The Metabolic Capability and Phylogenetic Diversity of Mono Lake During a Bloom of the Eukaryotic Phototroph *Picocystis* strain ML

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Running Head: Metabolism and Diversity of Mono Lake in Algal Bloom

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ABSTRACT

Algal blooms in lakes are often associated with anthropogenic eutrophication; however, they can occur naturally. In Spring of 2016 Mono Lake, a hyperalkaline lake in California, was near the height of a rare bloom of the algae Picocystis strain ML and at the apex of a multi-year long drought. These conditions presented a unique sampling opportunity to investigate microbiological dynamics during an intense natural bloom. We conducted a comprehensive molecular analysis along a depth transect near the center of the lake from surface to 25 m depth during June 2016. Across sampled depths, rRNA gene sequencing revealed that Picocystis associated chloroplast were found at 40-50 % relative abundance, greater than values recorded previously. Despite the presence of the photosynthetic oxygenic algal genus Picocystis, oxygen declined below detectible limits below 15 m depth, corresponding with an increase in microorganisms known to be anaerobic. In contrast to previously sampled years, metagenomic and metatranscriptomic data suggested a loss of sulfate reducing microorganisms throughout the lake's water column. Gene transcripts associated with Photosystem I and II were expressed at both 2 m and 25 m, suggesting that limited oxygen production may occur at extremely low light levels at depth within the lake. Oxygenic photosynthesis under low light conditions, in the absence of potential grazing by the brine shrimp Artemia, may allow for a cryptic redox cycle to occur in an otherwise anoxic setting at depth in the lake with the following effects: enhanced productivity, reduced grazing pressure on Picocystis, and an exacerbation of bloom.

IMPORTANCE

Mono Lake, California provides habitat to a unique ecological community that is heavily stressed due to recent human water diversions and a period of extended drought. To date, no baseline

69 information exists about Mono Lake to understand how the microbial community responds to
70 drought, bloom, and what genetic functions are lost in the water column. While previously
71 identified anaerobic members of the microbial community disappear from the water column
72 during drought and bloom, sediment samples suggest these microorganisms seek refuge at lake
73 bottom or in the subsurface. Thus, the sediments may represent a type of seed bank which could
74 restore the microbial community as a bloom subsides. Our work also sheds light on the activity
75 of the halotolerant algae Picocystis strain ML during a bloom at Mono Lake, its ability to
76 potentially produce oxygen via photosynthesis even under extreme low-light conditions, and how
77 the remainder of the microbial community responds.
78

79 ## Introduction

80

81 Mono Lake is a large hypersaline alkaline lake with a maximum depth of ≈ 50 m in the Mono
82 Basin near the eastern foothills of the Sierra Nevada Mountains, California (Figure 1). It formed
83 from the remnant of Paleolake Russell (a Pleistocene glacial lake) and has existed as a closed
84 basin for at least 50,000 years (1). Diversion of tributary streams to Mono Lake by the city of
85 Los Angeles began in 1941, and resulted in a drop of over 13 m in lake level by 1978 (2) with a
86 corresponding increase in water salinity from 48 g/L to 81 g/L by the 1990s (3) and a current
87 alkalinity of 30,400 ppm HCO_3^- (4). The steep decline in lake level also resulted in increasing
88 concentrations of other solutes (including arsenic), resulting in unusual lake geochemistry and a
89 the absence of large macrofauna (e.g., fish) (5). Mono Lake is home to a photosynthetic
90 eukaryotic algae, *Picocystis* (6) that is the primary food source of a brine shrimp endemic to the
91 lake, *Artemia monica* (7). In turn, *Artemia* is a crucial food source for birds along the North

American Pacific Flyway (8, 9) where Mono Lake's microbial / eukaryotic ecosystem serves a unique, multi-compartment, interlinked ecosystem role.

Beyond the visible macrofauna that overfly and nest at Mono Lake, the water and sediment both contain high concentrations of arsenic that have made the lake a prime location to study arsenic cycling in a natural setting (10, 11). Populations of *Gammaproteobacteria* from the family Helicobacteraceae, capable of phototrophic arsenate reduction, are commonly found within Mono Lake (12, 13). Recently, genes associated with sulfate reduction were identified below the oxycline (≈ 15 m) in Mono Lake while the lake was meromictic (i.e., stratified) (11). Prior to 2017, microbial community surveys of the lake during meromixis were only carried out using 16S rRNA gene clone library sequencing or denaturing gradient gel electrophoresis (DGGE) (14). Clone library sequencing using dideoxy chain terminator sequencing (15) is limited by sequencing coverage, and recent work using 454 Pyrosequencing (16) at Mono Lake provided additional diversity information for the lake during the onset of meromixis. However, the PCR primers chosen for previous high-throughput and clone library based amplicon surveys at Mono Lake were potentially biased (17, 18), and more recent primers for Illumina based amplicon sequencing (18) could provide a more accurate representation of lake microbial community. Furthermore, community distribution and profiling within Mono Lake during monomixis, or mixing of lake waters during a single time in a year, has yet to occur. Transcriptional profiling was recently carried out (11) with the same samples sequenced for rRNA gene analyses in another recent study (16) that describes the microbial activity from surface to below the oxycline at Mono Lake (11). A thorough description of the eukaryote responsible for much of the primary productivity in Mono Lake however, remains lacking from recent research. Such description for

how, for example, the algae responsible for this primary productivity, *Picocystis strain ML*, is distributed within Mono Lake during a bloom and its impact on the ecophysiology of the lake is of crucial importance to ensure that a critical food source for migratory macrofauna is not lost.

Picocystis is a genus of phototrophic algae, previously characterized in other saline or alkaline environments (19, 20). *Picocystis* strain ML identified in Mono Lake (6), is a near relative of *Picocystis salinarium*, isolated from the San Francisco Salt Works in a high-salinity (~ 85 ‰) pond (19). In addition to *P. salinarium*, other near relatives have been identified from hypersaline environments in inner Mongolia (21). *Picocystis* strain ML at Mono Lake is responsible for 100 mmol C m^{-2} d^{-1} of the primary productivity in the lake (6). Although *Picocystis* is nitrogen limited, if sufficient concentrations of ammonia are present during lake mixing and turnover (5), a bloom can occur often coinciding with periods of lake anoxia (6). It is also unknown, but possible that dissolved organic nitrogen (DON) may be a N source utilized by this eukaryote. The population density of *Picocystis* varies throughout the year, often reaching a maximum in early spring before falling as *Artemia* graze on them, reproduce and greatly reduce their number as measured by cell count (5). A possible key strategy for survival is that *Picocystis* strain ML is adapted to low-light conditions and anoxia near the bottom of Mono Lake, which prevents overgrazing by *Artemia* (22), or population decline when overgrowth reduces light transmission. Elevated concentrations of chlorophyll *a* and *Picocystis* are commonly detected below the oxycline (6). Yet, it is unknown if *Picocystis* is actively producing photosynthetic pigments and photosynthesizing under low-light conditions *in situ*, at depth. If *Picocystis* is capable of phototrophic growth below the oxycline, localized production of oxygen may disrupt localized anaerobic microbial communities in the bottom waters of Mono Lake. During a recent

study, sulfate reducing microorganisms were identified alongside strictly anaerobic Clostridia at a depth of 15 m below the oxycline (11), yet conditions may not be conducive for anaerobic microbial sulfate reduction during a bloom of phototrophic algae that produce's oxygen throughout the water column.

Mono Lake entered into a period of monomixis in 2012 corresponding to the onset of a near-record drought in the Eastern Sierra, resulting in a subsequent bloom of *Picocystis* in 2013 that failed to subside over the subsequent three years (23) and corresponded with a near record low of *Artemia* present within the lake in 2015. Lake clarity was at near-record lows and measured chlorophyll *a* concentrations were high in 2016 (23). Here, we describe the effects of an algal bloom during a period of intense drought within Mono Lake during the summer of 2016 on the distribution and abundance of the bacterial, archaeal, and eukaryotic planktonic microbial community and compare this to previously sampled years within the lake (11, 16). The possibility that the microbial community within Mono Lake could be re-populated by the sediment, groundwater, and streams that feed Mono Lake is also addressed. Finally, we determined if *Picocystis* strain ML is transcriptionally active under extremely low light levels in the lake.

Results

Major Ion Chemistry and Microbial rRNA Gene Copy Number within Mono Lake

At depths between 5 and 15 m, the water temperature decreased from ≈15 to ≈7 ˚C. Dissolved oxygen and photosynthetically active radiation (PAR) declined rapidly within the first 10 m, yet, fluorescence was above detectable limits throughout the sampled depths (Figure 2a). Microbial

density estimated by bacterial and archaeal 16S rRNA gene copy number varied by less than 10% from 2 to 25 m. In contrast, a eukaryotic 18S rRNA gene copy number maximum was present at 20 and 25 m (Figure 2b). Major anions including sodium (Na^+) were consistent, and near previously reported values (Table 1). Only minimal differences in anion or cation concentrations were detected within Mono Lake. Nitrate, nitrite, and sulfate were elevated at 10 m relative to 2, 20, and 25 m. No phosphate was detectable by ion chromatography (IC) from 2 to 25 m within Mono Lake, though surface water taken near shore had an average value of 0.02 mM (Table 1). Total dissolved phosphorus (potentially including phosphate and organophosphorus) measured by ICP-AES ranged from 0.59 to 0.63 mM ($\pm$0.08 mM) from surface to 25 m depth, respectively (Table 1). Most major anions and cations, and dissolved inorganic carbon, were below detectable limits in the sampled stream water and well water, with the exception of calcium which was elevated relative to Mono Lake water samples (Table 1). Individual replicate results for ICP-AES and IC are shown in supplemental table S1.

Bacterial and Eukaryotic Microbial Community of Mono Lake, Sediment, and Surrounding Streams

After quality control a total of 694,948 DNA sequence reads were obtained, clustering into 831 operational taxonomic units (OTUs). Additional summary statistics are found in Supplementary Table S2. Chloroplast sequences were abundant across all lake water samples and were removed from further analysis. The bacterial and archaeal community differed in structure above and below the oxycline (Figure 3a). Samples taken from sediment at 10 m depth near the water sampling site also were distinct in bacterial, archaeal, and eukaryotic community structure from those in the sampled water column. Two OTUs most closely related to genera within the order

184 Bacteroidetes decreased in relative abundance steadily with depth: *Psychroflexus* and ML602M-
185 17, whereas unclassified Bacteroidetes remained relatively constant in abundance throughout the
186 water column (Figure 3a). An OTU most closely related to the genus *Thioalkalivibrio* increased
187 in abundance as depth increased. Unique to the sediment were the Euryarchaeota and the
188 bacterial genus *Desulfonatroibacter*. An increase in the relative abundance of chloroplast
189 sequence was noted at 20 m, increasing from 39.7 at the surface to 48.4 at 10 m, and then to 61.9
190 percent relative abundance at 20 m (Supplemental Figure S1). Well water taken to compare to
191 lakewater samples contained an abundant population of OTUs most closely related to sulfur
192 oxidizing Proteobacteria including *Thiothrix* and *Thiobacillus*, as well as Actinobacteria
193 (*Rhodococcus*), and an abundant unclassified OTU within the Hydrogenophilaceae. Mono Lake
194 influent stream water samples collected and examined from Rush, Mill, Lee Vining, and Wilson
195 were distinct from samples taken from the lake itself, with the Flavobacteria, *Sediminibacterium*,
196 and the hgcI clade of the Actinobacteria being the most abundant OTUs across all stream
197 samples (Figure 3a). Mill was an outlier to other stream water samples, lacking abundant
198 populations of Actinobacteria (candidatus *Planktophila*, and hgcI clade) and a lower abundance
199 of the *Sediminibacterium* relative to Lee Vining, Rush, and Wilson streams. Community
200 membership and distribution in the lake water column profile samples were significantly
201 influenced ($p = 0.002$, $R^2 = 0.90$) by depth and the transition to anoxia visualized by weighted
202 UniFrac PCoA ordination and a corresponding ADONIS test (Figure 4a).
203
204 Compared to the observed bacterial and archaeal community, the eukaryotic community
205 contained far fewer OTUs. Within the water column at Mono Lake, an almost homogenous
206 distribution of OTUs most closely related to the genus *Picocystis* was observed at all depths,

with a maximum of to 97.9% relative abundance at 10 m depth (Fig 3b) during the sampled bloom event of 2016. Within the sediment, an OTU of unclassified Branchiopoda was most abundant, comprising 90.9% of all sediment eukaryotic sequence. BLAST results of this OTU suggest it is most likely *Artemia monica*, endemic to Mono Lake, although because of the short sequence read length of 250 bp the identification is ambiguous. Influent stream water samples were distinct from the water and sediment of Mono Lake, with few overlapping OTUs among the samples (Fig 3b). Specifically, multiple OTUs most closely related to the Ochrophyta (Heterokont algae), Ciliophora, and Chytridiomycota were unevenly distributed across the stream and well water sampled. Community membership and distribution within the water column at Mono Lake was significantly influenced (0.017, $R^2 = 0.61$) by depth and the transition to anoxia visualized by weighted UniFrac PCoA ordination and a corresponding ADONIS test, although less significantly than the bacteria and archaeal community (Figure 4b).

Metagenomic and Transcriptomic Profiling of Mono Lake and Sediments

A summary of assembly statistics for sediment and water samples are available in Table S3. The abundance of sulfate (> 100 mM) and the lack of oxygen beg the question of whether active sulfate reduction is occurring in the dissolved organic carbon (DOC) rich waters of Mono Lake. No sulfite oxidase genes (*sox*) were identified, however genes for the complete reduction of sulfate to sulfide were identified in the sediment metagenome, and genes for reverse-dissimilatory sulfite reductases (*dsrA*) were identified in water metagenomes. No true reductive *dsrA* genes were identified in the water metagenomes. Dissimilatory sulfite reductase genes within the sediment metagenome had high (> 80 %) homology to known Deltaproteobacterial sulfate reducing microorganisms. Reductive *dsrA/B* genes were identified within the water

column, identified putatively via BLAST that most closely related to known *Thioalkalivibrio* *dsrA/B* genes. Sulfite reductase genes did not appear to be expressed within the metatranscriptome (Table S4). Nitrate and nitrite reductases were found at 20, 25 m and within the sediment, while nitric oxide reductase (*nor*) was only identified within the sediment (Table S3). Genes associated with nitrogen fixation, including *nifH, D,* and *K* were found at 20 m within the water, and within the sediment metagenome. No genes associated with ammonium oxidation by bacteria or archaea (AOB/AOA) were identified. Formate-dependent nitrite reductases were identified as both genes and transcripts (Supplemental Table S4). A comprehensive list of identified transcripts is avalible as supplemental table S4 at the 10.6084/m9.figshare.6272159.

Metagenome Assembled Genomes of Mono Lake and Sediment

After refinement binning, a metagenomic analysis identified 80 metagenome assembled genomes (MAGs) of varying completion and contamination (Supplementary Table S3). Of the 80 identified MAGs, 38 were greater than 50 percent complete, and less than 10 percent contaminated with other DNA sequence. A subset of XX of these MAGs contained rRNA gene sequence, and a putative identification was produced from these data (Supplemental figure S2). Like the rRNA gene sequencing data, MAGs indicate that microbial community composition shifted by depth and correlated to the decline in oxygen at 10 m (Figure 5). A large number of MAGs were unique to the sediment, including a Euryarchaeon (Figure 5). No archaea were found in abundance throughout the sampled water column. However, no genes associated with the production of methane were identified. Multiple MAGs were recovered from uncultivated orders within the Actinobacteria, Gammaproteobacteria, and Bacteroidetes (Table S3) including MAGs with 16S rRNA gene sequence previously identified by rRNA gene clone library

253 sequencing at Mono Lake such as ML602J-51 (14). A summary of each genome is available in
254 Supplementary Table S3, and figure 5. No MAGs were identified with the genes required for
255 sulfate reduction, with only reverse-dsr genes found in MAGs. Nitrogen fixation genes (*nifH, D,*
256 and *K*) were identified within 3 MAGs, two within the Gammaproteobacteria (Bin 10 and 23), as
257 well as a single unclassified bin (Bin_11_2). One bin (Bin 45) contained photosystem II
258 associated genes, identified within the Epsilonproteobacteria (Table S3, Figure 5). Three MAGs
259 were identified in the EukRep filtered metagenomic sequence. A single MAG was identified
260 with 18S rRNA gene sequence closely related to that of *Picocystis* strain ML (Supplemental
261 Figure S2). However, this MAG appears to be contaminated with bacterial sequence, although
262 putative searching of the identified sequence returns homology to other known algae. While the
263 MAG should be interpreted with caution, it represents a partial genome sequence of *Picocystis* sp.
264 from Mono Lake. The annotated genome contained no genes related to sulfur cycling, and other
265 incomplete metabolic cycles (Supplemental Figure S3).
266
267 *Metatranscriptomics Suggested Photosynthesis was Active at 25 m*
268 Assembly of transcriptomes from 2 m and 25 m resulted in 113,202 coding sequences, and
269 111,709 annotated protein coding genes. No transcripts were identified with homology to known
270 dissimilatory sulfite reductases. A total of 3,117 genes were differentially expressed ($p < 0.05$
271 false discovery rate (FDR) corrected) between 2 m and 25 m (Supplemental Table S4). More
272 transcripts identified within the co-assembled metatranscriptome were significantly upregulated
273 at 25 m relative to 2 m (Figure 6). Genes associated with Photosystem I and II pathways were
274 expressed at both sampled depths (Supplemental table S4, Table 2). Expression values for
275 photosystem I and II transcripts including *psaA/B*, *psbA/B*, and *psbC* were significantly

upregulated at 25 m relative to 2 m depth (Table 2). In addition, several light-independent protochlorophyllide reductase transcripts were significantly upregulated at 25 m, while no transcripts related to chlorophyll production were significantly upregulated at 2 m (Supplementary table S4).

Discussion

Beginning in late 2012, Mono Lake exhibited signs of persistent *Picocystis* blooms. Subsequently, from 2013 to 2016 both lake clarity and *Artemia* abundance declined dramatically (23). Surface concentrations of chlorophyll *a* averaged 3.8 µM in July (1994-2013), yet 2016 concentrations were ten times higher, 33.9 µM (23). The elevated chlorophyll *a* concentration and Secchi disk values (indicative of lake clarity) above 1 m suggest that Mono Lake was well within a bloom of *Picocystis*. The relative abundances of microorganisms presented here and the well-mixed major ions of Mono Lake relative to previous work (11, 14), indicated that our sampling represents the first high-throughput molecular study of Mono Lake during a *Picocystis* bloom and concurrent monomixis. Genes required for sulfate reduction to sulfide were detected only in the sequenced lake sediment, while both metagenomic and 16S rRNA gene sequencing indicated a near complete loss of the anaerobic sulfate reducing potential within the water column of Mono Lake. Instead, a mixed algal and facultatively anaerobic microbial community was present below the detectable oxycline, more similar to the near-surface microbial community than previously reported (11). It is yet unknown how the microbial community of Mono Lake will rebound after such a significant algal bloom and a decline in the population of *Artemia* within the lake.

Our survey allowed for a comprehensive evaluation of the genomic potential, and expressed genes associated with metabolic processes throughout the water column. Dissimilatory nitrate reduction to ammonium (DNRA) appeared active, with formate-dependent cytochrome c nitrite reductases detected within the transcriptome (Table S4) and formate-dependent nitrite reductase subunits within the assembled metagenomes (Table S3). No genes associated with ammonium oxidation (AOB) were identified in contrast to previous years (24) in either the transcriptome or metagenome, suggesting that the ammonia produced within the lake was assimilated, likely by the dense population of growing *Picocystis*. In addition to nitrate reduction another key anaerobic respiratory process, sulfate reduction, was largely absent from the water column.

Previous work during meromixis/non-bloom intervals has shown that sulfate reduction is a key respiratory process in Mono Lake, supporting the growth of multiple species of sulfide oxidizing aerobic microorganisms above the oxycline (11). We found that microorganisms capable of sulfate reduction were only identified in sediment metagenomic samples during the bloom. Dissimilatory-type reverse sulfite reductases associated with sulfur oxidizing gammaproteobacterial (25) taxa were identified at 20 and 25 m, but no true reductive sulfite reductases were found in sequenced water samples. Taxa known to reduce sulfate were also only identified by 16S rRNA gene sequencing in stark contrast to previously sampled years (11, 26). Instead, the most abundant microorganisms with identifiable *dsrA/B* gene clusters were reverse-*dsr* type reductases identified previously in the Gammaproteobacterium genus *Thioalkalivibrio* (25). While lake sulfate reduction rates are typically very low (27) our data suggest a complete loss of sulfate reducing activity in the water column during a bloom. It is likely that during a bloom, sulfate reduction is repressed as more oxidizing conditions are present throughout the

323 water column due to an increased abundance of oxygenic photosynthetic algae. Members of the
324 Bacteroidetes were in high abundance throughout the water column, including OTUs most
325 closely related to ML310M-34, which remained abundant through the water column and
326 *Psychroflexus*, which decreased in abundance from 2 to 25 m as oxygen levels declined. The
327 eukaryotic microbial community was more evenly distributed throughout the water, with
328 *Picocystis* detected in near equivalent relative abundance throughout the water column (Figure
329 3b), agreeing with reported chlorophyll levels (23), as well as fluorescence values measured as a
330 part of this study (Figure 2a).
331
332 Eukaryotic 18S rRNA gene copy number was greater at 20 and 25 m than above the oxycline by
333 approximately 40 percent. The results were similar to previous estimates of *Picocystis* biomass
334 during bloom events (6). *Artemia* grazing pressure was unusually low during 2016, likely
335 allowing for the increase in *Picocystis* abundance throughout the sampled water column and
336 accounting for the similarly low visibility (Secchi disk) readings. Additional primary
337 productivity in the lake could also account for the oxycline shallowing from 15 m depth in 2013
338 (11, 14) to 10 m depth in July 2016. This expansion of anoxic waters likely limits *Artemia*
339 populations from grazing on *Picocystis.* Lake temperature decreases at the surface relative to
340 previous studies may also slow the metabolism of *Artemia,* resulting in reduced fecundity and
341 increased mortality (22, 28). A decline in *Artemia* population could also impact bird mortality,
342 though this was outside the scope of this study, and should be investigated at a later date.
343
344 A key finding of this study is the confirmation that *Picocystis* strain ML appears capable of
345 photosynthesis under very low light conditions near the bottom of Mono Lake. Previous work

suggested that *Picocystis* strain ML is capable of growth under very low light conditions, and showed elevated concentrations of chlorophyll below 15 m at Mono Lake (6). Chloroplast 16S rRNA gene sequence was most abundant at 20 m, corresponding to a peak in total 16S rRNA copy number (Figure 2b). 18S rRNA gene sequence identified as *Picocystis* were most abundant at 10 m, yet chloroplast relative abundance peaked at 20 m, near previously recorded peak depths in other recorded bloom events (6). Despite the high relative abundance of *Picocystis* throughout the water column, isolation and characterization of the *Picocystis* genome remains elusive. Binning resulted in a partial MAG with an incomplete 18S rRNA gene fragment with high similarity to the published sequence of *Picocystis* strain ML. Genome sequencing of *Picocystis*, recently isolated and sequenced twice independently (Ronald Oremland, personal communication), will allow for its genome to be removed from subsequent sequencing efforts which will simplify assembly, and enhance the resolution of bacterial and archaeal binning efforts in the future, yielding a better understanding of the microbial community responsible for the diverse metabolic potential in both the sediments and water of Mono Lake. Despite the lack of a reference genome, our transcriptomic sequencing was able to recover *Picocystis* chloroplast associated transcripts. At 25 m depth, a significant upregulation of Photosystem II was observed (Table 1, Supplemental Table S4). This, combined with the 40 percent increase in the number of 18S rRNA gene copies at 25 m relative to 2 m suggest that there is, at a minimum, a near equivalent amount of transcription of photosynthesis-associated genes throughout the water column. Recently, photosynthesis in a microbial mat was shown to be capable under extremely low light concentrations, although in a bacterial system (29). Still, the presented data suggest that under extreme low light conditions, photosynthesis may still occur. This is the first

368 transcriptomic evidence from Mono Lake to support previous laboratory observations of
369 *Picocystis* growing under low light conditions (6).
370
371 Our study represents the first study of Mono Lake during the height of an algal bloom and
372 suggests significant shifts in both the bacterial and archaeal microbial community and its
373 metabolic potential from non-bloom years (11, 16). *Picocystis* was present throughout the water
374 column, and apparently carrying out oxygenic photosynthesis even at extremely low levels of
375 light at depth within the lake. While Picocystis bloomed throughout Mono Lake, there was also a
376 loss of sulfate reducing microorganisms. The lack of sulfate reduction at and below 20 m within
377 Mono Lake is in contrast to previous work and is possibly linked to the intense drought
378 experienced by Mono Lake from 2012 to 2016. During such a drought anaerobic microorganisms
379 may seek refuge within the underlying sediment. By sequencing nearby sediment, we have
380 shown that even if sulfate reduction is temporarily lost in the planktonic community of Mono
381 Lake, the sediment may act as a "seed bank" or refugia for organisms capable of this, and likely
382 other necessary metabolisms dependent upon overlying water / lake conditions (30).
383 Alternatively, the sulfate reducing microorganisms may find a better reduced substrate or fewer
384 inhibitors in the sedimentary environment. Furthermore, the recovery of microbial populations
385 within Mono Lake must come from its' sediment or underlying groundwater, not from the
386 streams that feed it as no overlapping taxa exist. Establishing if, and how, the chemistry and
387 microbiota of Mono Lake recover after monomixis, drought, and algal bloom should be the focus
388 of future work. Such research can be compared against our metagenomic and transcriptomic
389 during bloom as well as previous metatranscriptomic sequencing (11) to better understand how,

or if, the microbial community of Mono Lake returns to its previous state after extended periods of both monomixis and algal bloom.

Materials and Methods

Sampling

A vertical profile of PAR (LiCor 2π quantum sensor, 400-700 nm, E m^{-2} s^{-2}), dissolved oxygen (SBE 43, mg/L^{-1}), and attenuation coefficient (WetLabs transmissometer, 600 nm wavelength light source, 10 cm path length, m-1) from surface (0 m) to ~30 m was taken using a SeaBird SBE 19 Conductivity, Temperature, and Depth (CTD) probe calibrated for use at Mono Lake. After measurements were obtained water was pumped from depth to the surface at station 6 (37.95739,-119.0316, Figure 1), sampled at 2 m, 10 m, 20 m, and 25 m the following day (due to lake conditions) using a submersible well-pump. Water was allowed to flow from the measured depth for 1 to two minutes to clear any residual water from the lines prior to sampling. *Artemia* were removed from water samples using clean cheese cloth prior to filling 1 L sterile high-density polyethylene containers. Samples were stored in a dark cooler until filtration occurred. Sediment was sampled at 10 m depth (37.9800, -119.1048) using a box-core sampling device. Well water (38.0922,-118.9919) was sampled by allowing the wellhead to flow for approximately 5 minutes before filling a 5 L HDPE container completely. For influent stream water, 1 L of water was taken from each location (Mill: 38.0230,-119.1333, Rush: 37.8883,-119.0936, Wilson: 38.0430,-119.1191) into a sterile HDPE container. Lee Vining (37.9422, -119.1194) and was sampled with the use of a submersible pump (as above) into a sterile 1 L HDPE container.

413 *Geochemical Water Analysis*

414 To characterize the water samples taken from 2 m to 25 m, major anions were measured using a
415 Dionex ICS-90 ion chromatography system running an AS14A (4 × 250 mm) column. Major
416 cations were also measured using a Perkin-Elmer Optima 5300 DV Inductively Coupled Plasma
417 Optical Emission Spectrometer (ICP-OES). Both IC and ICP were conducted in the Department
418 of Chemistry at the Colorado School of Mines. All sediment samples were extracted for ion
419 chromatography (IC) and ICP analysis following the Florida Department of Environmental
420 Protection method #NU-044-3.12. All fluid samples were filtered in the field using 0.22 µm PES
421 filters. All ICP samples were acidified with trace-metal grade nitric acid as per standard
422 procedure to ensure stabilization of all metal cations.
423
424 *Environmental Sampling, Field Preservation, and DNA/RNA Extraction of Samples*
425 Immediately after sampling concluded, water from Mono Lake and surrounding streams were
426 filtered onto 25 mm 0.22 µm polyether sulfone filters (Merck Milipore Corp., Billerica, MA) in
427 triplicate. Separate triplicate filters were obtained from each water sample for DNA and RNA
428 extraction respectively. Filter volumes are available in Supplemental Table S1. After filtration,
429 samples were immediately suspended in 750 µL DNA/RNA shield (Zymo Research Co., Irvine,
430 CA), and homogenized on-site using a custom designed lysis head for 1 m using a reciprocating
431 saw. Sediment samples were immediately preserved on-site by adding sediment directly to
432 DNA/RNA shield as above. Preserved samples were maintained on dry ice, and then stored at –
433 80 ˚C (RNA) or –20 ˚C (DNA) until extractions were performed. DNA extraction was carried
434 out using the Zymo Xpedition DNA mini kit (Zymo Research Co.), and samples were eluted into

435 a final volume of 100 µL. RNA extraction was performed using the Zymo QuickRNA Mini Prep
436 (Zymo Research Co.) according to manufacturer's instructions.
437
438 *rRNA Gene Sequencing Library Preparation*
439 Libraries of bacterial, archaeal, and eukaryotic SSU rRNA gene fragments were amplified from
440 each DNA extraction using PCR with primers (Integrated DNA Technologies Co., Coralville,
441 IA) that spanned the ribosomal RNA gene V4 hypervariable region between position 515 and
442 926 (*E. coli* numbering) that produced a ~400 bp fragment for bacteria and archaea, and a 600 bp
443 fragment for the eukaryotes. These primers evenly represent a broad distribution of all three
444 domains of life (18). The forward primer 515F-Y (**GTA AAA CGA CGG CCA G** CCG TGY
445 CAG CMG CCG CGG TAA-3') contains the M13 forward primer (in bold) fused to the ssuRNA
446 gene specific forward primer (underlined) while the reverse primer 926R (5'-CCG YCA ATT
447 YMT TTR AGT TT-3') was unmodified from Parada et. al 2015. 5 PRIME HOT master mix (5
448 PRIME Inc., Gaithersburg, MD) was used for all reactions at a final volume of 50 µL. Reactions
449 were purified using AmpureXP paramagnetic beads (Beckman Coulter Inc., Indianapolis, IN) at
450 a final concentration of 0.8 x v/v. After purification, 4 µL of PCR product was used in a
451 barcoding reaction, cleaned, concentrated, and pooled in equimolar amounts as previously
452 described (31). The pooled, prepared library was then submitted for sequencing on the Illumina
453 MiSeq platform (Illumina Inc., San Diego, CA) using V2 PE250 chemistry.

454 *Quantitative PCR*
455 Total bacterial/archaeal and eukaryotic small subunit (SSU) rRNA gene count within the water
456 column was obtained using two TaqMan based probe assays as previously described (32, 33).
457 Briefly, both assays were carried out using 25 µL reactions containing 1x final concentration of

458 Platinum™ Quantitative PCR SuperMix-UDG w/ROX (Thermo Fisher Scientific Inc.), 1.8 µM
459 of each primer, and 225 nM of either the bacterial/archaeal, or eukaryotic probe.

460 *SSU rRNA Gene Analysis*

461 Sequence reads were demultiplexed in QIIME version 1.9.1 (34), and filtered at a minimum Q
462 score of 20 prior to clustering. Sequence reads were first denoised and then clustered into
463 operational taxonomic units (OTUs) using UPARSE (35). After clustering, OTUs were assigned
464 taxonomy using mothur (36) against the SILVA database (r128, (37)). Each OTU was then
465 aligned against the SILVA r128 database using pyNAST (38), filtered to remove uninformative
466 bases, and then a tree was created using the maximum likelihood method and the Jukes Cantor
467 evolutionary model within FastTree 2 (39). A BIOM formatted file (40) was then produce for use
468 in analyses downstream. To limit OTUs originating from contaminating microorganisms found
469 in extraction and PCR reagents (41) all extraction blanks and PCR controls were processed
470 separately and a core microbiome was computed. Any OTU found in 95% of controls was
471 filtered from the overall dataset. Differences in community composition were estimated using the
472 weighted UniFrac index (42). The effect of depth was tested using an adonis using the R package
473 Vegan (43) within QIIME. Taxa heatmaps and ordination plots were generated using phyloseq
474 (44) and AmpVis (45).
475
476 Sequencing reads for all samples are available under the project PRJNA387610. A mapping file
477 is available both in supplemental table S2. The mapping file, as well as BIOM files used for
478 analyses are available at 10.5281/zenodo.1247529 including an R Markdown notebook including
479 the necessary steps to automate initial demultiplexing, quality filtering, and OTU clustering, as
480 well as reproduce figures associated with the rRNA gene analyses.

481

482 *Metagenomic/Transcriptomic Sequencing*

483 Metagenomic and metatranscriptomic samples were prepared using the Nextera XT library
484 preparation protocol. Prior to library preparation, first strand cDNA synthesis was carried out
485 using the ProtoScript cDNA synthesis kit (New England Biolabs, Ipswich, MA), followed by
486 second strand synthesis using the NEBNext mRNA second strand synthesis module (New
487 England Biolabs). A mixture of random hexamer and poly-A primers was using during first
488 strand synthesis. After conversion to cDNA, samples were quantified using the QuBit HS Assay,
489 and then prepared for DNA sequencing. Briefly, 1 ng of DNA or cDNA was used as input into
490 the NexteraXT protocol (Illumina, Inc.) following manufacturer's instructions. After
491 amplification, libraries were cleaned using AmpureXP paramagnetic beads, and normalized
492 following the NexteraXT protocol. All metagenomic and transcriptomic samples were then
493 sequenced on the Illumina NextSeq 500 Instrument using PE150 chemistry (Illumina, Inc.).

494

495 *Metagenomic Assembly and Binning*

496 Prior to assembly, metagenomic libraries were quality filtered and adapters removed using PEAT
497 (46). A co-assembly was produced using MEGAHIT (47) with a minimum contig length of 5000
498 basepair. After assembly, quality filtered reads from individual samples were mapped to the co-
499 assembly using Bowtie2 (44). Assembled contigs greater than 5 kb in length were first filtered to
500 remove eukaryotic sequence using EukRep (48) and then binned into MAGs using CONCOCT
501 (49) and refined using Anvi'o (50), in an attempt to manually reduce potential contamination or
502 redundancy within each bin. Finally, bin quality was assessed using CheckM (51).

503

CheckM was also used to identify possible SSU rRNA gene fragments within each bin. Putatively identified SSU rRNA gene fragments were aligned against the SILVA 132 database (37) using SINA (52). After alignment, sequences were added to the SILVA tree by SINA, and near relatives were included to give a putative identification of MAGs containing SSU sequence. The identities of each MAG with SSU sequence are available in Supplementary Table 2.

Metatranscriptomic Analysis

Metatranscriptome libraries were first filtered for quality and adapter removal using PEAT (46). After quality control, sequence files were concatenated into a single set of paired-end reads in FASTQ format, and then assembled *de novo* using Trinity (53). Post-assembly the Trinotate package (https://trinotate.github.io/) was used to annotate assembled transcripts. After assembly, reads were mapped against transcripts using Bowtie2 (54), and differential significance was assessed using DEseq2 (55). Assembly, annotation, mapping, and statistical analyses were carried out using XSEDE compute resources (56).

Data Availability

Sequence data are available in the NCBI sequence read archive under the BioProject accession PRJNA387610.

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References

1. **Reheis MC**, **STINE S**, **Sarna-Wojcicki AM**. 2002. Drainage reversals in Mono Basin during the late Pliocene and Pleistocene. Geol Soc Am Bull **114**:991–1006.

2. **Cloud P**, **Lajoie KR**. 1980. Calcite-impregnated Defluidization Structures in Littoral Sands of Mono Lake, California. Science **210**:1009–1012.

3. **Jellison R**, **Melack JM**. 2003. Meromixis in hypersaline Mono Lake, California. 1. Stratification and vertical mixing during the onset, persistence, and breakdown of meromixis. Limnol Oceanogr **38**:1008–1019.

4. **Nielsen LC**, **DePaolo DJ**. 2013. Ca isotope fractionation in a high-alkalinity lake system: Mono Lake, California. Geochim Cosmochim Acta **118**:276–294.

5. **Melack JM**, **Jellison R**, **MacIntyre S**, **Hollibaugh JT**. 2017. Mono Lake: Plankton Dynamics over Three Decades of Meromixis or Monomixis, pp. 325–351. *In* Ecology of Meromictic Lakes. Springer International Publishing, Cham.

6. **Roesler CS**, **Culbertson CW**, **Etheridge SM**, **Goericke R**, **Kiene RP**, **Miller LG**, **Oremland RS**. 2002. Distribution, production, and ecophysiology of Picocystis strain ML in Mono Lake, California. Limnology and Oceanography **47**:440–452.

7. **Warnock N**. 2010. Stopping vs. staging: the difference between a hop and a jump. J Avian Biol **41**:621–626.

8. **Jehl JR**. 1988. Biology of the Eared Grebe and Wilson's Phalarope in the nonbreeding season: a study of adaptations to saline lakes. Studies in Avian Biology **12:**1-74

9. **Jehl JR**. 1997. Cyclical Changes in Body Composition in the Annual Cycle and Migration of the Eared Grebe Podiceps nigricollis. J Avian Biol **28**:132.

10. **Oremland RS**, **Hoeft SE**, **Santini JM**, **Bano N**, **Hollibaugh RA**, **Hollibaugh JT**. 2002. Anaerobic oxidation of arsenite in Mono Lake water and by a facultative, arsenite-oxidizing chemoautotroph, strain MLHE-1. Appl Environ Microbiol **68**:4795–4802.

11. **Edwardson CF**, **Hollibaugh JT**. 2017. Metatranscriptomic analysis of prokaryotic communities active in sulfur and arsenic cycling in Mono Lake, California, USA. ISME J **54**:103.

12. **Hoeft SE**, **Blum JS**, **Stolz JF**, **Tabita FR**, **Witte B**, **King GM**, **Santini JM**, **Oremland RS**. 2007. Alkalilimnicola ehrlichii sp. nov., a novel, arsenite-oxidizing haloalkaliphilic gammaproteobacterium capable of chemoautotrophic or heterotrophic growth with nitrate or oxygen as the electron acceptor. Int J Syst Evol Microbiol **57**:504–512.

13. **Hoeft McCann S**, **Boren A**, **Hernandez-Maldonado J**, **Stoneburner B**, **Saltikov CW**, **Stolz JF**, **Oremland RS**. 2016. Arsenite as an Electron Donor for Anoxygenic Photosynthesis: Description of Three Strains of Ectothiorhodospira from Mono Lake, California and Big Soda Lake, Nevada. Life (Basel) **7**:1.

14. **Humayoun SB**, **Bano N**, **Hollibaugh JT**. 2003. Depth distribution of microbial diversity in Mono Lake, a meromictic soda lake in California. Appl Environ Microbiol **69**:1030–1042.

15. **Sanger, F, Nicklen, S, and Coulson, AR** (1977). DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci U S A **74**:5463–5467.

16. **Edwardson CF**, **Hollibaugh JT**. 2018. Composition and Activity of Microbial Communities along the Redox Gradient of an Alkaline, Hypersaline, Lake. Frontiers in Microbiology **9**:403.

17. **Klindworth A**, **Pruesse E**, **Schweer T**, **Peplies J**, **Quast C**, **Horn M**, **Glöckner FO**. 2013. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. Nucleic Acids Research **41**:e1.

18. **Parada A**, **Needham DM**, **Fuhrman JA**. 2015. Every base matters: assessing small subunit rRNA primers for marine microbiomes with mock communities, time-series and global field samples. Environ Microbiol.

19. **Lewin RA**, **Krienitz L**, **Goericke R**, **Takeda H**, **Hepperle D**. 2000. Picocystis salinarum gen. et sp. nov. (Chlorophyta) – a new picoplanktonic green alga. Phycologia **39**:560–565.

20. **Krienitz L**, **Bock C**, **Kotut K**, **Luo W**. 2012. Picocystis salinarum (Chlorophyta) in saline lakes and hot springs of East Africa. Phycologia **51**:22–32.

21. **Fanjing K**, **Qinxian J**, **and EJNR**, **2009**. Characterization of a eukaryotic picoplankton alga, strain DGN-Z1, isolated from a soda lake in Inner Mongolia, China. NREI **15:**38

22. **Wear RG**, **Haslett SJ**. 1986. Effects of temperature and salinity on the biology of Artemia fransiscana Kellogg from Lake Grassmere, New Zealand. 1. Growth and mortality. J Exp Mar Bio Ecol **98**:153–166.

23. **Collins AG**. 2017. In Response to the State Water Resources Control Board Order Nos. 98-05 and 98-07 COMPLIANCE REPORTING.

24. **Ward BB**, **Martino DP**, **Diaz MC**, **Joye SB**. 2000. Analysis of ammonia-oxidizing bacteria from hypersaline Mono Lake, California, on the basis of 16S rRNA sequences. Appl Environ Microbiol **66**:2873–2881.

25. **Sorokin DY**, **Muntyan MS**, **Panteleeva AN**, **Muyzer G**. 2012. Thioalkalivibrio sulfidiphilus sp. nov., a haloalkaliphilic, sulfur-oxidizing gammaproteobacterium from alkaline habitats. Int J Syst Evol Microbiol **62**:1884–1889.

26. **Scholten JCM**, **Joye SB**, **Hollibaugh JT**, **Murrell JC**. 2005. Molecular analysis of the sulfate reducing and archaeal community in a meromictic soda lake (Mono Lake, California) by targeting 16S rRNA, mcrA, apsA, and dsrAB genes. Microb Ecol **50**:29–39.

27. **Oremland RS**, **Miller LG**, **Whiticar MJ**. 1987. Sources and flux of natural gases from Mono Lake, California. Geochimica et Cosmochimica Acta **51**:2915–2929.

28. **Wear RG**, **Haslett SJ**, **Alexander NL**. 1986. Effects of temperature and salinity on the biology of Artemia fransiscana Kellogg from Lake Grassmere, New Zealand. 2. Maturation, fecundity, and generation times. J Exp Mar Bio Ecol **98**:167–183.

29. **Haas S**, **de Beer D**, **Klatt JM**, **Fink A**, **Rench RM**, **Hamilton TL**, **Meyer V**, **Kakuk B**, **Macalady JL**. 2018. Low-Light Anoxygenic Photosynthesis and Fe-S-Biogeochemistry in a Microbial Mat. Frontiers in Microbiology **9**:982.

30. **Gibbons SM**, **Gilbert JA**. 2015. Microbial diversity—exploration of natural ecosystems and microbiomes. Curr Opin Genet Dev **35**:66–72.

31. **Stamps BW**, **Lyles CN**, **Suflita JM**, **Masoner JR**, **Cozzarelli IM**, **Kolpin DW**, **Stevenson BS**. 2016. Municipal Solid Waste Landfills Harbor Distinct Microbiomes. Frontiers in Microbiology **7**:534.

625 32. **Liu CM**, **Aziz M**, **Kachur S**, **Hsueh P-R**, **Huang Y-T**, **Keim P**, **Price LB**. 2012.
626 BactQuant: an enhanced broad-coverage bacterial quantitative real-time PCR assay.
627 BMC Microbiol **12**:56.

628 33. **Liu CM**, **Kachur S**, **Dwan MG**, **Abraham AG**, **Aziz M**, **Hsueh P-R**, **Huang Y-T**,
629 **Busch JD**, **Lamit LJ**, **Gehring CA**, **Keim P**, **Price LB**. 2012. FungiQuant: a broad-
630 coverage fungal quantitative real-time PCR assay. BMC Microbiol **12**:255.

631 34. **Caporaso JG**, **Kuczynski J**, **Stombaugh J**, **Bittinger K**, **Bushman FD**, **Costello EK**,
632 **Fierer N**, **Peña AG**, **Goodrich JK**, **Gordon JI**, **Huttley GA**, **Kelley ST**, **Knights D**,
633 **Koenig JE**, **Ley RE**, **Lozupone CA**, **McDonald D**, **Muegge BD**, **Pirrung M**, **Reeder**
634 **J**, **Sevinsky JR**, **Turnbaugh PJ**, **Walters WA**, **Widmann J**, **Yatsunenko T**,
635 **Zaneveld J**, **Knight R**. 2010. QIIME allows analysis of high-throughput community
636 sequencing data. Nature Publishing Group **7**:335–336.

637 35. **Edgar, RC** (2013). UPARSE: highly accurate OTU sequences from microbial
638 amplicon reads. Nat Methods 10, 996–998.

639 36. **Schloss PD**, **Westcott SL**, **Ryabin T**, **Hall JR**, **Hartmann M**, **Hollister EB**,
640 **Lesniewski RA**, **Oakley BB**, **Parks DH**, **Robinson CJ**, **Sahl JW**, **Stres B**,
641 **Thallinger GG**, **Van Horn DJ**, **Weber CF**. 2009. Introducing mothur: open-source,
642 platform-independent, community-supported software for describing and comparing
643 microbial communities. Appl Environ Microbiol **75**:7537–7541.

644 37. **Pruesse E**, **Quast C**, **Knittel K**, **Fuchs BM**, **Ludwig W**, **Peplies J**, **Glöckner FO**.
645 2007. SILVA: a comprehensive online resource for quality checked and aligned

ribosomal RNA sequence data compatible with ARB. Nucleic Acids Res **35**:7188–7196.

38. **Caporaso JG**, **Bittinger K**, **Bushman FD**, **DeSantis TZ**, **Andersen GL**, **Knight R**. 2010. PyNAST: a flexible tool for aligning sequences to a template alignment. Bioinformatics **26**:266–267.

39. **Price MN**, **Dehal PS**, **Arkin AP**. 2010. FastTree 2 – Approximately Maximum-Likelihood Trees for Large Alignments. PLoS One **5**:e9490.

40. **McDonald D**, **Clemente JC**, **Kuczynski J**, **Rideout JR**, **Stombaugh J**, **Wendel D**, **Wilke A**, **Huse S**, **Hufnagle J**, **Meyer F**, **Knight R**, **Caporaso JG**. 2012. The Biological Observation Matrix (BIOM) format or: how I learned to stop worrying and love the ome-ome. GigaScience **1**:7.

41. **Salter, SJ, Cox, MJ, Turek, EM, Calus, ST, Cookson, WO, Moffatt, MF, Turner P, Parkhill J, Loman NJ, Walker AW.** (2014). Reagent and laboratory contamination can critically impact sequence-based microbiome analyses. BMC Biol 12.

42. **Lozupone C**, **Knight R**. 2005. UniFrac: a New Phylogenetic Method for Comparing Microbial Communities. Appl Environ Microbiol **71**:8228–8235.

43. **Dixon P**. 2003. VEGAN, a package of R functions for community ecology. J Veg Sci **14**:927–930.

665 44. **McMurdie PJ**, **Holmes S**. 2013. phyloseq: An R Package for Reproducible Interactive
666 Analysis and Graphics of Microbiome Census Data. PLoS One **8**:e61217.

667 45. **Albertsen M**, **Karst SM**, **Ziegler AS**, **Kirkegaard RH**, **Nielsen PH**. 2015. Back to
668 Basics – The Influence of DNA Extraction and Primer Choice on Phylogenetic
669 Analysis of Activated Sludge Communities. PLoS One **10**:e0132783.

670 46. **Li Y-L**, **Weng J-C**, **Hsiao C-C**, **Chou M-T**, **Tseng C-W**, **Hung J-H**. 2015. PEAT: an
671 intelligent and efficient paired-end sequencing adapter trimming algorithm. BMC
672 Bioinformatics **16 Suppl 1**:S2.

673 47. **Li D**, **Liu CM**, **Luo R**, **Sadakane K**, **Lam TW**. 2015. MEGAHIT: an ultra-fast
674 single-node solution for large and complex metagenomics assembly via succinct de
675 Bruijn graph. Bioinformatics **31**:1674–1676.

676 48. **West, P. T., Probst, A. J., Grigoriev, I. V., Thomas, B. C., and Banfield, J. F.**
677 (2018). Genome-reconstruction for eukaryotes from complex natural microbial
678 communities. Genome Res 28, 569–580.

679 49. **Alneberg J**, **Bjarnason BS**, **de Bruijn I**, **Schirmer M**, **Quick J**, **Ijaz UZ**, **Lahti L**,
680 **Loman NJ**, **Andersson AF**, **Quince C**. 2014. Binning metagenomic contigs by
681 coverage and composition. Nat Meth **11**:1144–1146.

682 50. **Eren AM**, **Esen ÖC**, **Quince C**, **Vineis JH**, **Morrison HG**, **Sogin ML**, **Delmont TO**.
683 2015. Anvi'o: an advanced analysis and visualization platform for 'omics data. PeerJ
684 **3**:e1319.

685 51. **Parks DH**, **Imelfort M**, **Skennerton CT**, **Hugenholtz P**, **Tyson GW**. 2015. CheckM:
686 assessing the quality of microbial genomes recovered from isolates, single cells, and
687 metagenomes. Genome Res **25**:1043–1055.

688 52. **Pruesse E**, **Peplies J**, **Glöckner FO**. 2012. SINA: accurate high-throughput multiple
689 sequence alignment of ribosomal RNA genes. Bioinformatics **28**:1823–1829.

690 53. **Grabherr MG**, **Haas BJ**, **Yassour M**, **Levin JZ**, **Thompson DA**, **Amit I**, **Adiconis**
691 **X**, **Fan L**, **Raychowdhury R**, **Zeng Q**, **Chen Z**, **Mauceli E**, **Hacohen N**, **Gnirke A**,
692 **Rhind N**, **di Palma F**, **Birren BW**, **Nusbaum C**, **Lindblad-Toh K**, **Friedman N**,
693 **Regev A**. 2011. Full-length transcriptome assembly from RNA-Seq data without a
694 reference genome. Nat Biotechnol **29**:644–652.

695 54. **Langmead B**, **Salzberg SL**. 2012. Fast gapped-read alignment with Bowtie 2. Nat
696 Methods **9**:357–359.

697 55. **Love MI**, **Huber W**, **Anders S**. 2014. Moderated estimation of fold change and
698 dispersion for RNA-seq data with DESeq2. Genome Biol **15**:1–21.

699 56. **Towns J**, **Cockerill T**, **Dahan M**, **Foster I**, **Gaither K**, **Grimshaw A**, **Hazlewood V**,
700 **Lathrop S**, **Lifka D**, **Peterson GD**, **Roskies R**, **Scott JR**, **Wilkens-Diehr N**. 2014.
701 XSEDE: Accelerating Scientific Discovery. Computing in Science & Engineering
702 **16**:62–74.

703

704 ## Figure Legends and Tables

705 Figure 1. Overview of North Eastern California, with Mono Lake inset. Approximate sampling
706 location is shown by the white circle/cross. Scale is given in kilometers. Overview image
707 captured from Google Earth/Landsat. Inset image modified from U.S. Geological Survey
708 Miscellaneous Field Studies Map MF-2393 (Raumann et. al 2002).
709
710 Figure 2. CTD measurements taken during 2016 sampling (A), with salinity (squares),
711 fluorescence (circles), and PAR (crosses) shown on the upper axis, and temperature (triangles)
712 and dissolved oxygen (diamonds) shown on the lower axis. Points are half-meter averages, with
713 standard deviation shown. For clarity, lines connecting temperature and PAR are dashed.
714 Quantification of 16S and 18S rRNA gene copy number (B) at discrete sampling depths of 2, 10,
715 20, and 25 m. 16S rRNA gene copy number is shown by closed circles, and 18S by closed
716 squares, with error bars representing the mean standard deviation of triplicate biological and
717 triplicate technical replicates.
718
719 Figure 3. Heatmap of the top 25 OTUs within the bacteria/archaea (A) or eukarya (B). OTUs are
720 named by Phyla, and the most likely genera.
721
722 Figure 4. Principal component (PCoA) ordination of bacteria/archaeal (A) and eukaryotic (B)
723 communities of water samples taken at Mono Lake. Ordination based on a weighted UniFrac
724 distance matrix.
725
726 Figure 5. Overview of detected MAGs across sampled metagenomes and metatranscriptomes
727 (denoted as cDNA within the figure). Color intensity from grey to blue corresponds to the

728 coverage of each MAG within each sample. Estimates of GC content, completeness, and
729 contamination of each MAG are also given. Presence (black) of key genes related to sulfur,
730 nitrogen, and carbon cycling, as well as respiration are also shown. *fcc* = Sulfide dehydrogenase ,
731 *sqr* = Sulfide-quinone reductase, *sat* = sulfate adenylyltransferase, *apr* = adenosine-5-
732 phosphosulfate reductase, *dsr* = Dissimilatory sulfite reductase, *nap* = periplasmic nitrate
733 reductase, *nar* = nitrate reductase, nrf = nitrite reductase, *nir* = nitrite reductase, *nor* = nitric
734 oxide reductase, *nos* = nitric oxide synthase, *nifD* = Nitrogenase molybdenum-iron protein alpha
735 chain, *nifH* = nitrogenase iron protein 1, *nifK* = Nitrogenase molybdenum-iron protein beta
736 chain , PSII = photosystem II, *cbb* = ribulose 1,5-bisphosphate carboxylase/oxygenase, *bic* =
737 bicarbonate transporter, *acc* = acetyl-CoA carboxylase, *pcc* = propionyl-CoA carboxylase, *fad* =
738 Long-chain-fatty-acid--CoA ligase , *fadE* = Acyl-coenzyme A dehydrogenase , *cox* =
739 cytochrome c oxidase, *hyd* = Hydrogenase I, *hyf* = Hydrogenase-4, *hoxS* = bidirectional NiFe
740 Hydrogenase.
741
742 Figure 6. Normalized and centered expression values of *de novo* assembled transcripts
743 significantly (FDR corrected p value < 0.05) expressed at either 2 or 25 m.

744 Table 1. Measured geochemical parameters from the water column, as well as nearby streams and well water, representing subsurface
745 water below Mono Lake. All values reported in millimolar (mM) as the average of triplicate samples, unless otherwise noted.

Depth (In m)	Surface	2	10	20	25	Well	Lee Vining	Mill	Rush	Wilson
Analyte										
As[a]	0.19±0.01	0.18±0.00	0.19±0.01	0.17±0.00	0.20±0.03	BDL	BDL	BDL	BDL	BDL
Br[a]	0.76±0.09	0.98±0.00	1.10±0.05	1.97[d]	0.96±0.01	BDL	BDL	BDL	BDL	BDL
Ca[a]	BDL	BDL	BDL	BDL	BDL	3.2±3.4	2.7[d]	1.1±1.7	4.5±4.0	1.6±2.4
Cl^{-}[,b]	578 ± 4.1	588±1.9	695±35	585±7.6	577±7.3	0.24±0.01	0.02±0.00	0.01±0.00	0.07±0.00	0.01±0.00
F^{-}[,b]	2.7±0.34	3.6±0.02	4.1±0.17	3.5±0.03	3.5±0.06	0.02±0.00	BDL	0.01[d]	BDL	BDL
Fe[a]	0.01	0.01	BDL	BDL	BDL	0.02	BDL	BDL	BDL	0.01
K[a]	37±7.1	39±2.0	38±4.0	37±5.5	41±3.8	0.24±0.21	BDL	BDL	BDL	BDL
Mg[a]	1.9±1.4	1.7±1.2	1.0±0.02	1.0±0.10	1.4±0.30	2.8±4.2	1.5[d]	0.14[d]	0.34[d]	5.1[d]
Na[a]	1030±10	874±13	866±74	696±286	892±59	4.2±0.31	0.29±0.26	0.33[d]	0.37±0.17	0.55±0.60
NO_2[b]	0.37[d]	BDL	0.77[d]	BDL	BDL	BDL	BDL	BDL	BDL	BDL
NO_3[b]	0.01[d]	0.03[d]	0.15[d]	0.03[d]	0.09[d]	0.01±0.00	0.01±0.00	BDL	BDL	BDL
P[a]	0.59±0.08	0.62±0.05	0.61±0.03	0.59±0.04	0.63±0.07	BDL	BDL	BDL	BDL	BDL
PO_4[b]	0.02[d]	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
S[a]	111±13	125±1.2	124.68±2.21	120.48±3.6	130±4.7	0.29±0.11	0.05±0.04	0.17±0.06	0.16±0.12	0.25±0.10
SO_4[b]	122±1.1	113±0.71	136.73±9.30	113.62±1.7	112±1.1	0.25±0.01	0.05±0.00	0.13±0.00	0.05±0.00	0.15±0.00
DIC[c]	NA	313	300	322	318	5.19	NA	NA	NA	NA

746 [a] Measured using ICP-AES. BDL, Below Detectible Limit.

747 [b] Measured using Ion Chromatography (IC). BDL, Below Detectible Limit.

[c] Dissolved inorganic carbon (DIC) reported from a single sample per site.

[d] Measurement from one or two samples. No standard deviation was calculated. NA indicates sample not measured.

750 Table 2. Mean expression values of select genes identified associated with photosynthesis.

751

Gene	Mean Exp. 25 m	Mean Exp. 2 m	Log_2 Fold Change	p value (FDR)
psaA	155.4	80.4	1.0	0.008
psaA	209.7	86.8	1.3	0.0001
psaB	247.0	123.0	1.0	0.002
psbA	563.7	176.7	1.7	< 0.0001
psbB	241.1	97.4	1.3	< 0.0001
psbB	165.4	74.8	1.1	0.001
psbC	183.5	84.1	1.1	< 0.0001
psbY	23.5	18.4	0.3	> 0.05
gyrA[a]	4.2	4.4	-0.03	-
gyrB[a]	5.2	5.5	-0.04	-

752 [a]Gyrase shown as an average expression value of all annotated *gyrA/B* transcripts at each
753 sampled depth.

754

755