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1 **Title:**

2 # Transient exposure to oxygen or nitrate reveals ecophysiology of fermentative and sulfate-
3 # reducing benthic microbial populations

4
5

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26
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34 **Short summary**

35 Fermentation coupled to sulfate reduction is a globally important process for the remineralization
36 of organic carbon in marine sediments. The present study uses long-term, replicated continuous
37 culture bioreactors and meta-omics to investigate the ecophysiology of the involved microbial
38 populations at an unprecedented resolution. We reveal complex trophic networks, in which
39 fermenters and sulfate reducers coexist with nitrate- and oxygen respirers, we indicate strategies
40 and niches of the microbial populations, and describe a novel and widespread, yet uncultured
41 fermentative organism. These insights are crucial to understand fermentation coupled to sulfate
42 reduction and relevant to assess microbial dynamics and community-level responses in coastal
43 ecosystems.

44

45 **Abstract**

46 For the anaerobic remineralization of organic matter in marine sediments, sulfate reduction
47 coupled to fermentation plays a key role. Here, we enriched sulfate-reducing/fermentative
48 communities from intertidal sediments under defined conditions in continuous culture. We
49 transiently exposed the cultures to oxygen or nitrate twice daily and investigated the community
50 response. Chemical measurements, provisional genomes and transcriptomic profiles revealed
51 trophic networks of microbial populations. Sulfate reducers coexisted with facultative nitrate
52 reducers or aerobes enabling the community to adjust to nitrate or oxygen pulses. Exposure to
53 oxygen and nitrate impacted the community structure, but did not suppress fermentation or sulfate
54 reduction as community functions, highlighting their stability under dynamic conditions. The most
55 abundant sulfate reducer in all cultures, related to *Desulfotignum balticum*, appeared to have
56 coupled acetate oxidation to sulfate reduction. We described a novel representative of the
57 widespread uncultured phylum *Candidatus* Fermentibacteria (formerly candidate division Hyd24-
58 12). For this strictly anaerobic, obligate fermentative bacterium, we propose the name *Ca.*
59 "Sabulitectum silens" and identify it as a partner of sulfate reducers in marine sediments. Overall,
60 we provide insights into the metabolic network of fermentative and sulfate-reducing microbial
61 populations, their niches, and adaptations to a dynamic environment.

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62 **Introduction**

63 Around 30% of the total oceanic phytoplankton-derived primary production occurs along the
64 continental margins (Walsh, 1991) and up to 50% of this organic matter reaches the surface of
65 shallow coastal sediments. This organic matter can be re-mineralized by the microorganisms in
66 the surface sediment using a broad suite of electron acceptors, such as oxygen, nitrate, metal oxides
67 and sulfate (Henrichs and Reeburgh, 1987; Canfield *et al.*, 1993; Janssen *et al.*, 2005). It has been
68 estimated that about 50% of the total organic carbon mineralization in shallow sediments
69 (Jørgensen, 1982) and salt marsh sediments (Howes *et al.*, 1984) and up to 35% of the total
70 mineralization in intertidal flats (Billerbeck *et al.*, 2006) is coupled to sulfate reduction. Yet,
71 despite the global importance of sulfate reduction, the ecophysiology of the involved
72 microorganisms and their environmental controls are poorly constrained.

73

74 The sulfate-reducing microbial populations in the surface sediments of intertidal flats are exposed
75 to pulses of oxygen approximately twice daily, because of tidal cycling. In addition, the
76 communities may be regularly exposed to pulses of nitrogen from riverine sources (van Beusekom,
77 2005; Boyer *et al.*, 2006). It is thus very likely that sulfate reducers and also other key anaerobic
78 functional types, such as fermenters, are adapted to these ecosystem dynamics and survive
79 exposure to oxygen and nitrate. Generally, the availability of oxygen leads to a lower relative
80 importance of sulfate reduction, because electron acceptors tend to be consumed in a
81 thermodynamically determined order (the redox cascade). According to this order, oxygen is used
82 first, followed by nitrate, manganese and iron oxides, and finally sulfate (Froelich *et al.*, 1979).
83 Hence, sulfate is thought to be the predominant electron acceptor only in the anoxic layers after
84 other electron acceptors are depleted. Sulfate-reducing bacteria are often strict anaerobes and
85 couple the oxidation of molecular hydrogen or organic compounds to the complete reduction of
86 sulfate to sulfide (Muyzer and Stams, 2008; Rabus *et al.*, 2013). Nevertheless, sulfate-reducing
87 bacteria were detected throughout the whole sediment of an intertidal flat, including the aerobic
88 and denitrifying zones (Llobet-Brossa *et al.*, 2002; Mußmann *et al.*, 2005; Gittel *et al.*, 2008). In
89 addition, it was found that intertidal flats are a sink for riverine and atmospheric nitrogen (Gao *et*

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90 *al.*, 2012), with the microbial conversion of nitrate to ammonium or dinitrogen (Marchant *et al.*,
91 2014) and the internal storage of nitrate in benthic diatoms (Stief *et al.*, 2013) being widespread
92 and important processes. Also, nitrite is common in intertidal flats and it was found that some
93 sulfate reducers, like *Desulfovibrio desulfuricans*, are able to grow on hydrogen coupled to
94 ammonification of nitrate or nitrite (Dalsgaard and Bak, 1994). Although, much progress has been
95 made in understanding the key processes and populations in intertidal sediments, e.g. elucidating
96 the environmental controls of nitrate respiration (Kraft *et al.*, 2014) and the impact of chemical
97 gradients on community structure (Chen *et al.*, 2017), the trophic network defining combined
98 fermentation and sulfate reduction remains largely unknown.
99
100 A major challenge in microbial ecology in general is to understand the dynamics of an ecosystem
101 and its impact on the microbial communities (Widder *et al.*, 2016). This can be addressed e.g. by
102 investigating the resistance and resilience of microbial communities to perturbations (Shade *et al.*,
103 2012; Lee *et al.*, 2017), or by investigating the response of microbial communities to recurring
104 events (Ward *et al.*, 2017). Simple model systems are a promising tool to disentangle community
105 dynamics and constrain cause and effect (Widder *et al.*, 2016). To investigate the effect of the tidal
106 cycle on fermentation coupled to sulfate reduction as a community function, we set up defined
107 continuous cultures and created a homogeneous microbial habitat that selected for communities of
108 sulfate-reducing and fermentative bacteria. We inoculated the cultures with biomass from tidal flat
109 sediments that were exposed to a tidal cycle. The effect of diurnal exposure to oxygen or nitrate
110 on the microbial activity and community structure was examined by combined chemical,
111 metagenomic and transcriptomic analyses. Using this setup, we gained insights into fermentation
112 coupled to sulfate reduction and the involved trophic networks, as well as into the ecophysiology
113 of an uncultured candidate phylum.
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118 **Results and Discussion**

119

120 **Physiology of the continuous cultures**

121 Six cultures were inoculated with cell suspensions obtained from intertidal sediments of the
122 Janssand tidal flat. The cultures were continuously supplied with sulfate as electron acceptor and
123 a mixture of glucose, seven different amino acids and acetate as electron donors. This mixture was
124 chosen to stimulate the growth of a wide range of organisms, and represents compounds of
125 decaying biomass, which is the main organic carbon source in marine sediments. After two days,
126 sulfide was detected in all cultures and increased during the first 150 days, to a concentration of 2
127 – 6 mM (Fig. 1). All six cultures were incubated for 20 days under identical sulfate-only conditions
128 to establish anaerobic communities that carry out fermentation-coupled sulfate reduction. From
129 day 21 onward, four of the cultures were treated with oxygen or nitrate pulses, while two cultures
130 remained untreated. Oxygen was supplied to two replicate cultures (Oxy-1 and Oxy-2) for 30
131 minutes twice daily, by sparging the cultures with air. Two replicate cultures (Nit-1 and Nit-2)
132 were supplied with a nitrate solution for seven minutes twice daily. The final two replicate cultures
133 (Con-1 and Con-2) did not receive any additional electron acceptor and served as an untreated
134 control. The biomass in each of the cultures remained stable during the entire experiment (OD_{600}:
135 ~0.15; Fig. S2). The sulfide concentrations in the cultures, in combination with the nature of the
136 provided carbon sources, indicated that we selected for a syntrophic community of fermenters and
137 sulfate reducers. It was expected that the fermenting bacteria convert glucose and amino acids to
138 short-chain fatty acids, lactate, alcohols, or hydrogen. These could then be used as carbon sources
139 and/or electron donors by the sulfate-reducing bacteria (Rabus *et al.*, 2013).

140

141 We characterized the cultures in detail on day 311 (Oxy-1 and Oxy-2), day 327 (Nit-1 and Nit-2)
142 and day 300 (Con-1 and Con-2). During the air supply, the oxygen concentration was stable at
143 around 1.3% air saturation (3.1 μM), while the sulfide concentration decreased by 0.7±0.4 mM
144 (Fig. S3A). In the cultures supplied with nitrate, sulfide concentrations did not decrease (Fig. S3B)
145 and the nitrate was metabolized within ~200 min after termination of the supply (Fig. S3D). In

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146 both treatments we observed the transient production of elemental sulfur. In the oxygen treatment
147 we measured sulfur concentrations of up to 0.8 mM immediately after the start of aeration,
148 decreasing to ~0.1 mM within 2-4 hours (Fig. S3E). In the nitrate cultures, sulfur was increasing
149 from ~0.1 mM to up to 0.4 mM within 2 hours, and decreased to ~0.1 mM within 4 hours after the
150 start of the treatment (Fig. S3F). Using ^{15}N-labeled nitrate we found no production of ^{15}N-labeled
151 N_2, which indicated that ammonia may have been the end product of nitrate reduction. Ammonia
152 production could not be assessed directly because of the high background ammonia concentration
153 that resulted from ammonification of the supplied amino acids. Over the one year incubation,
154 transient oxygen supply yielded the lowest average sulfide concentrations (2.3±0.3 mM; Fig. 1A),
155 followed by the cultures that received nitrate (4.2±0.6 mM, Fig. 1B) and the untreated control
156 cultures (6.3±0.7 mM, Fig. 1C). Fluctuations in sulfide concentration were highest in the nitrate
157 treatment and lowest in the cultures that were not exposed to oxygen or nitrate. Yet, the cyclic
158 exposure to oxygen or nitrate did not suppress sulfide production (Fig. S3), and thus sulfate
159 reduction, as a community function. Aerobic respiration and ammonification coincided with a
160 decreased magnitude and stability of the sulfide concentration, likely due to microbial re-oxidation
161 of sulfide, or due to competition between sulfate reducers, aerobes or nitrate ammonifiers.
162

163 **Microbial communities and their response to cyclic exposure to oxygen and nitrate**

164 After around 300 days of cultivation, we sequenced the metagenomes of the six continuous
165 cultures. We hypothesized that cyclical exposure to oxygen or nitrate alters resource access to
166 create ecological niches that resemble those present in permeable intertidal sediments. Each
167 treatment would thus select for a different microbial community. Indeed, the community structure
168 was different between the treatments (Fig. 2, S4). Yet, all treatments and cultures had a similar
169 microbial community composition (Fig. 2). The nitrate treatment favored fermentative organisms
170 that were less abundant in other treatments, such as *Defluviitaleaceae* (bin K) and certain
171 *Spirochaeta* (bin M/bin N) (Fig. 2, 4A, S5). Moreover, each of the replicate cultures Nit-1 and Nit-
172 2 selected for communities of different structure, although they experienced the same selective
173 pressure. The cultures exposed to oxygen were also different from each other, based on the relative

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174 abundances of *Clostridia* (bin H, bin J) and *Psychromonas* (bin B). In contrast, the communities
175 in the untreated replicate controls Con-1 and Con-2 had a nearly identical community structure
176 after 300 days of cultivation (Fig. 2, S4). Despite the different communities in the nitrate and
177 oxygen treated cultures, fermentation coupled to sulfate reduction was not greatly affected as a
178 community function, as inferred from gene expression (Fig. 3, Dataset 1) and the production of
179 sulfide (Fig. 1). This functional similarity may be explained by the presence of fermentative
180 populations that are phylogenetically different, but perform similar metabolisms (Allison and
181 Martiny, 2008).(Burke *et al.*, 2011)
182
183 To study the communities in detail, we focused on metagenomic bins with relatively long contigs
184 with relatively equal coverage distribution and a consistent taxonomic signature (Table 1). These
185 bins can be considered provisional genome sequences that represented the genetic repertoire of the
186 most abundant populations (Table 1, Fig. 2), which were all present in the inoculum as well (Table
187 1). The 16S rRNA gene sequences of fifteen of these bins were used for a detailed analysis of their
188 taxonomic and phylogenetic affiliation (Fig. S6-S9). *Proteobacteria*, *Firmicutes*, and *Spirochaeta*
189 were the phyla with the overall highest relative abundance (Fig. 2) and dominated the
190 metagenomes of each treatment. Of the 18 most sequence abundant bins, only bin D affiliating
191 with sulfate-reducing *Desulfotignum* had a high relative abundance across all treatments and
192 cultures, while the other 17 bins were either always relatively low abundant or thrived only under
193 certain conditions (Fig. 2). Most organisms were predicted to have a fermentative (bin G-P) or
194 sulfate-reducing (bin D and F) lifestyle (Fig. 3). Bin A-C, E, Q and R belonged to heterotrophs
195 that were selected in the oxygen or nitrate treatments and hence a respiratory lifestyle is most
196 likely. For the bins C (*Thioalkalispira*), Q, and R (*Bacteroidetes*) meaningful metabolic inferences
197 were impossible because the binned metagenomic data was too scarce (Table 1), bin J was not
198 further analyzed due to a high percentage of contamination (40%).
199
200 To infer the metabolic activity of key organisms in the cultures and in response to the applied
201 treatment, we sequenced ten metatranscriptomes after 300 (control), 311 (oxygen treatment) and

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202 327 (nitrate treatment) days of incubation. We sampled one hour before the treatment and directly
203 after the treatment subsided (Fig. S3C, D). This enabled us to sketch a trophic network in the
204 cultures (Fig. 4A) and look at differences in their gene expression caused by the treatments. The
205 transcriptional activity mirrored relative abundance, such that populations that were abundant in a
206 treatment, were also most active. The differences in overall gene transcription before and after the
207 treatment were not very pronounced. The relative transcription of most genes involved in
208 anabolism, catabolism and energy metabolism showed minor changes, suggesting that after 300
209 days the key organisms were very well adapted to the provided cyclic environment. Consistent and
210 large differences caused by the treatment were mainly detected for genes involved in oxidative
211 (Fig 4B) and general (Fig 4C) stress protection. The genes that were transcribed by the populations
212 indicated that each population had a slightly different strategy to cope with stress.
213

214 ***Sulfate reducers***

215 Most of the sulfate reduction was likely performed by *Deltaproteobacteria* affiliating with
216 *Desulfotignum balticum* (bin D, Fig. S6) and *Desulfovibrio profundus* (bin F, Fig. S6). Both
217 organisms constitutively expressed bd-type terminal oxidases to respire oxygen and protect
218 oxygen-sensitive enzymes (Ramel *et al.*, 2013). Genes encoding for sulfate adenylyltransferase
219 (*sat*), adenylyl-sulfate reductase (*aps*) and dissimilatory sulfite reductase (*dsr*) were also
220 transcribed by both organisms (Fig. 3, Dataset 1). *Desulfotignum* dominated all conditions based
221 on relative abundance, yet *Desulfovibrio* seemed to have a higher relative transcription of *dsr* genes
222 than *Desulfotignum* in the nitrate-supplied and untreated cultures (Fig. S5). *Desulfotignum*
223 transcribed NiFe(Se)-hydrogenases (e.g. *hyb*) and c-type cytochromes, which are needed to use
224 hydrogen as an electron donor (Heidelberg *et al.*, 2004). *Desulfotignum* also constitutively
225 expressed carbon monoxide dehydrogenase and acetyl-CoA synthase (*cooS/acsA*), the key genes
226 in the acetyl-CoA pathway for acetate oxidation or carbon dioxide fixation (Fig. 3). Both,
227 autotrophic growth and heterotrophic growth using genes of the acetyl-CoA pathway has been
228 previously described for *D. balticum* (Kuever *et al.*, 2001). Together, the high transcriptional
229 activity of these genes (Fig. 3, Dataset 1) indicated that the organisms most likely fermented

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230 acetate to H_2 and CO_2, and then used the H_2 for sulfate reduction (Kuever *et al.*, 2001). However,
231 it cannot be ruled out that the organisms grew chemolithoautotrophically, despite the excess of
232 organic carbon sources, whichwould be counter-intuitive and merits further investigation. The
233 *Desulfovibrio* population (bin F) also transcribed Ni/Fe hydrogenases (*hyb/hyd*) and appeared to
234 consume hydrogen. It also transcribed genes for formate-hydrogen lyase (*hycE*) and formate
235 oxidation (Fig. 3), consistent with the physiology of many *Desulfovibrio* species (Barton and
236 Fauque, 2009). The coexistence of *Desulfotignum* and *Desulfovibrio* populations in each treatment
237 of the experiment, revealed two stable ecological niches for sulfate reducers in our cultures.
238

239 ***Obligate fermenters***

240 All *Clostridiales* (bin G-K, Fig. S7), *Spirochaetales* (bin L-M, Fig. S7), and *Anaerolineales* (bin
241 P) were strictly fermentative, based on their gene content and transcriptional activity. They
242 transcribed thioredoxins, peroxiredoxins and rubredoxins to protect their enzymes against
243 oxidative stress during oxygen or nitrate treatments (Fig. 4B). The organisms transcribed
244 hydrogen-producing hydrogenases and their associated electron transfer apparatus, but lacked a
245 respiratory chain. All fermenters transcribed genes for electron transport complexes (*rnf*), which
246 apparently enabled them to harness a proton/sodium motive force to reduce ferredoxins by
247 oxidizing NADH. Glucose and amino acids supplied with the medium were the main substrates,
248 as shown by highly transcribed sugar and amino acid transporters (Dataset 1). All *Firmicutes* (bin
249 G – K) transcribed V-type and F-type ATP synthases. It was shown that F-type ATP synthases act
250 as sodium pumps in certain *Clostridia* (Ferguson *et al.*, 2006), so it is unclear whether these
251 organisms harnessed a proton motive force to generate ATP. The three Spirochaetes only encoded
252 a vacuolar type ATP synthase and are thus likely dependent on substrate level phosphorylation
253 during fermentation. Transcription of acyl phosphatase and formate acetyltransferase (pyruvate-
254 formate lyase) suggested that acetate and formate were end products of fermentation, in addition
255 to hydrogen. All three end products seemed to be used by the two sulfate reducers, suggesting a
256 syntrophic relationship between fermenters and sulfate reducers. The uncultured *Spirochaeta* bin
257 L (Fig. S8) also transcribed genes to metabolize a large number of carbohydrates. The

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258 transcriptional activity indicated that this organism is able to import diverse sugars, into the cell
259 and shuttle them into glycolysis or the pentose phosphate way (Fig. S10). Based on the
260 transcription of key metabolic genes, the organisms affiliating with *Clostridiales* (bin G-I) seemed
261 to have very similar physiologies, which was also the case for the organisms affiliating with
262 *Spirochaeta* (bin L-N) (Fig. 3).
263
264 However, each population appeared to use slightly different glycosyl hydrolases (Table S1), and
265 sets of genes involved in fermentation and energy conversion. For instance, the *Clostridiales*
266 transcribed pyruvate synthase (*porC*), lactate dehydrogenase (*ldh*) and nitrite reductase (*nasD*),
267 which the *Spirochaeta* did not transcribe. In turn, the Spirochaetes seemed to have a much higher
268 expression of citrate synthase (*citA*) and isocitrate dehydrogenase (*icd*), key genes involved in the
269 citric acid cycle (Fig. 3). The *Firmicutes* bin G exhibited high numbers of transcripts for amino
270 acid importers and amino acid metabolism (e.g. glutamate dehydrogenase), whereas the *Firmicutes*
271 bin K exhibited mainly transcripts of sugar importers and glycolysis. These differences in gene
272 transcription may explain the observed coexistence of these organisms and hint towards metabolic
273 complementation within the fermentative network.
274
275 ***Facultative aerobes and nitrate respirers***
276 Populations affiliating with *Alphaproteobacteria* and *Gammaproteobacteria* (Fig. S9, bins A-C)
277 were detected in the transient oxygen and nitrate cultures and were minor constituents in the
278 sulfate-only cultures (Fig. 2). The transient exposure to oxygen and nitrate apparently selected for
279 these organisms, which were capable of respiration. Genes encoding respiratory complexes I-IV
280 and genes of the citric acid cycle were present and actively transcribed in the *Rhodobacterales* (bin
281 A) and *Alteromonadales* (bin B). Compared to the fermenters, the respiratory organisms showed
282 low transcriptional activity of sugar and amino acid transporters. Thus, it is likely that the
283 respiratory organisms mainly used fermentation products, such as acetate, as electron donors.
284 Hydrogen did not seem to be a major energy source for these organisms, as transcriptional activity
285 of hydrogenases was not detected. In contrast, the *Rhodobacterales* actively transcribed all *sox*

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286 genes that are needed for sulfide and sulfur oxidation. Both organisms transcribed genes involved
287 in polyhydroxybutyrate (PHB) and polyphosphate metabolisms. This indicates that PHB may have
288 accumulated under anoxic conditions driven by polyphosphate hydrolysis, and was oxidized under
289 oxic conditions, a well-known strategy for biological phosphorus removal (Wu *et al.*, 2010).
290 Indeed, in the *Rhodobacterales*, polyphosphate kinase and poly-beta-hydroxybutyrate polymerase
291 were down-regulated during the period of air supply (Dataset 1).
292
293 The population related to *Desulfuromusa bakii* (bin E), did not have or transcribe *dsr* genes and
294 was apparently not performing sulfate reduction. This organism were only selected in cultures with
295 transient nitrate supply and showed a strong global transcriptional response to nitrate availability.
296 In response to nitrate, it transcribed genes for citric acid cycle enzymes, complex I, nitrate-induced
297 formate dehydrogenase (*fdn*), periplasmic nitrate reductase (*nap*), and pentaheme nitrite reductase
298 (*nrf*). It likely performed nitrate ammonification with substrates such as amino acids, acetate and
299 formate. *Desulfuromusa bakii* and related bacteria are known as sulfur-reducing, and often
300 facultatively fermentative bacteria (Liesack and Finster, 1994). Hence, in the absence of nitrate
301 the organisms selected here may also have performed fermentation of amino acids and/or
302 dicarboxylates.
303

304 **Ecophysiology of *Candidatus* Sabulitectum silens**

305 We also detected an organism (bin O) that affiliated with the candidate phylum *Fermentibacteria*
306 (formerly candidate division Hyd24-12) (Kirkegaard *et al.*, 2016). These organisms were present
307 in all cultures, but were only abundant in the untreated cultures that were not exposed to oxygen
308 or nitrate (Fig. 2). The contigs of this bin were very long (up to 538 kb; N50: 222 kb), the
309 provisional genome had a size of 2.9 Mb and was inferred to be 77% complete (Table S2).
310 Annotation of the genes encoded on the contigs of bin O suggested that the organisms have a
311 typical gram-negative cell envelope with a complete peptidoglycan biosynthesis pathway and an
312 active outer membrane transport system (*tonB*/*exbBD*). Glycolysis and the non-oxidative pentose
313 phosphate pathway were complete (Fig. 5). The presence of largely complete operons coding for

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314 genes involved in lipid biosynthesis, cofactor biosynthesis, amino acid metabolism, and nucleotide
315 metabolism indicated that these bacteria are likely not dependent on others for the generation of
316 the major cellular building blocks. The organism transcribed an H^+/Na^+-translocating V-type ATP
317 synthase as well as numerous protein complexes that translocate sodium ions across the cell
318 membrane, such as an electron transport complex protein (*rnf*), a NADH-oxidoreductase (*ndh*),
319 and a Na^+-translocating decarboxylase (*oad/gcd*). This combination of proteins indicated that the
320 organism was able to synthesize ATP using a sodium motive force (Mulkidjanian *et al.*, 2008).
321 However, the organism lacked a complete citric acid cycle and a respiratory chain. Single genes
322 for flagellar biosynthesis and twitching motility were transcribed, yet the pathways for motility
323 were incomplete (Fig. 5). Bin O lacked many of the mechanisms for oxidative and general stress
324 protection (Fig 4B), which may explain its low abundance in the oxygen and nitrate treated
325 cultures. The metagenome and metatranscriptome indicated that the organism is a non-motile,
326 strictly anaerobic, obligate fermenter. We propose to name it *Candidatus* Sabulitectum silens (gen.
327 *et* sp. nov.; *sabulum* (lat.) – sand; *tectus* (lat.) – covered, roofed; *silens* (lat.) – still, silent). In
328 addition, we propose the new family *Ca.* Sabulitectaceae (fam. nov.) within the order *Ca.*
329 Fermentibacterales (Fig. 6). The phylum *Fermentibacteria* belongs to the Fibrobacteres-Chlorobi-
330 Bacteroidetes superphylum (Fig. S11). The *Fermentibacteria* comprise one class, one order, four
331 families and at least nine genera (Fig. 6). The four families were previously indicated as four
332 distinct clades (Kirkegaard *et al.*, 2016).
333
334 The nearest relative of *Ca*. Sabulitectum silens is the recently described *Ca.* Fermentibacter
335 daniensis, an anaerobic fermenter that is possibly involved in the sulfur-cycle (Kirkegaard *et al.*,
336 2016). In contrast to *Ca.* Fermentibacter, *Ca*. Sabulitectum did not seem to possess or transcribe
337 genes for sulfhydrogenases, despite the presence of sulfur in the cultures. Overall, both organisms
338 appear to have similar lifestyles based on their transcriptional activity, despite their phylogenetic
339 distance, suggesting that this lifestyle might be common among the phylum *Fermentibacteria*.
340 Thus, it is not surprising that, so far, the phylum comprises sequences that almost exclusively
341 originated from anoxic, organic and/or methane-rich ecosystems (Fig. 6), including sulfidic cave

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342 biofilms (Macalady *et al.*, 2006), sulfur-rich springs (Elshahed *et al.*, 2007), methane seeps (Ruff
343 *et al.*, 2015; McKay *et al.*, 2016; Trembath-Reichert *et al.*, 2016), mud volcanoes (Pachiadaki *et*
344 *al.*, 2011; Chang *et al.*, 2012), methane hydrates (Mills *et al.*, 2005), marine sediments (Schauer
345 *et al.*, 2011), coral reef sands (Schöttner *et al.*, 2011), microbial mats (Harris *et al.*, 2013; Schneider
346 *et al.*, 2013), marine sponges (Simister *et al.*, 2012) and anaerobic digesters (Nelson *et al.*, 2012;
347 Kirkegaard *et al.*, 2016). The physiology that *Ca*. Sabulitectum exhibited in our cultures (Fig. 3,
348 4B, 5) suggests that *Fermentibacteria* are strict anaerobes that produce hydrogen and acetate from
349 the fermentation of amino acids and sugars, in these ecosystems.
350

351 ## Conclusion

352 The transient exposure to oxygen or nitrate changed the microbial community structure, and
353 impacted the magnitude of net sulfide production as a community function, yet had a minor effect
354 on microbial community composition. This shows that the communities of Janssand intertidal
355 sediments contained organisms that were well adjusted for each of these scenarios, diverting the
356 flow of carbon and energy through the trophic network based on the available electron acceptors.
357 The treatment with oxygen or nitrate did not cause the community to shift to an alternative stable
358 state (Shade *et al.*, 2012). Community stability during the exposure to oxygen or nitrate was
359 enabled by the increased expression of genes involved in oxidative and general stress protection.
360 The stable coexistence of several fermenters and sulfate reducers with nitrate reducers or aerobic
361 respirers supports the recent finding that microbial communities are assembled based on rules that
362 go beyond those of the classical redox tower (Chen et al., 2017).
363

364 ## Materials and Methods

365

366 ### Sampling site and inoculum for enrichment experiments

367 Sediment was sampled from the upper part of the intertidal back-barrier flat Janssand, in the
368 German Wadden Sea (53.73515 N, 07.69913 E) in June 2012. The top 2 cm of sediment was
369 collected with a flat trowel during low tide. After transport of the sediment to the laboratory, an

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370 equal volume of sterile artificial seawater (Red Sea Salt, 33.4 g l^{-1}; http://www.redseafish.com)
371 was added to the sediment and stirred vigorously. The sediment was allowed to settle briefly, and
372 the liquid was transferred into (1 l) glass bottles that were closed with rubber stoppers and of which
373 the headspace was exchanged with argon. The liquid was kept at 4°C for 2 days and then used as
374 inoculum.
375

376 **Continuous culture setup and medium**

377 Six continuous cultures were set up and maintained for 350 days. Each glass vessel (DURAN,
378 GLS 80, 500 ml) was filled with 0.4 l inoculum, fitted with tubes for in- and outflowing medium
379 as well as in- and outflowing gas, and was stirred at 200 to 400 rpm. The medium supply rate was
380 0.17 l day^{-1}, resulting in a dilution rate of 0.36 - 0.4 day^{-1}. The anoxic medium consisted of Red
381 Sea Salt artificial seawater (33.4 g l^{-1}), containing 28 mM sulfate, supplemented with 20 C-mM
382 organic carbon (1.1 mM D-glucose, 1.7 mM acetic acid, and 0.4 mM amino acids), 0.2 mM
383 phosphate and trace elements (for details see Supporting Information). The culture pH was
384 measured off line (Mettler Toledo, Five Easy ™) and was in the range of pH 7.5 to 7.8. The OD_{600}
385 of all cultures was monitored off-line spectrophotometrically (Thermo Scientific Genesys 10S UV-
386 Vis). Sulfide concentration in the culture was measured using the Cline method (1969). After 21
387 days, the headspace of two of the cultures (Oxy-1 and Oxy-2) was oxygenated twice daily, by
388 supplying air (1 l min^{-1}) for 5 min. The air was removed after 30 min by supplying Argon (1 l min^{-}
389 ^{1}) for 5 min. This procedure was repeated every 12 h for the remainder of the experiment. In
390 parallel, nitrate was supplied twice daily to two cultures (Nit-1 and Nit-2) by supplying a nitrate
391 solution (1.4 ml min^{-1}, 20 mM $NaNO_3$ dissolved in artificial seawater) for 7 min, every 12 hours.
392 Two other cultures (Con-1 and Con-2) only received sulfate as electron acceptor. During aeration,
393 the oxygen concentration in the culture liquid was measured with Optical Oxygen Meter –
394 FireSting O_2. At the same time, we measured off-line the hydrogen sulfide (Cline, 1969) and sulfur
395 concentrations (Kamyshny Jr and Ferdelman, 2010) in the cultures. In addition, after 327 days the
396 nitrate in the medium was replaced with ^{15}N-nitrate by direct injection of 10 ml of 20 mM ^{15}N-
397 nitrate and the production of ^{15}N-nitrogen gas was measured off-line by mass spectrometry (GAM

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398 400, InProcess Instruments, Bremen, Germany) using 0.5 ml headspace samples. Nitrate in the
399 culture liquid was determined as previously described (Hanke *et al.*, 2014).
400
401 **Metagenomics**
402 On day 311 (Oxy-1 and Oxy-2), day 327 (Nit-1 and Nit-2), and day 300 (Con-1 and Con-2) of the
403 experiment, we extracted nucleic acids from 10 ml samples of all six cultures as previously
404 described (Zhou *et al.*, 1996), after incubation with lysozyme (2.5 mg ml^{-1}) and RNAse (0.1 mg
405 ml^{-1}). For metagenome shotgun sequencing, 1.5 µg of the extracted DNA was mechanically
406 fragmented using Nebulizers (Roche; 32 psi; 3 min, 500 µl nebulization buffer). The fragmented
407 DNA was purified using MinElute PCR purification columns (Qiagen) and eluted in 50 µl Tris-
408 EDTA buffer (Life Technologies). The entire eluate was used for the preparation of barcoded
409 Personal Genome Machine (PGM) sequencing libraries with the Ion Xpress™ Plus gDNA
410 Fragment Library Preparation kit (Life Technologies). Library insert sizes were between 350 and
411 400 base pairs (bp). The libraries were sequenced with the PGM on a 318 Chip, using the chemistry
412 for 400 bp libraries. Base calling was performed with the Torrent Suite software v3.6 or v4.0.2,
413 with default settings. Sequence reads were assembled with the Newbler assembler v2.8 with
414 default settings for genomic DNA assembly for non-paired reads. Assembled DNA sequences
415 were binned based on multivariate statistics of tetranucleotide frequencies with MetaWatt v2.1
416 (Strous *et al.*, 2012) (Fig. S1, Table 1). Phylogenetic profiles of the bins were obtained by
417 analyzing all open reading frames encoded on the contigs using blast and a database that contained
418 a representative of every genus with a publicly available, complete or draft whole genome
419 sequence. Genome completeness and contamination were evaluated by detection of a set of 139
420 conserved single copy genes (Campbell *et al.*, 2013) with Hidden Markov Models (HMMER 3.1)
421 and by detection of transfer RNA genes (Laslett and Canback, 2004). Percentage completeness
422 was calculated as the number of conserved single copy genes (CSCG) detected, divided by the
423 total number of CSCG. Percentage contamination was calculated as the number of CSCG present
424 in >1 copy, divided by the number of CSCG detected. Due to frameshift errors resulting from Ion
425 Torrent sequencing it was not possible to use CheckM (Parks *et al.*, 2015) to estimate genome

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426 completeness and contamination. Note that, using the above described method, the completeness
427 values reported likely underestimate the actual completeness of the bins. Genes present in each bin
428 were annotated with Prokka v1.9 (Seemann, 2014). Each bin constitutes a provisional whole
429 genome sequence of a microbial population (Fig. S1). Bins denoted by the same letter across all
430 cultures (e.g. bin D) represent a genetically identical population, or nearly identical populations,
431 since all six continuous cultures were inoculated with the same sediment sample. Bin abundances
432 over all samples were estimated based on coverage and bin size, by mapping the sequence reads
433 to the contigs that made up each associated bin. The abundance of the enriched organisms in the
434 inoculum was estimated by mapping the reads of four Janssand sediment metagenomes (Sequence
435 Read Archive accession numbers SRR577219, SRR577220, SRR577221, SRR577224) to the bins
436 using BBMap (github.com/BioInfoTools/BBMap) and the parameters "maxlen 500, minid 0.98".
437
438 **Metatranscriptomics**
439 Parallel to DNA extraction, RNA was extracted from a 2 ml sample of all six cultures (for details
440 see Supporting Information) on day 311 (oxygen treated cultures), on day 327 (nitrate treated
441 cultures) and on day 300 (untreated control cultures). For those cultures with cyclic oxygen or
442 nitrate supply, RNA was extracted an hour before the treatment and immediately after the
443 treatment subsided, i.e. when oxygen and nitrate concentrations had decreased to background
444 values, 30 min and 240 min after the treatment commenced, respectively (Fig. S3C, D). Ribosomal
445 RNA was depleted from purified RNA (3–5µg) using the Ribo-Zero rRNA removal kit (Bacteria,
446 Epicentre, Madison,WI, USA). Libraries were prepared with the Ion Total RNA-Seq Kit v2 (Life
447 Technologies) following the protocol for whole transcriptome library preparation. Transcriptional
448 activities for each gene (Dataset 1) were determined by Ion Torrent sequencing of cDNA obtained
449 from extracted RNA and subsequent mapping of the cDNA reads to the annotated contigs with
450 BBMap v32 (http://sourceforge.net/projects/bbmap/). Reported activities were calculated by
451 dividing the number of mapped reads/gene length by the total number of reads mapped to coding
452 sequences of the bin/total length of all coding sequences of the bin.
453

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$$\frac{\left(\frac{\textit{Number of mapped reads}}{\textit{Length of coding sequence}}\right)}{\left(\frac{\textit{Total number of reads mapped to coding sequences of the bin}}{\textit{Total length of coding sequences of the bin}}\right)}$$

454
455
456 This way, the average transcriptional activity equals 1.0 and hence the bins can be compared within
457 and between the treatments. 16S rRNA gene sequences were detected with Hidden Markov Models
458 (www.github.com/Victorian-Bioinformatics-Consortium/barrnap) and, independently,
459 reconstructed with Emirge (Miller *et al.*, 2011). 16S rRNA gene sequences were linked to bins as
460 previously described (Kraft *et al.*, 2014).
461
462 **Phylogenetic tree reconstruction**
463 The 16S rRNA gene based phylogenetic trees were generated using near full-length sequences
464 (>1300 bases) of the non-redundant SILVA small subunit reference database (release 123.1; March
465 2016) (Quast *et al.*, 2013) and the software ARB (Ludwig *et al.*, 2004). Sequences were aligned
466 using SINA (Pruesse *et al.*, 2012) and the alignment was manually optimized according to the
467 rRNA secondary structure, resulting in high-quality alignments of 1267-1287 bases length. We
468 used a maximum-likelihood algorithm (PHYML) with a positional variability filter, excluding
469 highly variable regions, and 100 bootstrap iterations. Phylogenetic levels were calculated based on
470 phylogenetic distance using the clustering tool as implemented in ARB. Threshold sequence
471 identity for genus (94.5%), family (86.5%), order (82.0%), class (78.5%) and phylum (75.0%)
472 were chosen according to the latest taxonomic threshold recommendations (Yarza *et al.*, 2014).
473
474 **Sequence data accession**
475 16S rRNA gene sequences are archived under the accession numbers KX550146 - KX550265
476 (Janssand continuous cultures) and KX539512-KX539546 (Seep sediment enrichments).
477 Metagenomic and metatranscriptomic sequencing raw data, as well as the assembled contigs and

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478 the *Ca.* Sabulitectum silens draft genome, are archived under the SRA Bioproject PRJNA305678
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480

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491 **References**

492

493 Allison, S.D. and Martiny, J.B.H. (2008) Resistance, resilience, and redundancy in microbial
494 communities. *Proc. Natl. Acad. Sci. U. S. A.* **105**: 11512–11519.
495 Barton, L.L. and Fauque, G.D. (2009) Chapter 2: Biochemistry, Physiology and Biotechnology of
496 Sulfate-Reducing Bacteria. In, *Advances in Applied Microbiology*. Academic Press, pp. 41–
497 98.
498 van Beusekom, J.E.E. (2005) A historic perspective on Wadden Sea eutrophication. *Helgol. Mar.*
499 *Res.* **59**: 45–54.
500 Billerbeck, M., Werner, U., Polerecky, L., Walpersdorf, E., deBeer, D., and Huettel, M. (2006)
501 Surficial and deep pore water circulation governs spatial and temporal scales of nutrient
502 recycling in intertidal sand flat sediment. *Mar. Ecol. Prog. Ser.* **326**: 61–76.
503 Boyer, E.W., Howarth, R.W., Galloway, J.N., Dentener, F.J., Green, P.A., and Vörösmarty, C.J.
504 (2006) Riverine nitrogen export from the continents to the coasts. *Global Biogeochem.*
505 *Cycles* **20**: GB1S91.
506 Burke, C., Steinberg, P., Rusch, D., Kjelleberg, S., and Thomas, T. (2011) Bacterial community
507 assembly based on functional genes rather than species. *Proc. Natl. Acad. Sci. U. S. A.* **108**:
508 14288–14293.
509 Campbell, J.H., O'Donoghue, P., Campbell, A.G., Schwientek, P., Sczyrba, A., Woyke, T., et al.
510 (2013) UGA is an additional glycine codon in uncultured SR1 bacteria from the human
511 microbiota. *Proc. Natl. Acad. Sci. U. S. A.* **110**: 5540–5.
512 Canfield, D.E., Jørgensen, B.B., Fossing, H., Glud, R., Gundersen, J., Ramsing, N.B., et al. (1993)
513 Pathways of organic carbon oxidation in three continental margin sediments. *Mar. Geol.* **113**:
514 27–40.
515 Chang, Y.-H., Cheng, T.-W., Lai, W.-J., Tsai, W.-Y., Sun, C.-H., Lin, L.-H., and Wang, P.-L.
516 (2012) Microbial methane cycling in a terrestrial mud volcano in eastern Taiwan. *Environ.*
517 *Microbiol.* **14**: 895–908.
518 Chen, J., Hanke, A., Tegetmeyer, H.E., Kattelmann, I., Sharma, R., Hamann, E., et al. (2017)
519 Impacts of chemical gradients on microbial community structure. *ISME J.*
520 Cline, J.D. (1969) Spectrophotometric determination of hydrogen sulfide in natural waters.
521 *Limnol. Oceanogr.* **14**: 454–458.
522 Dalsgaard, T. and Bak, F. (1994) Nitrate Reduction in a Sulfate-Reducing Bacterium,
523 Desulfovibrio desulfuricans, Isolated from Rice Paddy Soil: Sulfide Inhibition, Kinetics, and
524 Regulation. *Appl. Environ. Microbiol.* **60**: 291–297.
525 Elshahed, M.S., Youssef, N.H., Luo, Q., Najar, F.Z., Roe, B.A., Sisk, T.M., et al. (2007)
526 Phylogenetic and Metabolic Diversity of Planctomycetes from Anaerobic, Sulfide- and
527 Sulfur-Rich Zodletone Spring, Oklahoma. *Appl. Environ. Microbiol.* **73**: 4707–4716.
528 Ferguson, S.A., Keis, S., and Cook, G.M. (2006) Biochemical and Molecular Characterization of
529 a Na+-Translocating F1Fo-ATPase from the Thermoalkaliphilic Bacterium Clostridium
530 paradoxum. *J. Bacteriol.* **188**: 5045–5054.
531 Froelich, P.N., Klinkhammer, G.P., Bender, M.L., Luedtke, N.A., Heath, G.R., Cullen, D., et al.
532 (1979) Early oxidation of organic matter in pelagic sediments of the eastern equatorial

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533 Atlantic: Suboxic diagenesis. *Geochim. Cosmochim. Acta* **43**: 1075–1090.
534 Gao, H., Matyka, M., Liu, B., Khalili, A., Kostka, J.E., Collins, G., et al. (2012) Intensive and
535 extensive nitrogen loss from intertidal permeable sediments of the Wadden Sea. *Limnol.*
536 *Oceanogr.* **57**: 185–198.
537 Gittel, A., Mußmann, M., Sass, H., Cypionka, H., and Könneke, M. (2008) Identity and abundance
538 of active sulfate-reducing bacteria in deep tidal flat sediments determined by directed
539 cultivation and CARD-FISH analysis. *Environ. Microbiol.* **10**: 2645–2658.
540 Hanke, A., Hamann, E., Sharma, R., Geelhoed, J.S., Hargesheimer, T., Kraft, B., et al. (2014)
541 Recoding of the stop codon UGA to glycine by a BD1-5/SN-2 bacterium and niche
542 partitioning between Alpha- and Gammaproteobacteria in a tidal sediment microbial
543 community naturally selected in a laboratory chemostat. *Front. Microbiol.* **5**: 231.
544 Harris, J.K., Caporaso, J.G., Walker, J.J., Spear, J.R., Gold, N.J., Robertson, C.E., et al. (2013)
545 Phylogenetic stratigraphy in the Guerrero Negro hypersaline microbial mat. *ISME J.* **7**: 50–
546 60.
547 Heidelberg, J.F., Seshadri, R., Haveman, S.A., Hemme, C.L., Paulsen, I.T., Kolonay, J.F., et al.
548 (2004) The genome sequence of the anaerobic, sulfate-reducing bacterium Desulfovibrio
549 vulgaris Hildenborough. *Nat. Biotechnol.* **22**: 554–559.
550 Henrichs, S.M. and Reeburgh, W.S. (1987) Anaerobic mineralization of marine sediment organic
551 matter: Rates and the role of anaerobic processes in the oceanic carbon economy.
552 *Geomicrobiol. J.* **5**: 191–237.
553 Howes, B.L., Dacey, J.W.H., and King, G.M. (1984) Carbon flow through oxygen and sulfate
554 reduction pathways in salt marsh sediments. *Limnol. Oceanogr.* **29**: 1037–1051.
555 Janssen, F., Huettel, M., and Witte, U. (2005) Pore-water advection and solute fluxes in permeable
556 marine sediments (II): Benthic respiration at three sandy sites with different permeabilities
557 (German Bight, North Sea). *Limnol. Oceanogr.* **50**: 779–792.
558 Jørgensen, B.B. (1982) Mineralization of organic matter in the sea bed - the role of sulphate
559 reduction. *Nature* **296**: 643–645.
560 Kamyshny Jr, A. and Ferdelman, T.G. (2010) Dynamics of zero-valent sulfur species including
561 polysulfides at seep sites on intertidal sand flats (Wadden Sea, North Sea). *Mar. Chem.* **121**:
562 17–26.
563 Kirkegaard, R.H., Dueholm, M.S., McIlroy, S.J., Nierychlo, M., Karst, S.M., Albertsen, M., and
564 Nielsen, P.H. (2016) Genomic insights into members of the candidate phylum Hyd24-12
565 common in mesophilic anaerobic digesters. *ISME J.* 1–13.
566 Kraft, B., Tegetmeyer, H.E., Meier, D., Geelhoed, J.S., and Strous, M. (2014) Rapid succession of
567 uncultured marine bacterial and archaeal populations in a denitrifying continuous culture.
568 *Environ. Microbiol.* **16**: 3275–3286.
569 Kuever, J., Könneke, M., Galushko, A., and Drzyzga, O. (2001) Reclassification of
570 Desulfobacterium phenolicum as Desulfobacula phenolica comb. nov. and description of
571 strain SaxT as Desulfotignum balticum gen. nov., sp. nov. *Int. J. Syst. Evol. Microbiol.* **51**:
572 171–177.
573 Laslett, D. and Canback, B. (2004) ARAGORN, a program to detect tRNA genes and tmRNA
574 genes in nucleotide sequences. *Nucleic Acids Res.* **32**: 11–16.

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575 Lee, S.-H., Sorensen, J.W., Grady, K.L., Tobin, T.C., and Shade, A. (2017) Divergent extremes
576 but convergent recovery of bacterial and archaeal soil communities to an ongoing
577 subterranean coal mine fire. *ISME J.*
578 Liesack, W. and Finster, K. (1994) Phylogenetic Analysis of Five Strains of Gram-Negative,
579 Obligately Anaerobic, Sulfur-Reducing Bacteria and Description of Desulfuromusa gen.
580 nov., Including Desulfuromusa kysingii sp. nov., Desulfuromusa bakii sp. nov., and
581 Desulfuromusa succinoxidans sp. *Int. J. Syst. Bacteriol.* **44**: 753–758.
582 Llobet-Brossa, E., Rabus, R., Böttcher, M.E., Könneke, M., Finke, N., Schramm, A., et al. (2002)
583 Community structure and activity of sulfate-reducing bacteria in an intertidal surface
584 sediment: a multi-method approach. *Aquat. Microb. Ecol.* **29**: 211–226.
585 Ludwig, W., Strunk, O., Westram, R., Richter, L., Meier, H., Yadhukumar, et al. (2004) ARB: a
586 software environment for sequence data. *Nucleic Acids Res.* **32**: 1363–1371.
587 Macalady, J.L., Lyon, E.H., Koffman, B., Albertson, L.K., Meyer, K., Galdenzi, S., and Mariani,
588 S. (2006) Dominant Microbial Populations in Limestone-Corroding Stream Biofilms,
589 Frasassi Cave System, Italy. *Appl. Environ. Microbiol.* **72**: 5596–5609.
590 Marchant, H.K., Lavik, G., Holtappels, M., and Kuypers, M.M.M. (2014) The Fate of Nitrate in
591 Intertidal Permeable Sediments. *PLoS One* **9**: e104517.
592 McKay, L., Klokman, V.W., Mendlovitz, H.P., LaRowe, D.E., Hoer, D.R., Albert, D., et al. (2016)
593 Thermal and geochemical influences on microbial biogeography in the hydrothermal
594 sediments of Guaymas Basin, Gulf of California. *Environ. Microbiol. Rep.* **8**: 150–161.
595 Miller, C.S., Baker, B.J., Thomas, B.C., Singer, S.W., and Banfield, J.F. (2011) EMIRGE:
596 reconstruction of full-length ribosomal genes from microbial community short read
597 sequencing data. *Genome Biol.* **12**: R44.
598 Mills, H.J., Martinez, R.J., Story, S., and Sobecky, P.A. (2005) Characterization of Microbial
599 Community Structure in Gulf of Mexico Gas Hydrates: Comparative Analysis of DNA- and
600 RNA-Derived Clone Libraries. *Appl. Environ. Microbiol.* **71**: 3235–3247.
601 Mulkidjanian, A.Y., Dibrov, P., and Galperin, M.Y. (2008) The past and present of sodium
602 energetics: May the sodium-motive force be with you. *Biochim. Biophys. Acta - Bioenerg.*
603 **1777**: 985–992.
604 Mußmann, M., Ishii, K., Rabus, R., and Amann, R. (2005) Diversity and vertical distribution of
605 cultured and uncultured Deltaproteobacteria in an intertidal mud flat of the Wadden Sea.
606 *Environ. Microbiol.* **7**: 405–418.
607 Muyzer, G. and Stams, A.J.M. (2008) The ecology and biotechnology of sulphate-reducing
608 bacteria. *Nat. Rev. Microbiol.* **6**: 441–454.
609 Nelson, M.C., Morrison, M., Schanbacher, F., and Yu, Z. (2012) Shifts in microbial community
610 structure of granular and liquid biomass in response to changes to infeed and digester design
611 in anaerobic digesters receiving food-processing wastes. *Bioresour. Technol.* **107**: 135–143.
612 Pachiadaki, M.G., Kallionaki, A., Dählmann, A., De Lange, G.J., and Kormas, K.A. (2011)
613 Diversity and Spatial Distribution of Prokaryotic Communities Along A Sediment Vertical
614 Profile of A Deep-Sea Mud Volcano. *Microb. Ecol.* **62**: 655–668.
615 Parks, D.H., Imelfort, M., Skennerton, C.T., Hugenholtz, P., and Tyson, G.W. (2015) CheckM:
616 assessing the quality of microbial genomes recovered from isolates, single cells, and

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617 metagenomes. *Genome Res.* **25**: 1043–1055.
618 Pruesse, E., Peplies, J., and Glockner, F.O. (2012) SINA: Accurate high-throughput multiple
619 sequence alignment of ribosomal RNA genes. *Bioinformatics* **28**: 1823–1829.
620 Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., et al. (2013) The SILVA
621 ribosomal RNA gene database project: improved data processing and web-based tools.
622 *Nucleic Acids Res.* **41**: D590–D596.
623 Rabus, R., Hansen, T.A., and Widdel, F. (2013) Dissimilatory Sulfate-and Sulfur-reducing
624 Prokaryotes. In, Rosenberg E.; Lory, S.; Stackebrandt, E. and Thompson F.,E.. D. (ed), *The*
625 *Prokaryotes*. Springer, Berlin, Heidelberg, pp. 309–404.
626 Ramel, F., Amrani, A., Pieulle, L., Lamrabet, O., Voordouw, G., Seddiki, N., et al. (2013)
627 Membrane-bound oxygen reductases of the anaerobic sulfate-reducing Desulfovibrio
628 vulgaris Hildenborough: roles in oxygen defence and electron link with periplasmic
629 hydrogen oxidation. *Microbiology* **159**: 2663–2673.
630 Ruff, S.E., Biddle, J.F., Teske, A.P., Knittel, K., Boetius, A., and Ramette, A. (2015) Global
631 dispersion and local diversification of the methane seep microbiome. *Proc. Natl. Acad. Sci.*
632 *U. S. A.* **112**: 4015–20.
633 Schauer, R., Røy, H., Augustin, N., Gennerich, H.-H., Peters, M., Wenzhoefer, F., et al. (2011)
634 Bacterial sulfur cycling shapes microbial communities in surface sediments of an ultramafic
635 hydrothermal vent field. *Environ. Microbiol.* **13**: no-no.
636 Schneider, D., Arp, G., Reimer, A., Reitner, J., and Daniel, R. (2013) Phylogenetic Analysis of a
637 Microbialite-Forming Microbial Mat from a Hypersaline Lake of the Kiritimati Atoll,
638 Central Pacific. *PLoS One* **8**: e66662.
639 Schöttner, S., Pfitzner, B., Grünke, S., Rasheed, M., Wild, C., and Ramette, A. (2011) Drivers of
640 bacterial diversity dynamics in permeable carbonate and silicate coral reef sands from the
641 Red Sea. *Environ. Microbiol.* **13**: 1815–1826.
642 Seemann, T. (2014) Prokka: rapid prokaryotic genome annotation. *Bioinformatics* **30**: 2068–2069.
643 Shade, A., Peter, H., Allison, S.D., Baho, D., Berga, M., Bürgmann, H., et al. (2012) Fundamentals
644 of microbial community resistance and resilience. *Front. Microbiol.* **3**:.
645 Simister, R.L., Deines, P., Botté, E.S., Webster, N.S., and Taylor, M.W. (2012) Sponge-specific
646 clusters revisited: a comprehensive phylogeny of sponge-associated microorganisms.
647 *Environ. Microbiol.* **14**: 517–524.
648 Stief, P., Kamp, A., and de Beer, D. (2013) Role of diatoms in the spatial-temporal distribution of
649 intracellular nitrate in intertidal sediment. *PLoS One* **8**: e73257.
650 Strous, M., Kraft, B., Bisdorf, R., and Tegetmeyer, H.E. (2012) The binning of metagenomic
651 contigs for microbial physiology of mixed cultures. *Front. Microbiol.* **3**: 1–11.
652 Trembath-Reichert, E., Case, D.H., and Orphan, V.J. (2016) Characterization of microbial
653 associations with methanotrophic archaea and sulfate-reducing bacteria through statistical
654 comparison of nested Magneto-FISH enrichments. *PeerJ* **4**: e1913.
655 Walsh, J.J. (1991) Importance of continental margins in the marine biogeochemical cycling of
656 carbon and nitrogen. *Nature* **350**: 53–55.
657 Ward, L., Taylor, M.W., Power, J.F., Scott, B.J., McDonald, I.R., and Stott, M.B. (2017) Microbial
658 community dynamics in Inferno Crater Lake, a thermally fluctuating geothermal spring.

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659 *ISME J.*
660 Widder, S., Allen, R.J., Pfeiffer, T., Curtis, T.P., Wiuf, C., Sloan, W.T., et al. (2016) Challenges
661 in microbial ecology: building predictive understanding of community function and
662 dynamics. *ISME J.*
663 Wu, C.-Y., Peng, Y.-Z., Wang, S.-Y., and Ma, Y. (2010) Enhanced biological phosphorus removal
664 by granular sludge: From macro- to micro-scale. *Water Res.* **44**: 807–814.
665 Yarza, P., Yilmaz, P., Pruesse, E., Glockner, F.O., Ludwig, W., Schleifer, K.-H., et al. (2014)
666 Uniting the classification of cultured and uncultured bacteria and archaea using 16S rRNA
667 gene sequences. *Nat. Rev. Microbiol.* **12**: 635–645.
668 Zhou, J., Bruns, M.A., and Tiedje, J.M. (1996) DNA recovery from soils of diverse composition.
669 *Appl. Environ. Microbiol.* **62**: 316–322.
670
671

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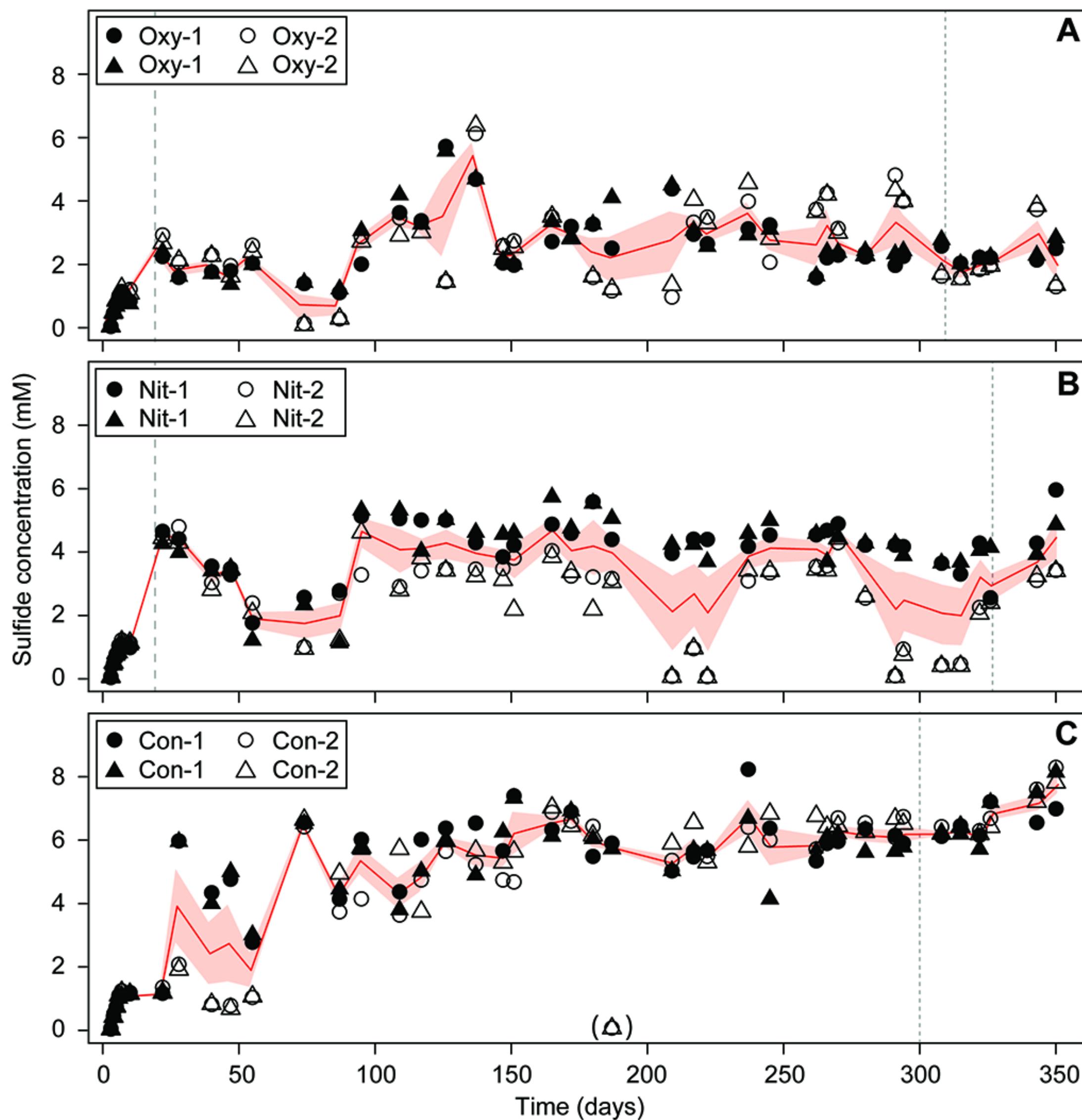
672 **Figures**

673

674 **Figure 1**



675

676 **Figure 1**: Sulfide concentrations in the replicate cultures treated with oxygen (A), treated with
677 nitrate (B), and in the untreated control with sulfate as sole electron acceptor (C). Duplicate
678 measurements of each culture are shown as triangles and circles, the red line depicts the mean of
679 four measurements, the red ribbon represents standard error of the mean. The start of the treatments
680 is indicated by dashed lines, sampling time points for metagenomics and metatranscriptomics are
681 indicated by dotted lines.

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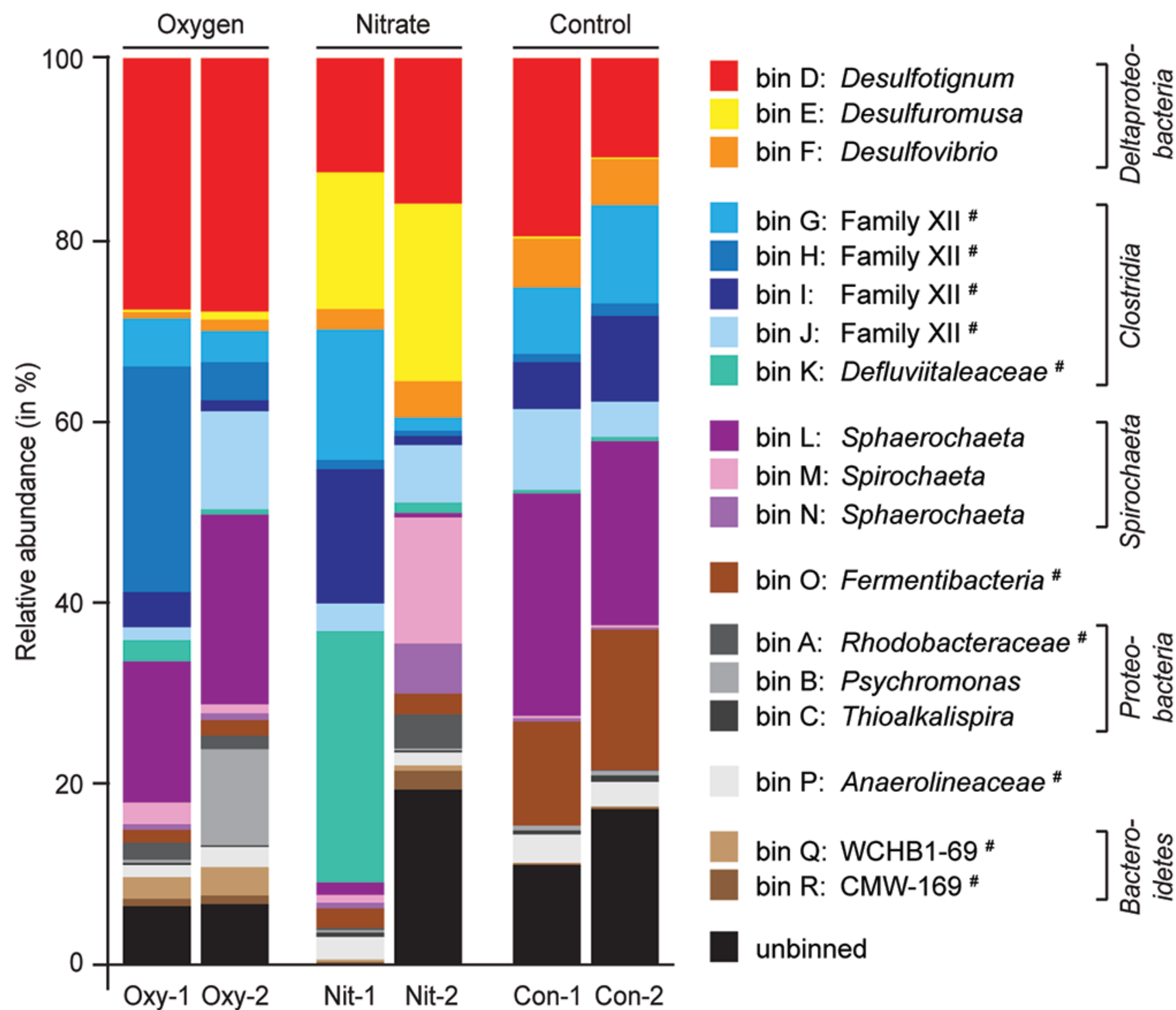
Figure 2



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683

684 **Figure 2**: Estimated relative abundances of bins in cultures treated with oxygen (Oxy-1, Oxy-2),
685 with nitrate (Nit-1, Nit-2) and in an untreated control (Con-1, Con-2). The bins were classified to
686 genus level. Populations that affiliated with genera lacking a cultured representative are marked
687 with #. For these bins we reported the closest taxonomically assigned, phylogenetic level, e.g. bin
688 K affiliates with an uncultured genus in the family *Defluviitaleaceae*. Taxonomic assignment is
689 based on the SILVA small ribosomal subunit reference database (SSURef, v123). Relative
690 abundances were obtained by mapping metagenomic sequence reads to the assembled contigs of
691 each bin. The phylogeny of most bins is provided (bin O: Fig. 6, bin A-N: Fig. S6-S9).

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692 **Figure 3**

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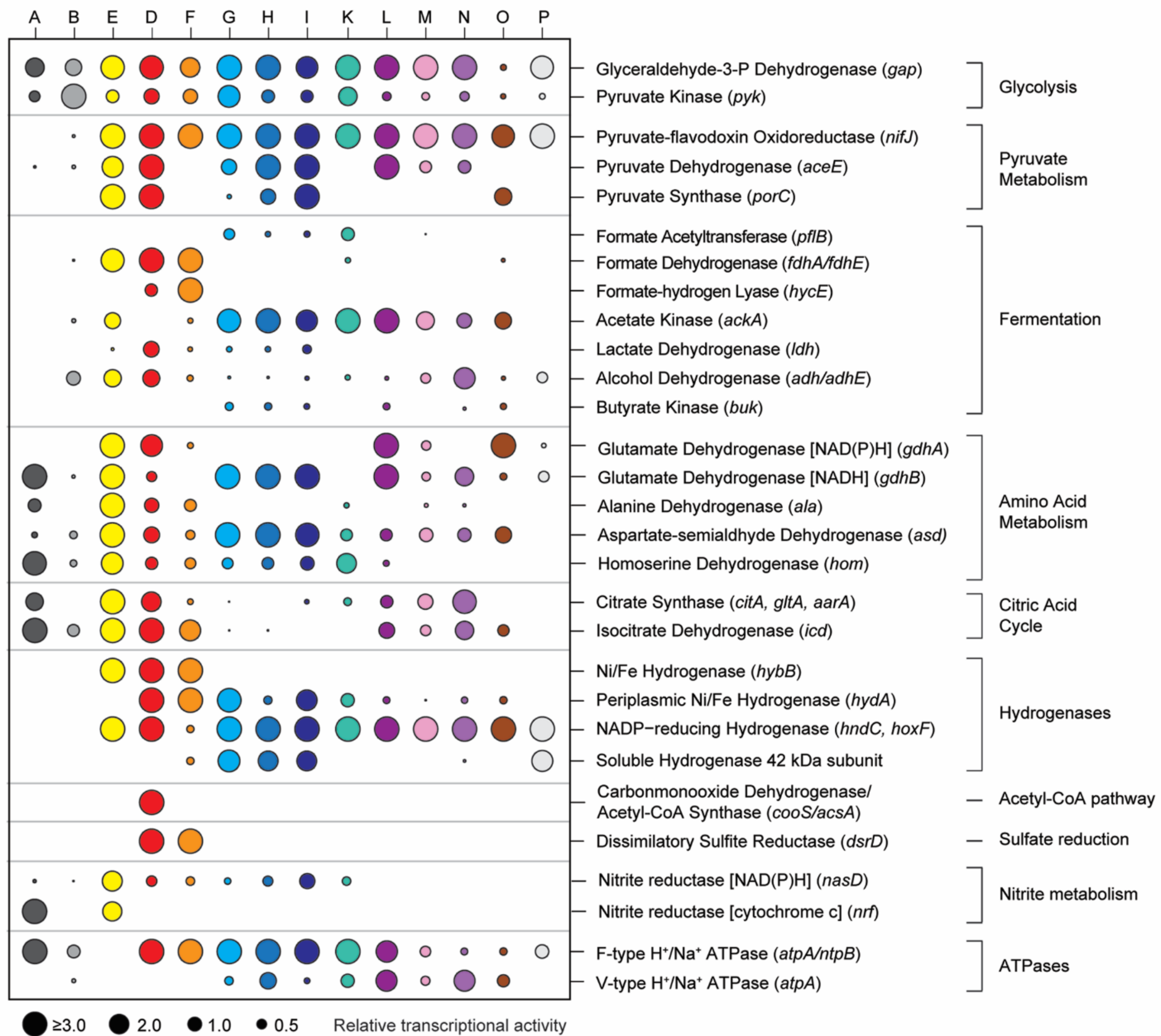

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696 **Figure 3**: Metabolic capabilities of the populations based on key genes. Circle size represents
697 relative transcriptional activity averaged over all ten transcriptomes. The absence of a circle shows
698 that gene transcription was not detected in any of the treatments and cultures. Note: For reasons of
699 visualization all relative transcriptional activities above 3.0 are shown as ≥3.0.

700

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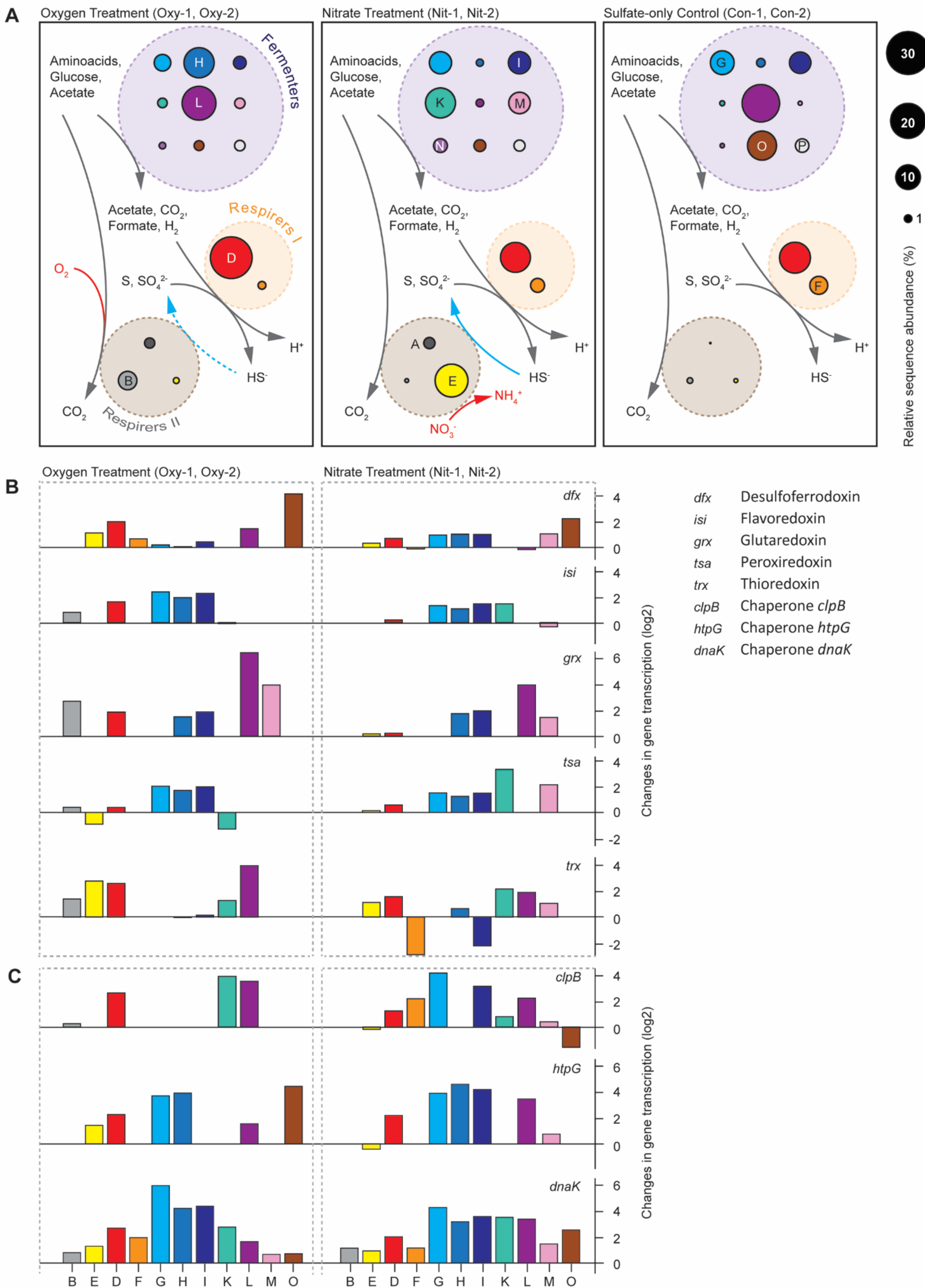
701 **Figure 4**



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703 **Figure 4**: Schematic of the trophic network of key populations, and transcriptional changes of
704 stress response genes. (A) Most abundant obligate fermentative heterotrophs (Fermenters), sulfate-
705 reducing bacteria (Respirers I) and associated respiratory heterotrophs (Respirers II) in the three
706 different conditions. The network is based on metagenomic and /–transcriptomic data. All 14
707 shown bins were present in all cultures. Circle size represents estimated relative abundance. Only
708 one organism (bin D) was abundant in all cultures. Arrows depict key pathways that occur in all
709 (grey), two (blue) or one condition (red). (B) Change of gene transcription caused by the treatment
710 with oxygen or nitrate. Values are log2-transformed ratios of gene transcription in replicate
711 cultures after and before the treatment, i.e. a value of 1 means that gene transcription was twice as
712 high after the treatment than before the treatment; a value of -2 means a four-fold decrease in
713 transcription.
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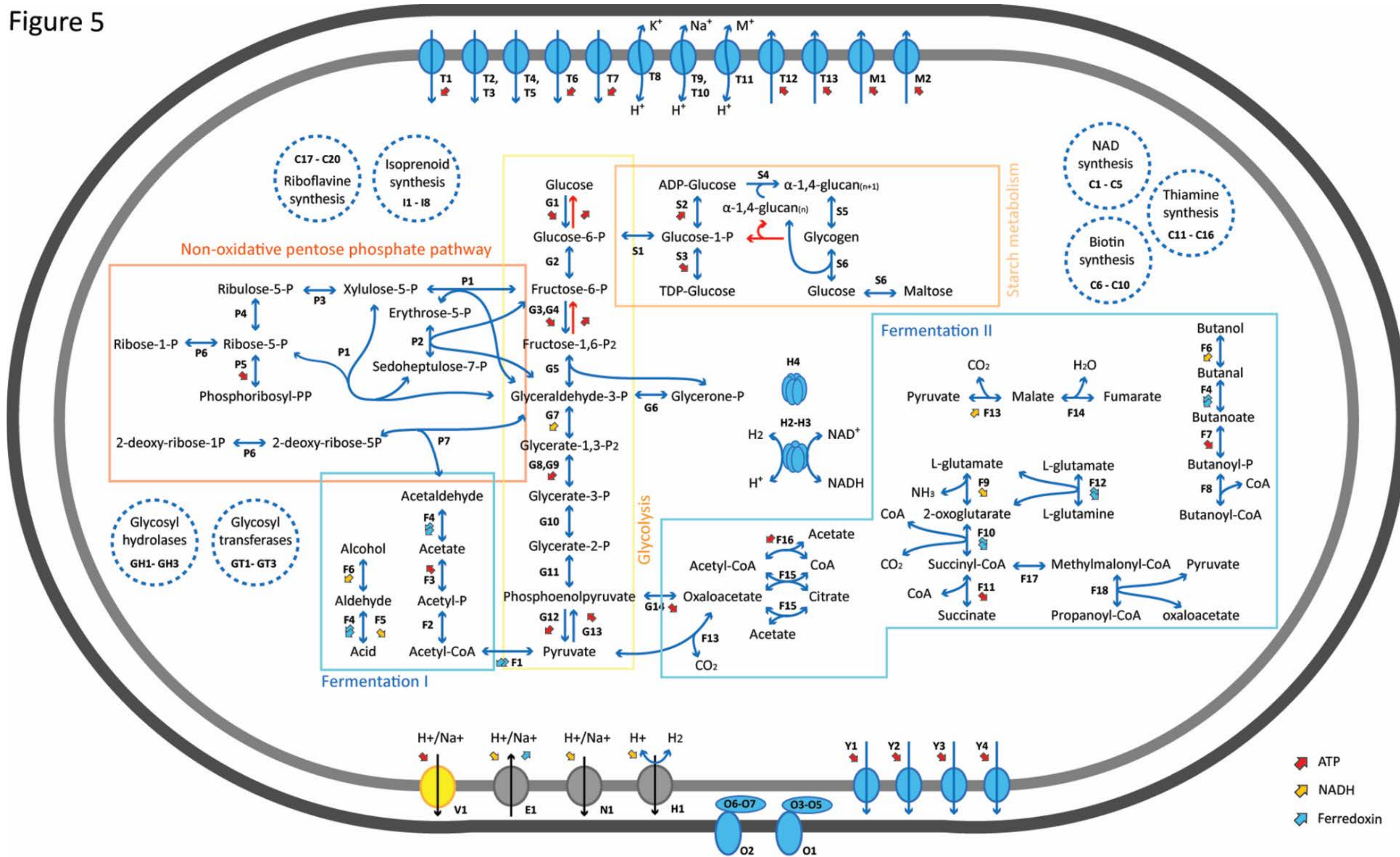

Figure 5: Metabolic map of *Ca.* Sabulitectum silens (bin O) showing central pathways that the organism transcribed in the sulfate-only treatment (Con-5, Con-6). Transcribed genes are shown as blue arrows, genes of annotated pathways that were not detected as red arrows. Enzymes are abbreviated with letters, the full list as well as further metabolic pathways are provided in Table S3. Dashed blue circles depict additional pathways that were detected.

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722 **Figure 6**

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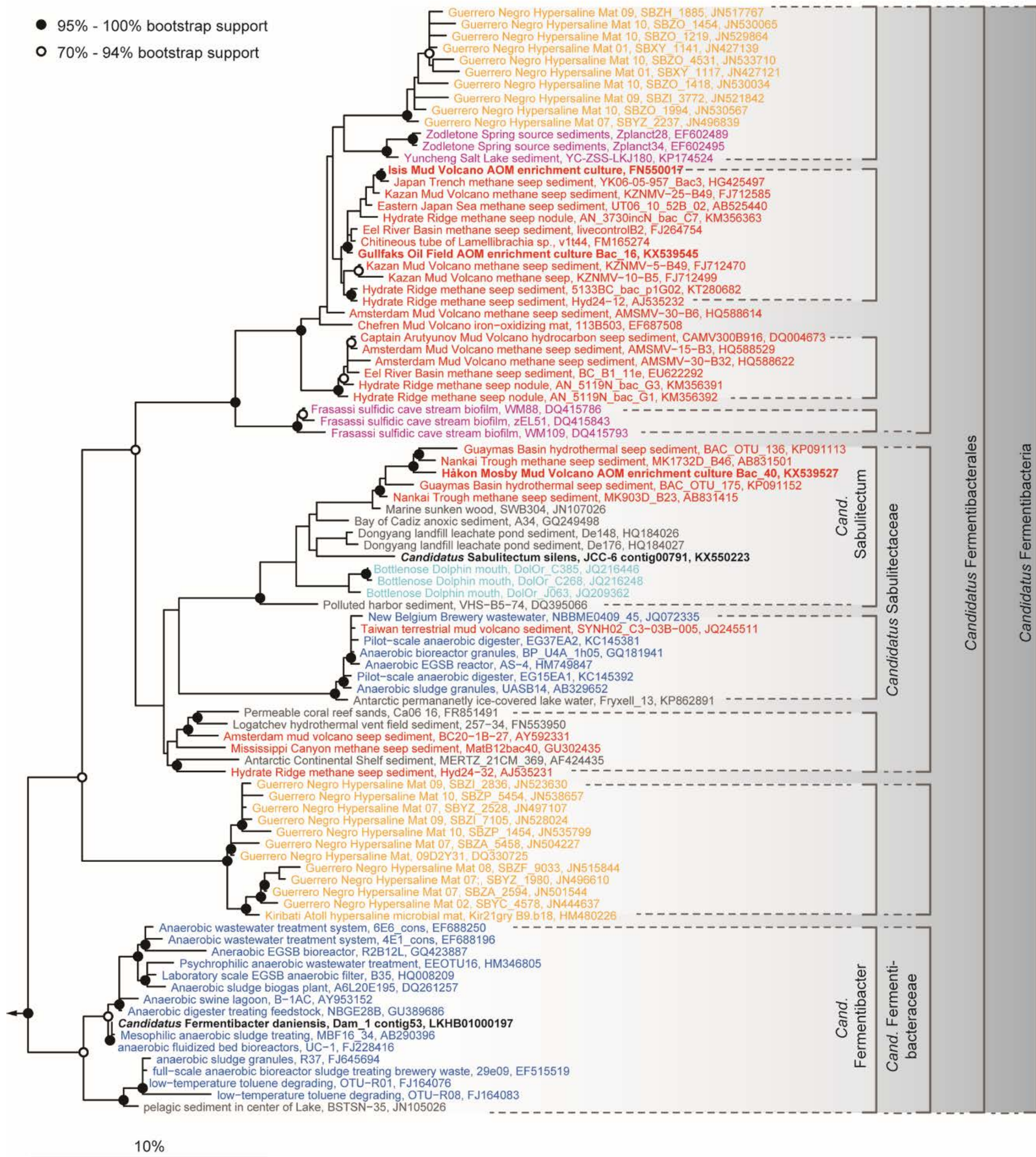

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726 **Figure 6**: Phylogenetic tree of the phylum "*Ca.* Fermentibacteria", showing the affiliation of all
727 publicly available, non-redundant 16S rRNA gene sequences, including the provisional species
728 *Ca.* Sabulitectum silens and *Ca.* Fermentibacter daniensis (black). The phylum comprises one class
729 (at a threshold sequence identity of 78.5%), one order (at 82%), four families (at 86.5%) and at
730 least nine genera (at 94.5%). The origin of the sequences is color-coded (red: methane seeps; red
731 bold: anaerobic methanotrophic enrichment cultures; orange: hypersaline mats; pink: springs; light
732 blue: dolphin, dark blue: anaerobic digesters: grey: other) and indicates niche-differentiation
733 among *Fermentibacteria*. An extensive list of ecosystems harboring *Fermentibacteria* is provided
734 in the Supplementary Results. Phylogeny is based on the SILVA small subunit ribosomal database
735 SSURef 123.1 (released 03/2016). The scale bar shows estimated sequence divergence.
736 *Fermentibacteria* sequence alignments and phylogeny are provided as an ARB database (Dataset
737 3). The parameters that were used to compile the sequence database are described in the Supporting
738 Information.
739
740

741 **Table 1. Properties of the 18 bins obtained from metagenomes of the six continuous cultures (Oxy-1/2, Nit-1/2, Con-1/2)**

742

Bin	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**
Affiliation	Rhodo bacterales	Altero monadales	Chromati ales	Desulfo bacterales	Desulfuro monadales	Desulfo vibrionales	Firmi cutes	Clostridi ales	Clostridi ales
Size (Mb)	3.85	3.08	1.55	4.77	4.10	4.11	4.27	4.45	4.59
Number of contigs	3272	1897	1650	594	237	885	2310	351	562
N50 contig length (kb)	1.5	2.5	1.1	126	58.9	14.6	2.8	27.6	107
GC content (%)	60.1	50.6	47.6	51.9	50.2	53.9	40.7	37.6	39.9
Number of CSCGs	131	115	71	132	131	138	121	112	119
Number of tRNAs	41	34	16	43	50	60	62	31	51
Completeness (%)	71.1	73.2	33.5	84.9	89.3	86	72.9	74.2	79.4
Contamination (%)	25	7	3	15	11	8	9	8	20
In situ relative abundance, Mean ± S.D. (%)	0.05±0.026	0.035±0.022	0.013±0.009	0.041±0.024	0.048±0.028	0.062±0.036	0.002±0.001	0.02±0.012	0.031±0.02

Bin	**J**	**K**	**L**	**M**	**N**	**O**	**P**	**Q**	**R**
Affiliation	Clostridi ales	Clostridi ales	Spiro chaetales	Spiro chaetales	Spiro chaetales	Fermenti bacteria	Anaero linea	Bacteroi detes	Bacteroi detes
Size (Mb)	6.69	3.86	3.36	3.57	3.78	2.92	2.51	1.68	2.30
Number of contigs	3459	248	40	317	691	96	1147	1661	2657
N50 contig length (kb)	3.3	118	144	160	10.1	222	6.0	1.2	0.99
GC content (%)	34.5	45.4	53.5	36.8	35.7	56.6	52.8	43.5	42.1
Number of CSCGs	182	126	129	88	93	98	128	69	100
Number of tRNAs	41	39	47	47	36	39	36	11	11
Completeness (%)	86.1	90.7	88	82.1	75.3	76.9	86.7	40.5	42.4
Contamination (%)	40	18	10	15	17	5	9	2	21
In situ relative abundance, Mean ± S.D. (%)	0.019±0.01	0.023±0.011	0.021±0.015	0.021±0.015	0.016±0.011	0.019±0.01	0.015±0.009	0.005±0.004	0.012±0.008

743
744 CSCG: Conserved single-copy gene
745 S.D.: Standard deviation of the mean
746