
666 clustering of taxa across the phylogeny (Helmus et al., 2007), where a PSC value
667 approaching zero indicates that the subset of taxa are clustered within the
668 phylogeny.

669 To estimate the two metrics, we used the rarified diversity matrices and the
670 corresponding phylogenetic trees for bacterial and fungal ASVs, separately. We

671 tested the observed patterns of PSC across samples against 1,000 replicates under
672 a null model and estimated standardized effect sizes (SES) for this index. This
673 allowed us to identify samples that had more or less phylogenetic clustering than
674 expected by chance alone. To construct the null model, we randomized the entries
675 of the rarified diversity matrices while keeping total sample diversity constant. In
676 addition, we estimated PD and PSC for the most abundant and rare ASVs within
677 each site. To define rare and abundant ASVs, we estimated the first and third
678 quartiles (1Q, 3Q) of the distribution of ASV abundances for each site separately.
679 We used the sites' 1Q and 3Q as the thresholds to define rare and abundant ASVs;
680 the sites' 1Qs ranged from 3 to 16 reads per ASV, whereas the 3Qs ranged from 37
681 to 306 reads per ASV. We estimated SES for PD and PSC as defined above, using
682 matrices for rare and abundant ASV (R code for all phylogenetic diversity analysis
683 is available at https://gitlab.com/lauasuar/archaean-domes-repository).
684 The phylogenetic tree was generated by QIIME 2 (Bolyen et al., 2018) for 16S data.
685 For the ITS data (Forward-Only database), a phylogeny was obtained by applying
686 an equivalent phylogenetic QIIME 2 methodology, using Maftt (Katoh & Toh, 2010)
687 and FastTree (Price et al., 2009) within Cipres Science Gateway (Miller et al., 2010).
688
689 *Pearson correlation estimate among abundant taxa*
690 We carried out an analysis of correlated presences and absences of microbial taxa
691 across communities to identify plausible interactions and ecological roles within
692 microbial genera (Karpinets et al., 2018). To do this, we used a normalized
693 abundance of six-sites matrix that included ASVs from both ITS databases and 16S
694 rRNA gene library (Suppl Table 1): <1% abundance classified the ASV 16S
695 database, 22 six-sites archaea ASVs, and <1% classified the ASV ITS database
696 (calculated without the first three high abundance ASVs). Data was Hellinger
697 transformed, with the *vegan* package (Oksanen et al., 2013) in R (R Core Team,
698 2020). This normalized matrix was used to calculate the correlogram with Pearson
699 correlation (positive and negative strong correlation ($r \geq 0.9$, $P \leq 0.05$)) using the R
700 Stats v3.6.1 library (R Core Team, 2020) and *corrplot* package for the graph (Taiyun
701 & Viliam, 2017).

702 *Metabolomics*

703 *Soluble compounds extraction and LC-MS/MS analysis*

704 The samples (10 g each) were extracted with 60 mL of $CHCl_3$-MeOH (1:1) in an
705 orbital shaker at 150 rpm for 24 hrs. The mixture was filtered, and the solvent was
706 evaporated under reduced pressure. The dried extracts were reconstituted in 60 mL
707 of CH_3CN-MeOH (1:1) and defatted with hexanes. Then the CH_3CN-MeOH layer
708 was dried under a vacuum and resuspended in MeOH (LC-MS grade) to yield a
709 concentration of 1 mg mL^{-1}. All samples were filtered with a 0.22 μm membrane and
710 analyzed using a Waters Acquity Ultraperformance Liquid Chromatography (UPLC)
711 system (Waters Corp.) coupled to a Thermo Q Exactive Plus (ThermoFisher) mass
712 spectrometer. The UPLC separations were performed using an Acquity BEH C_{18}
713 column (50 mm × 2.1 mm I.D., 1.7 μm; Waters) equilibrated at 40 °C and a flow rate
714 set at 0.3 mL min^{-1}. The mobile phase consisted of a linear CH_3CN-H_2O (acidified
715 with 0.1% formic acid) gradient starting at 15% to 100% of CH_3CN over 8 min. The
716 mobile phase was held for another 1.5 min at 100% CH_3CN before returning to the
717 starting conditions. High-resolution mass spectrometry (HRMS) data and MS/MS
718 spectra were collected in the positive/negative switching electrospray ionization
719 (ESI) mode at a full scan range of *m/z* 150−2000, with the following settings: capillary
720 voltage, 5 V; capillary temperature, 300°C; tube lens offset, 35 V; spray voltage 3.80
721 kV; sheath gas flow and auxiliary gas flow, 35 and 20 arbitrary units, respectively.

722

723 *Untargeted metabolomics*

724 The HRMS-MS/MS raw data was individually aligned and filtered with MZmine 2.17
725 software (http://mzmine.sourceforge.net/) (Pluskal et al., 2010). Peak detection was
726 achieved as follows: *m/z* values were detected within each spectrum above a
727 baseline, a chromatogram was constructed for each of the *m/z* values that spanned
728 longer than 0.1 min, and finally, deconvolution algorithms were applied to each
729 chromatogram to recognize the individual chromatographic peaks. The parameters
730 were set as follows for peak detection: noise level (absolute value) at 1×10^6,
731 minimum peak duration 0.5 s, tolerance for *m/z* variation 0.05, and tolerance for *m/z*
732 intensity variation 20%. Deisotoping, peak list filtering, and retention time alignment

algorithm packages were employed to refine peak detection. The join align algorithm compiled a peak table according to the following parameters: the balance between *m/z* and retention time was set at 10.0 each, *m/z* tolerance at 0.05, and retention time tolerance size was defined as 2 min. The spectral data matrix (comprised of *m/z*, retention time, and peak area for each peak) was imported to Excel (Microsoft) for heatmap analysis using *R* (R Core Team, 2020).

Molecular networking was performed from the mzML files using the standard Global Natural Products Social (GNPS) molecular networking platform workflow with the spectral clustering algorithm (http://gnps.ucsd.edu; Aron et al, 2020). For spectral networks, parent mass of 0.01 Da and fragment ion tolerance of 0.02 Da were considered. For edges construction, a cosine score over 0.70 was fitted with a minimum of 4 matched peaks, and two nodes at least in the top 10 cosine scores (K) and maximum of 100 connected the components. After that, the network spectra were searched against GNPS spectral libraries (full data, feature-based molecular networking can be accessed here: https://gnps.ucsd.edu/ProteoSAFe/status.jsp?task=547303b749f041bb88eee71145de5184) and graphic visualization of molecular networking was performed in Cytoscape 3.8.0 (Shannon et al., 2003). The chemical profiles were also dereplicated using UV-absorption maxima and HRMS-MS/MS data against the Dictionary of Natural Products v 29.1 and Dictionary of Marine Natural Products 2019 (Taylor and Francis Group), MarinLite (University of Canterbury, New Zealand) and SciFinder (CAS) databases as described by El-Elimat et al. (El-Elimat et al., 2013), for fungal and bacterial small molecules. For candidate search, exact mass accuracy was set to 5 ppm. Heatmap visualization of metabolomic data table was generated in MataboAnalyst (Pang et al., 2020).

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Competing interests

The authors declare that no competing interests exist.

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Figure legends

Figure 1

(A) The Cuatro Ciénegas Basin within México, and geographic location of Archaean Domes in Pozas Azules Ranch. (B) Detail of an Archaean Domes slice. (C) Domes when the sampling was conducted (note the size scale). Photos by David Jaramillo.

Figure 2

Genera relative abundance of taxa in Archaean Domes, Cuatro Ciénegas Basin sample. A) Bacteria (only showed the most common taxa, more than 1% of diversity) B) Fungi (using Merge database, only fungal ASVs. NA is for ASVs that were not assigned to any genera, but phylum only)

1275
1276 Figure 3
1277 Graphic representation of shared ASVs within Archaean Domes mats sampling
1278 sites. Number of shared ASVs is represented by black bars, showing the exact
1279 number on the top. Dots below bars denote sites where those ASVs are shared, and
1280 lines joining dots indicate the shared sites. Colored bars at bottom left of the main
1281 figure are for ASVs number within each site. Pays show taxonomy of shared ASVs
1282 within all sites (a few but abundant ASVs core).
1283 (A) 16S Archaea and Bacteria, rRNA gene database, (B) Fungi and other Eucarya,
1284 Forward-Only ITS database.
1285
1286 Figure 4
1287 Graph representing null model distribution of Phylogenetic Species Clustering
1288 (nmdPSC) values for all community, abundant and rare taxa from 16S rRNA
1289 database (A) and Forward-Only ITS database (B). Dotted lines are showing
1290 nmdPSC significant values: > +2 is consistent with a sample that had more
1291 phylogenetic overdispersion than expected by chance alone, and nmdPSC value <
1292 -2 indicates more phylogenetic clustering than expected by chance alone; values in
1293 the range +2 and -2 had a null model distribution. nmdPSC values are reported in
1294 Suppl. Table 5.
1295
1296 Figure 5
1297 Pearson correlation analysis within the most common (i.e., involving more than 1%
1298 of diversity) Bacteria, and all genera of Archaea and Fungi (from Merge ITS
1299 database). Colored squares are for significant correlations ($P \leq 0.05$), blue for positive
1300 ones and red for negatives.
1301
1302 Figure 6
1303 Molecular network of soil samples grouped metabolite features into 140 chemical
1304 families. Each node in the network represents one metabolite feature. Nodes
1305 connected to each other are structurally-related (cosine MS^2 similarity score ≥0.7).
1306
1307 Figure 7
1308 Structures of microbial metabolites tentatively annotated in samples S1-S10 (≤ 5
1309 ppm mass error).
1310
1311 Table1
1312 Alpha diversity indices for 16S rRNAgene and Forward-Only ITS region databases
1313 from Archaean Domes CCB microbial mats.
1314
1315 Suppl. Fig. 1
1316 (A) Phylum composition within 16S rRNA gene database (B) Kingdom composition
1317 (based on UNITE database eukaryotic taxonomy) within Forward-Only ITS region
1318 database.
1319
1320
1321

1322 Suppl. Fig. 2
1323 Rarefaction curves for 16S rRNA gene database (A) and ITS region Forward-Only
1324 ITS database (B).
1325
1326 Suppl. Fig. 3
1327 UPGMA dendrogram showing composition similarities among sampling sites
1328 (Forward-Only ITS database for Eucarya UPGMA).
1329 Branch length represent Bray-Curtis and Jaccard-1 dissimilarity coefficient, based
1330 on (A) abundance of 16S rDNA sequences ASVs, and (B) abundance of ITS region
1331 database ASVs. The numbers (height) are Bray-Curtis and Jaccard values.
1332
1333 Suppl. Figure 4
1334 Heatmap analysis showing relative abundance of metabolite features (MS ions in
1335 positive mode) in the Archaean Domes mats samples. On the scale bar, brick red
1336 color indicates increased metabolite levels, and blue color represents decreased
1337 levels. A shows non clustered samples and sites, B clustered samples and sites.
1338
1339 Suppl. Table 1.
1340 List of ASVs Blast hits with <90%, of non-fungal Merge ITS database.
1341
1342 Suppl. Table 2.
1343 Abundance tables (16S rRNA gene and ITS region taxa).
1344
1345 Suppl. Table 3.
1346 Counts of significant negative and positive correlations from Pearson Correlation
1347 Analysis (Figure 5).
1348
1349 Suppl. Table 4.
1350 Tentatively identified microbial metabolites in samples S1-S10 by GNPS and
1351 detailed metabolomic analysis.
1352
1353 Suppl. Table 5
1354 nmdPSC values