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3 **Impacts of Deep-Sea Mining on Microbial Ecosystem Services**

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30

31 **ABSTRACT**

32

33 Interest in extracting mineral resources from the seafloor through deep-sea mining has
34 accelerated substantially in the past decade, driven by increasing consumer demand for various
35 metals like copper, zinc, manganese, cobalt and rare earth elements. While there are many on-
36 going discussions and studies evaluating potential environmental impacts of deep-sea mining
37 activities, these focus primarily on impacts to animal biodiversity. The microscopic spectrum of
38 life on the seafloor and the services that this microbial realm provides in the deep sea are rarely
39 considered explicitly. In April 2018, a community of scientists met to define the microbial
40 ecosystem services that should be considered when assessing potential impacts of deep-sea
41 mining, and to provide recommendations for how to evaluate these services. Here we show that
42 the potential impacts of mining on microbial ecosystem services in the deep sea vary
43 substantially, from minimal expected impact to complete loss of services that cannot be remedied
44 by protected area offsets. We conclude by recommending that certain types of ecosystems should
45 be “off limits” until initial characterizations can be performed, and that baseline assessments of
46 microbial diversity, biomass, and biogeochemical function need to be considered in
47 environmental impact assessments of all potential instances of deep-sea mining.

48

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50 **KEYWORDS (5-8):** deep-sea mining, ecosystem services, hydrothermal vents, inactive
51 sulfides, ferromanganese nodules, cobalt crusts, seamounts, marine microbiology

52

53 1. INTRODUCTION

54

55 With increasing demand for rare and critical metals – such as cobalt, copper, manganese,
56 tellurium, and zinc – there is increasing interest in mining these resources from the seafloor
57 (Hein et al., 2013a; Wedding et al., 2015). The primary mineral resources in the deep sea that
58 attract attention fall into four categories (Figures 1, 2): (1) massive sulfide deposits created at
59 active high-temperature hydrothermal vent systems along mid-ocean ridges, back-arc spreading
60 centers, and volcanic arcs; (2) similar deposits at inactive hydrothermal vent sites; (3)
61 polymetallic “nodules” that form on the seafloor of the open ocean (often referred to as
62 manganese nodules); and (4) other polymetallic crusts that can form in the deep sea at
63 underwater mountains called seamounts (often referred to as cobalt crusts). Current areal
64 estimates of these resources range from 38 million km² for ferromanganese nodules, 3.2 million
65 km² for massive sulfides (combined active and inactive), and 1.7 million km² for polymetallic
66 crusts on seamounts (Petersen et al., 2016). Some of these resources occur within the Exclusive
67 Economic Zones (EEZ) of coastal nations, while others occur in international waters. In some
68 EEZs continental shelf sediments, additional commercial interests include diamond and
69 phosphorite deposits. Since these fall exclusively within national jurisdictions, they will not be a
70 focus of this article, although mining activities for these deposits are currently occurring (Miller
71 et al., 2018).

72

73 For seabed mineral resources in international waters beyond national jurisdiction (referred to as
74 “the Area”), access is only possible through the International Seabed Authority (the ISA) as
75 established in the United Nations Convention on the Law of the Sea (UNCLOS). The ISA awards
76 contracts of blocks of seafloor via sponsoring States to authorized contractors for resource
77 exploration activity. This is done following regulations established under the Mining Code,
78 which were established in 2000 and updated in 2013 for polymetallic nodules, in 2010 for
79 polymetallic sulfides, and in 2012 for cobalt-rich crusts. More than 1.3 million km² of
80 international seabed is currently set aside in 29 exploration contracts for mineral exploration in
81 the Pacific and Indian Oceans and along the Mid-Atlantic Ridge (Figure 1); another 1 million
82 km² of seabed has been licensed or applied for in waters under national jurisdiction (Cuyvers et
83 al., 2018). Currently, no country has an exploitation license to actively mine these resources in
84 the Area, but the ISA is developing international regulations to govern future exploitation
85 activities within the Area (International Seabed Authority, 2012; 2013). A few States have
86 already begun allowing resource exploration and extraction testing in their national waters
87 (Figure 1). Companies are developing and testing prototype mining equipment for this purpose.
88 Proponents of deep-sea mining argue that resource extraction from the deep-sea is more
89 environmentally friendly than mining on land for the same metals, but the environmental impacts
90 of these efforts are currently poorly understood. The UNCLOS stipulates that deep-sea mining
91 related activities on the international seabed within the Area must be carried out for the benefit of
92 mankind (UNCLOS Articles 136, 137 and 140; (Cuyvers et al., 2018)). Thus, it is imperative to
93 robustly and unbiasedly assess what the positive and negative impacts of deep-sea mining may
94 be, to determine the nature and extent of benefits and consequences for mankind.

95

96 Mineral resources on the seabed are also centerpieces of deep-sea ecosystems, functioning as
97 refugia and stepping stones for animal biodiversity. For example, polymetallic crusts in the deep-
98 sea serve as hard substrate for the attachment of sessile animal communities such as sponges, or

99 for egg-laying for mobile species like octopus which do not anchor in the soft sediment
100 surrounding the deposits. As another example, unique animal communities have evolved to
101 survive under the high temperature and extreme chemical conditions found at hydrothermal vents
102 where massive sulfide deposits form from the interaction of these hot, mineral-rich fluids with
103 surrounding cold seawater. Thus, these mineral deposits often host “hotspots” of animal life on
104 the otherwise barren seafloor. There have been several recent studies and syntheses on the
105 potential impacts of mining of these mineral resources on animal life (Boschen et al., 2013;
106 Vanreusel et al., 2016; Jones et al., 2017; Suzuki et al., 2018).

107
108 In addition to the visible animal life associated with these resources, diverse *microscopic* life
109 also flourishes in these systems. This nearly invisible microbial life is responsible for the
110 majority of the chemical cycling that occurs in these habitats, providing essential ecosystem
111 services for the deep-sea environments. Microbial life represents a vast and diverse genetic
112 reservoir with mostly unexplored potential for medical and commercial applications. Despite the
113 importance of the microscopic component of life to ecosystem services in the deep sea, this
114 category has been largely overlooked in current planning related to assessing and evaluating
115 possible environmental impacts related to deep-sea mining.

116
117 To address this gap in understanding and provide recommendations to policy makers about the
118 possible impacts to deep-sea ecosystem services provided explicitly by microscopic life, a
119 workshop of experts in deep-sea microbial ecology and geochemistry convened in April 2018 to
120 discuss these topics, with support from the Center for Dark Energy Biosphere Investigations, the
121 Deep Carbon Observatory, and the Bigelow Laboratory for Ocean Sciences. The outcomes of
122 this workshop are presented here. We provide an overview of the four mineral resource types
123 along with descriptions of the microbial ecosystems that they support, the ecosystem services
124 that these microbial communities provide, and an assessment of possible impacts to these
125 services from mining activity. We also provide recommendations for baseline assessment and
126 monitoring to evaluate the impact of mining activities on microbial ecosystem services.

127
128

129 **2. EVALUATING THE POTENTIAL IMPACT OF DEEP-SEA MINING ON** 130 **ECOSYSTEM SERVICES FROM MICROORGANISMS**

131

132 Seventy percent of the solid exterior of Earth lies under the ocean. While our perceptions of life
133 on Earth are skewed by our daily encounter with photosynthesis-supported life on land, the deep-
134 sea is a fundamentally different environment where sunlight does not penetrate. In deep-sea
135 environments, energy for life comes in two forms. The first is through the respiration of organic
136 matter delivered either in dissolved or particulate form (ranging from small particles up to large
137 “food falls” like dead whales) from the sunlit surface world that is ultimately sourced from
138 photosynthesis. The second source of energy is through generation of new organic matter (i.e.,
139 primary production) from a process known as *chemosynthesis*, where energy from inorganic
140 chemical reactions is used to convert dissolved carbon dioxide into the organic molecules
141 (sugars, fats, proteins, etc.) that are the building blocks of life. The ratio of these two energy
142 sources can vary significantly in the deep sea, with habitats like hydrothermal vents offering a
143 figurative buffet of chemical reactions that can fuel abundant chemosynthesis-driven microbial
144 life. Similarly, in the low-temperature mineral deposits like ferromanganese nodules and cobalt

145 crusts, chemosynthetic processes also occur (Orcutt et al., 2015), although the ratio of the two
146 energy sources is poorly constrained. In these ecosystems, the chemosynthetic microbial life can
147 form the base of the food web. Thus, disruption to the supply of chemical energy sources can
148 have consequences for the amount and type of life that can be supported (**Figure 3**). The
149 following sections describe how mining activities can upset the chemical energy supplies that
150 fuel microbial life in these ecosystems, and how this can result in a disruption of the ecosystem
151 services that microscopic life provides (**Figure 3**).

152

153 ***2.1. ACTIVE VENTS AND ACTIVE VENT FIELDS***

154

155 Hydrothermal vents are among the most dynamic environments on Earth, where hot, chemically
156 reduced fluids come into contact with cold, oxidized seawater, leading to the precipitation of
157 metal-rich deposits on and beneath the seafloor surrounding these vents (**Figure 2**). The high flux
158 of metal-rich fluids mixing with cold, oxic seawater is a natural mechanism for accumulating
159 iron, copper, zinc, and other economically viable elements within metal-sulfide rich mineral
160 deposits, which makes these areas conducive to supporting chemosynthetic life as well as bring
161 attractive targets for mining.

162

163 Any given vent ecosystem might only be the size of a football field, with a handful of 5-10 m
164 diameter concentrated deposits within that footprint, or alternatively with the entire area
165 consisting entirely of massive sulfide. Individual vent fields can be separated by 10s to 100s of
166 kilometers, depending on the geological setting (Hannington et al., 2011). Inactive sulfide-rich
167 mineral deposits often surround active vents where mineral deposition is occurring, in a
168 formation processes that can take thousands of years (Jamieson et al., 2013). The combined
169 global footprint of all active vent ecosystems is estimated to be up to 50 km², which is
170 <0.00001% of the planet's surface (Van Dover et al., 2018). Because of the difficulty in
171 separating active from inactive vent sites (see **Supplemental Materials**), the total amount of
172 mineable resources resulting from high temperature hydrothermal activity is covered in the next
173 section on inactive sulfides.

174

175 From their first discovery in the late 1970s (Corliss et al., 1979), hydrothermal vents have
176 attracted widespread public attention because they support unique and abundant animals that
177 thrive in these systems because of symbioses with chemosynthetic microorganisms (Dubilier et
178 al., 2008; Sievert and Vetriani, 2012). Geological and geochemical heterogeneity of vent fields
179 leads to localized differences in fluid and deposit chemistry (Fouquet et al., 2010; German et al.,
180 2016), which translates to animal endemism and biodiverse animal populations (Van Dover,
181 2000; Van Dover et al., 2018). Changes to hydrothermal venting chemistry or intensity could
182 have repercussions on the types of microbial life that can exist, and therefore on the animals that
183 can be supported.

184

185 **2.1.1. Possible impacts to biomass, primary production and microbial diversity at active 186 vents**

187

188 Microbes inhabit nearly every niche associated with active hydrothermal systems including the
189 rocks and fluids in the subseafloor, sulfide chimney walls and surfaces, and in animal
190 assemblages as internal and external symbionts (Fisher et al., 2007; Dubilier et al., 2008;

191 Schrenk et al., 2009). Microbes growing on and within hydrothermal chimneys often produce
192 thick biofilm mats easily visible to the naked eye, and even the extreme zones of high-
193 temperature chimneys where temperatures up to 122°C host millions of microbial cells per gram
194 of chimney material (Schrenk et al., 2003; Han et al., 2018). Nevertheless, the vast majority of
195 microbial biomass in hydrothermal systems probably resides in the porous subseafloor
196 underlying the chimneys. The amount of microbial biomass in the subseafloor of active
197 hydrothermal vent systems is poorly constrained, though, due to difficulties in accessing this
198 environment. Models of fluid circulation that assume a temperature limit of life of 122°C (Takai
199 et al., 2008) as the main limitation to life yield a wide range of results depending on the depth of
200 fluid circulation within the seafloor (Lowell et al., 2015), which can range from a few
201 centimeters to the full thickness of the highly permeable basalt layer (~ 500 m). Therefore,
202 mining activities risk removing the bulk of microbial biomass in areas where the habitable
203 crustal area is thin. Without being able to directly observe and sample this microbial habitat, the
204 diffuse fluids exiting cracks in the seafloor are considered to be windows into the subsurface of
205 active vent systems (Deming and Baross, 1993; Huber and Holden, 2008). Diffuse fluids contain
206 active microbial cells that are in one to two orders of magnitude greater abundance than that of
207 the surrounding seawater (Karl et al., 1980; Huber et al., 2002; Meyer et al., 2013) with diverse
208 metabolic capacities that affect global chemical cycling of carbon, nitrogen, iron, and sulfur
209 (Mehta and Baross, 2006; Wankel et al., 2011; Holden et al., 2012; Bourbonnais et al., 2014;
210 Fortunato et al., 2018).

211
212 The physiologically diverse microorganisms inhabiting active vent fields are considered to be
213 fast growing and highly productive. The annual global production of biomass is estimated to
214 reach 1.4 Tg carbon, significantly influencing deep-sea chemical cycling (McNichol et al.,
215 2018). Thus, despite the small size of active vent fields, the rates of microbial primary
216 productivity by microorganisms fueled by chemosynthesis in active vent systems can rival that of
217 coastal and open ocean photosynthetic systems, making them productivity hotspots in an
218 otherwise energy-starved deep sea (McNichol et al., 2018). As primary producers in the
219 ecosystem, microbes support nearly all life at vents, from microbial consumers to the abundant
220 and charismatic animals (Sievert and Vetriani, 2012). Animals can benefit from microbial
221 primary productivity directly by harboring endosymbionts (e.g., tube worms, mussels, and
222 clams) or indirectly by grazing microbial mats (e.g., *Rimicaris* shrimp)(Dubilier et al., 2008).
223 Therefore, a major disruption of the chemical conditions that permit microbial chemosynthesis
224 could have devastating consequences for all animals in that ecosystem.

225
226 Beyond their role in supporting primary productivity through chemosynthesis, many microbes in
227 hydrothermal ecosystems play essential roles for animals by providing cues for larvae to settle
228 (O'Brien et al., 2015). Microbial mats create barriers that slow the release of diffuse fluids and
229 concentrate the energy-rich chemicals used in chemosynthesis. Growth of the microbial mat
230 attracts microscopic and macroscopic grazers and traps more fluids, resulting in a rich ecosystem
231 that supports dense and diverse assemblages of animals and microbes (Fisher et al., 2007). The
232 concentration of energy and nutrients in complex, microbially-created habitats with strong spatial
233 gradients fosters the evolution of highly diverse microbial communities, making hydrothermal
234 vent systems hotspots of microbial diversity on the seafloor (Campbell et al., 2006; Schrenk et
235 al., 2009; Olins et al., 2013; Meier et al., 2017).

236

237 In addition to the microscopic Bacteria and Archaea that are the base of the food web,
238 hydrothermal systems also host abundant microscopic Eukarya, including protists and fungi
239 (Edgcomb et al., 2002; López-García et al., 2007), as well as viruses (Ortmann and Suttle, 2005;
240 Williamson et al., 2008; Anderson et al., 2011; He et al., 2017). The diversity, distribution, and
241 ecological roles of both groups are poorly constrained. Therefore, although hydrothermal
242 systems are one of the better studied deep-sea environments (Figure 4), there is still much to
243 learn about their microbial communities and their role in maintaining ecosystem functions.
244

245 In summary, microbial life at active hydrothermal vents are the dominant base of the food web at
246 these sites, supporting abundant and diverse animal life at distinct “oases” on the seafloor. These
247 microbial ecosystems comprise abundant standing stock of life that is diverse and highly
248 productive, fueled by the abundant chemical energy supplies in these active vent systems.
249 Mining activities near active vents could disrupt the nature of fluid flow, and therefore the
250 availability of chemical energy to these ecosystems, potentially causing a cascade effect on the
251 size, production, and diversity of these ecosystems (Figure 3) (Van Dover et al., 2018).
252

253 **2.1.2. Potential loss of genetic resources from active vent ecosystems**

254

255 In addition to being biological hot spots, hydrothermal vents are also targets for natural products
256 discovery owing to the unique genetic resources that some of the microbes contain (Thornburg et
257 al., 2010). According to the Convention on Biological Diversity, the term “genetic resources” is
258 defined as genetic material (i.e., any material of plant, animal, microbial, or other origin
259 containing functional units of heredity) of actual or potential value. They may be used in
260 biotechnological development of pharmaceutical drugs, research-based enzymes, food
261 processing enzymes, cosmetic products, and other potential applications. Natural products from
262 marine animals and microbes are already being marketed as anti-cancer and anti-viral drugs as
263 well as various “cosmeceuticals” – cosmetic products with medicinal properties (Martins et al.,
264 2014). For example, a novel benzoquinone compound isolated from a thermophilic bacterium
265 from a deep-sea hydrothermal vent had anti-tumor activity by triggering cell death of cancer cells
266 (Xu et al., 2017), and recently a novel antibiotic was identified from a vent microorganism (Shi
267 et al., 2017). DNA polymerases Vent® and Deep Vent®, both isolated from hyperthermophilic
268 vent microorganisms, are marketed for research applications in molecular biology.

269 Cosmeceuticals such as Abyssine® and RefirMAR® capitalize on excretions and internal
270 proteins from hydrothermal vent bacteria and are marketed as reducing irritation in sensitive skin
271 and reducing wrinkles. Numerous additional bioactive compounds from hydrothermal vent
272 microorganisms are in research and development phases, and awareness of the potential of
273 hydrothermal vents, other extreme environments, and the deep seafloor in general, as sources
274 of natural products is growing (Navarri et al., 2016; Zhang et al., 2018). The prevalence of
275 symbiotic relationships among hydrothermal vent species may also be a source of untapped
276 potential given that some bioactive compounds isolated from marine animals have now been
277 attributed to their microbial symbionts (Piel, 2009; Penesyan et al., 2010).
278

279 Because each active vent site most likely contains endemic microbial species that are unique to
280 the particular environmental conditions at that site (e.g. (Huber et al., 2010)), disruption of any
281 vent site is likely to have some level of negative impacts on humanity's ability to discover and
282 utilize these genetic resources. The ability of these unique microbial ecosystems to reset after

283 anthropogenic disturbance is poorly known, although there is evidence of recovery after natural
284 disturbances such as volcanic eruptions (Opatkiewicz et al., 2009; Fortunato et al., 2018), so it is
285 not clear if these impacts would be permanent, long-lasting, or ephemeral (Figure 3). Very few
286 active vent systems have been studied in any detail, so a primary concern about mining of these
287 systems is that their unique biodiversity and genetic resources could be lost before they are ever
288 discovered.

289

290 **2.1.3 Impacts on other microbial ecosystem services at active vents**

291

292 In addition to the metals that are sourced from hydrothermal vents, other reducing substrates
293 such as methane – a potent greenhouse gas – are highly enriched at active vent sites (Holden et
294 al., 2012). Some specialized microbes use methane as their primary energy source and convert it
295 into the less potent greenhouse gas carbon dioxide. Microbial consumption of methane and other
296 chemicals has a measurable impact on the flux of these chemicals from hydrothermal systems
297 into the ocean (Wankel et al., 2011; Wankel et al., 2012), but more exploration of these systems
298 is required for accurate estimates of their global contribution to the carbon cycle. Irrespective of
299 its global significance, disruption of the vigorous microbial activity in hydrothermal systems is
300 likely to have unpredictable consequences for nearby deep-sea habitats, which may be exposed
301 to chemicals such as methane, hydrogen, and hydrogen sulfide that were previously removed in
302 the vents (Figure 3).

303

304 Active vent systems also have incalculable value as representations of habitats that were likely to
305 be prevalent on the ancient Earth and perhaps even acted as the cradle for the evolution of
306 microbial life (Baross and Hoffman, 1985; Martin et al., 2008). Similar environmental conditions
307 that promote vigorous microbial activity in vents today could have also promoted the origin and
308 early evolution of life on ancient Earth (e.g. (Baaske et al., 2007)). Furthermore, the isolation of
309 deep-sea vents from the surface would have enabled them to act as refugia for early lifeforms
310 when conditions at the surface of the planet were not hospitable (Nisbet and Sleep, 2001). Many
311 of the enzymes and metabolic pathways used by vent microbes today appear to contain clues
312 about the nature of the first biological molecules (Russell and Martin, 2004) and key
313 evolutionary milestones (Nasir et al., 2015). Therefore, the microbial diversity of active vents is
314 not only important for modern ecosystem functions but also as natural wonders and precious
315 cultural and educational resources that connect us to our ancient origins on this planet (Figure 3).

316

317 Finally, it must be emphasized that the vast majority of microbial life at hydrothermal vents has
318 not been explored (Figure 4), despite increasing improvements in access to the deep ocean and
319 new analytical tools (Xie et al., 2011; Fortunato and Huber, 2016; Fortunato et al., 2018).

320 Therefore, many of the ecosystem services that microbes provide in these ecosystems are not yet
321 known to science. Thus, the cultural heritage and educational services that active hydrothermal
322 vents provide, both known and unknown, could be lost from mining activities.

323

324 In summary, the ecosystem services described above (Figure 3) highlight the importance of
325 active hydrothermal vents. As argued elsewhere (Niner et al., 2018; Van Dover et al., 2018),
326 given the substantial challenges associated with the meaning and measurement of “no net loss”
327 guidelines – e.g., distinguishing between different types of biodiversity, the central importance of
328 function in addition to identity, and consideration of spatial and time scales involved – we argue

329 that active systems should not be mined. This view is bolstered by recent recommendations
330 (IUCN, 2016; OECD, 2016; Cuyvers et al., 2018), which note that perturbation coupled with
331 biodiversity offsets is not an acceptable interpretation of the ISA's remit to protect the marine
332 environment for the common benefit of humanity, and other recent studies that try to model the
333 efficacy of offsets for this type of resource (Dunn et al., 2018). Caution is particularly prudent
334 when there is uncertainty around an ecosystem's vulnerability and recoverability (Donohue and
335 al., 2016), as with microbial communities at active hydrothermal vents. Given this stance, it is
336 troubling that a large fraction of known active vents are within areas of the seabed that have
337 already been contracted for exploration and possible exploitation (Figures 3, 4).

338

339 **2.2. INACTIVE VENT FIELDS WITH MASSIVE SULFIDE DEPOSITS**

340

341 Inactive vent fields are the remnants of prior active hydrothermal circulation (Figure 2). Current
342 volumetric estimates and ore percentages of seafloor massive sulfide deposits approximate those
343 of terrestrial ores, though the size of individual deposits is up to 20 Mt as opposed to opposed to
344 50-60 Mt for terrestrial equivalents (Hoagland et al., 2010; Hannington et al., 2011; Petersen et
345 al., 2016). 90% of known deposits are less than 2 Mt, with only 10% above the current 2 Mt
346 threshold of economic interest (Petersen et al., 2016). Moreover, mineral content, and therefore
347 economic value, also varies greatly between and within deposits, with those of largest volume
348 not necessarily being the most valuable (Petersen et al., 2016). Finally, these are rough estimates
349 and they do not distinguish between active and inactive hydrothermal vent fields, which could
350 differ greatly in the challenges they present to mining operations and in the potential impacts to
351 biological communities.

352

353 Inactive vent fields may be much more amenable to mining operations than active vents due to
354 their size and absence of high-temperature acidic fluids. A complication, however, is that
355 systems with no observable surficial venting may reveal underlying activity when disturbed by
356 mining. Individual quiescent chimneys in a still-active vent fields are not a truly inactive
357 hydrothermal system. Any indication of even minor venting of warm fluid could be indicative of
358 high temperature fluids at depth, with any disruption of surface material from mining activities
359 having operational and environmental consequences. Information is required regarding
360 underlying hydrology and microbial colonization patterns before any predictions can be made
361 regarding the potential unintended consequences of disturbing the microbial communities of
362 inactive vent fields.

363

364 **2.2.1. Possible impacts to microbial biomass, primary production and diversity at inactive 365 vent fields**

366

367 Inactive vent fields are not currently known to host many endemic *animals*, though this may be
368 due to a lack of exploration (Figure 4). Inactive vent fields represent a broad transition zone
369 between actively venting hydrothermal systems and non-hydrothermal seafloor environments
370 and are therefore expected to share features of each (Levin et al., 2016a; Levin et al., 2016b). As
371 in active vents, many animal taxa in inactive vent fields obtain their nutrition in association with
372 chemoautotrophic microbial symbionts (Erickson et al., 2009). Many animals from the next
373 generation recruit their symbionts from the environment independently of the previous
374 generation, so disruptions to the composition of the ambient seawater microbial communities

375 could affect the ability of these animals to persist in areas adjacent to mining activities even if
376 their own habitat is not directly affected (Figure 3).

377
378 By contrast, inactive hydrothermal vent fields are home to *microbial* species that are distinct
379 from those of active hydrothermal sites (Suzuki et al., 2004; Erickson et al., 2009; Sylvan et al.,
380 2012; Toner et al., 2013). The overall microbial community composition of inactive vent fields
381 can be similar to that of the surrounding seafloor (Kato et al., 2010), indicating that inactive
382 fields may not host as many unique and endemic populations as active vents do (Figure 3). Any
383 generalized descriptions of inactive vent fields are premature, however, considering that very
384 few examples have been detected and studied (Figure 4) (Boschen et al., 2013; Vare et al., 2018).
385 Furthermore, very few studies have attempted to characterize the microbial communities of
386 inactive vent fields, their roles in local and global biogeochemical cycling, or as refugia and
387 seed-banks for the more dynamic active vent fields. Inactive hydrothermal systems may lack
388 vigorous hydrothermal venting, but they nevertheless contain complex subsurface habitats with
389 unknown microbial ecosystems. Ecosystem services that these subsurface microbial communities
390 could potentially provide include primary production, secondary production, element cycling,
391 and unique genetic resources, although knowledge of these services is poorly constrained due to
392 very limited sampling (Figures 3, 4).

393 394 **2.2.2. Potential loss of habitat and creation of acidic conditions from mining massive sulfide** 395 **deposits at inactive vent fields**

396
397 A few categories of the potential impacts of mining on inactive sulfide-associated microbial
398 communities are highlighted here. Our estimates mainly come from activities occurring within
399 the national boundaries of Papua New Guinea, which is the most well-known mining project in a
400 seafloor hydrothermal system, led by Nautilus Minerals Ltd. (Coffey Natural Systems, 2008).
401 There have been recent reports of newer mining tests offshore Japan, but less information is
402 publicly available from this site.

403
404 Mining seafloor massive sulfide deposits is a form of strip mining, where the top layer of
405 sediment and crust is removed as overburden, and the exposed ore is removed in successive
406 layers until the deposit is completely removed or “mined out”. By comparison to terrestrial
407 mining sites, one can expect that exposure of massive sulfide deposits will start a cascade of
408 abiotic and microbially catalyzed reactions, due to the exposure of the deposits to oxygenated
409 seawater. Pyrite – an iron sulfide mineral – is the main constituent of massive sulfide deposits,
410 and the overall oxidation reaction that occurs when this mineral is exposed to oxygen and water
411 generates protons. Where the local environmental buffering capacity is unable to absorb these
412 additional protons, a feedback system takes effect that causes a pH decrease. A change in pH
413 causes changes in the type and speed of chemical reactions that occur (Bethke et al., 2011; Jin
414 and Kirk, 2018), leading to changes in metal and oxygen dissolution properties in addition to
415 changes in biology. This process and its effects are termed acid mine drainage or acid rock
416 drainage in terrestrial systems (Schippers et al., 2010; Nordstrom, 2011), where many studies
417 have been conducted on the pivotal roles that microbes play in contributing to these processes.

418
419 In terrestrial systems, exhausted strip mines create terraced open pits that can slowly fill with
420 lakes or groundwater of altered chemistry, as any remaining metal-rich sulfides react with

421 exposure to water and oxygen to create acidic conditions. In a marine sulfide system, such a pit
422 will be permanently exposed to the oxic deep seawater long after extraction ceases, also allowing
423 for the creation of acidic conditions. Although seawater has a higher pH buffering capacity than
424 freshwater on land, a recent study on treatment of acid mine drainage from a terrestrial massive
425 sulfide deposit (itself an ancient hydrothermal vent site) showed that a ratio of 1 part acid mine
426 drainage to 90 parts seawater was required to neutralize the acid conditions (Sapsford et al.,
427 2015). Biotic catalysis in the form of microbes may be a key factor in determining how exposed
428 sulfide deposits will react to bottom seawater, but no studies have directly investigated the role
429 of biological catalysis in marine environments affected by mining.

430
431 The consequences of the complete destruction and permanent loss of seafloor habitat caused by
432 deep-sea mining are difficult to predict (Figure 3), since there is no precedent for such activities
433 in the deep sea. One speculative scenario is that water in a deep mining pit on the seafloor may
434 become sufficiently isolated from actively flowing seawater to stagnate and create a potentially
435 permanent acidic, anoxic condition, but research is needed to investigate this possibility. Even
436 minimal fluxes of material out of the pit could be sufficient to propagate acid mine drainage
437 reactions to surrounding areas. Where there is local recharge of bottom seawater into ocean crust
438 (Fisher and Wheat, 2010), which itself would likely be affected by changes in seafloor
439 topography, the polluted water may also be entrained into the seafloor and transported from the
440 point source farther than predicted. The degree to which acidic mining pits might influence
441 surrounding ecosystems and the roles of microbial communities in these acid-generating
442 reactions requires investigation.

443 444 **2.2.3. Generation of tailings plumes from mining massive sulfide deposits at inactive vent** 445 **fields**

446
447 In addition to the loss of habitat directly caused by mining the seafloor, mining activities will
448 produce a plume of waste material that will disperse and fall on the surrounding seafloor, which
449 is expected to nearly double the total area of seafloor impacted by mining (Boschen et al., 2013;
450 Fallon et al., 2017). The environmental impact of this plume of waste material will depend on
451 several factors, but perhaps most importantly, on the proximity of the waste plume to active
452 hydrothermal systems. If the active vents are close enough to the mining area, they could become
453 buried in the mining plume. Determination of this critical distance should be studied prior to any
454 mining activities (Dunn et al., 2018).

455
456 Furthermore, because inactive vent fields are poorly explored and their possible level of
457 hydrothermal activity is difficult to ascertain without high resolution seafloor surveys, there is a
458 high likelihood that undiscovered active vents could be associated with apparently inactive
459 fields. If these vents are not discovered prior to mining activities, they could become buried in
460 the plume of waste material before there is any opportunity to explore their ecosystem services
461 and potential scientific value. In addition, burial of seafloor habitat, even if it is not
462 hydrothermally active, could disrupt the ability of animal larvae to sense seafloor conditions and
463 to respond to environmental cues of where to attach and colonize (Gollner et al., 2010; Gollner et
464 al., 2015).

465

466 There is also the potential impact of plumes of mining tailings closer to the ocean surface.
467 Current mining operation designs propose to transport mined seafloor material to a surface ship
468 for processing, returning the waste fluids to the ocean. This tailings waste stream, consisting of
469 rock/ore fragments of small size and initial treatment chemicals as well as elevated
470 concentrations of dissolved metals from the mining process, will create plumes of debris in the
471 water column (Nath et al., 2012; Boschen et al., 2013; Fallon et al., 2017). Tailings may extend
472 affected areas up to 80% from the point-source of pollution, though this can be difficult to
473 predict, particularly in the deep sea where baseline information is scarce (Boschen et al., 2013;
474 Hughes et al., 2015; Fallon et al., 2017; Vare et al., 2018). Studies on the effects and magnitude
475 of potential metal leachate concentrations indicate significant local effects, particularly in areas
476 of low or stagnant flow, and have the potential to remain in solution despite extensive mixing
477 (Sapsford et al., 2015; Fallon et al., 2017; Fallon et al., 2018). Any substantial chemical
478 amendments will likely have dramatic consequences for community structure and function, as
479 observed with hydraulic fracturing on land (Murali Mohan et al., 2013).

480
481 Legislation currently requires treatment of any mine tailings on land to minimize this historically
482 problematic waste (Dold, 2014; Hughes et al., 2015; Ma et al., 2017; Vare et al., 2018). Tailings
483 often still contain elevated concentrations of acid-generating sulfides and heavy metals, and thus
484 represent a significant additional source of mine drainage. Current deep-sea mining proposals
485 indicate disposal of rock over the side of the mining vessel at various depths (Schriever and
486 Thiel, 2013), which may eventually form deposits of sulfide-containing rock on the seafloor.
487 This is similar to Deep-Sea Tailings Disposal, a strategy already used by a small number of
488 terrestrial mines (Jones and Ellis, 1995; Schriever and Thiel, 2013; Dold, 2014; Vare et al.,
489 2018). Measurable impacts from these tailings dumps include elevated concentrations of various
490 transition metals in sediment, blanketing of the seabed by compacted precipitates, and release of
491 elevated concentrations of sulfur and transition metals into the water column (Kline and Stekoll,
492 2001; Shimmield et al., 2007; Ramirez-Llodra et al., 2015; Hauton et al., 2017).

493
494 A few studies have investigated the various effects of these tailings plumes on animals (Kline
495 and Stekoll, 2001; Mestre et al., 2017). Mining waste is known to affect microbial
496 biogeochemical cycling and the rates and success of community recovery in shallow coastal sites
497 (Pedersen, 1984; Pedersen and Losher, 1988; Almeida et al., 2007). However, no studies have
498 explored the impacts of tailings plumes on deep-sea microbial communities. Natural plumes
499 emitted from hydrothermal systems are known to have profound implications for the
500 composition and activity of deep-sea microbial communities (Anantharaman et al., 2013; Dick et
501 al., 2013; Levin et al., 2016a), therefore the potential impacts of tailings plumes can also be
502 expected to be significant.

503
504 One concern is that disruption of natural microbial communities and stimulation of heavy metal-
505 metabolizing microbes, in particular, will have far-reaching consequences for element cycling in
506 the deep sea (Figure 3). Some metals may enter solution due to microbial activity, thus spreading
507 the effect to a larger area and making the metals more bioavailable and increasing their toxicity.
508 Others may precipitate out of solution more readily, causing issues such as blanketing areas of
509 the seafloor with amorphous metal-rich precipitates. There is currently no research on the
510 relevant thresholds over which some level of mining activity might begin to impact marine
511 element cycling on a regional level.

512
513 Lessons from terrestrial massive sulfide mining show that environmental change brought about
514 by these activities persists long after mining activity has ceased, including cases where point-
515 source remediation measures are in place (Bird, 2016). However, remediation strategies that
516 might be applied to operationally challenging deep-sea environments are poorly developed,
517 though some studies have made tentative recommendations (Ramirez-Llodra et al., 2015; Vare et
518 al., 2018). An industry report proposes that active sites will regenerate themselves by generating
519 new mineral cover from already-present geochemical reactions and biology re-seeded from
520 nearby refugia (Coffey Natural Systems, 2008), though the report does not specify how long this
521 might take and whether it will require active human management. Even if this prediction is
522 reasonable for active vent fields, it is not applicable to inactive vent fields. We speculate that
523 taking no remedial action will likely result in acid mine drainage conditions over many decades,
524 but research is need to assess this. Remediation strategies are likely to involve either natural
525 dilution of the mining pit with seawater or else capping and permanent isolation of the pit. Both
526 strategies have potential consequences and require extensive investigation. Depending on the
527 local buffering capacities, natural dilution of the pit may not be sufficient to completely
528 neutralize the acid-generating chemical reactions, potentially resulting in spreading acid-mine
529 drainage across a much broader area of the seafloor. Capping the pit would require development
530 of new technology capable of permanently isolating a large deep-sea pit, and failure of the cap
531 could have devastating consequences for nearby ecosystems, potentially resulting in run-away
532 acid mine drainage reactions within the capped region.

533

534

535 **2.3. FERROMANGANESE NODULES**

536

537 Ferromanganese nodules form in sediment underlying organic-poor regions of the global ocean,
538 often at water depths > 4,000 meters (Figures 1, 2). In addition to iron and manganese, nodules
539 incorporate high concentrations of economically valuable metals such as nickel, cobalt, and
540 copper (Hein et al., 2013a). Nodule size ranges from microscopic particles to several cm in
541 diameter and occur dispersed across nodule fields. Nodule growth is extremely slow (mm to cm
542 accumulation per million years; (Ku and Broecker, 1965; Bender et al., 1966; Boltenkov, 2012)),
543 and surrounding pelagic sediments accumulate Mn at approximately similar rates as nodules (< 5
544 mg cm⁻³ per 1000 years; (Bender et al., 1970)). Nodules can acquire manganese from sediment
545 pore waters or from the overlying water column. The growth mechanism is mediated by the
546 redox state of overlying waters and, in some environments, growth can be supported by
547 hydrothermal influence and may change throughout the growth history of the manganese nodule
548 (Mewes et al., 2014; Wegorzewski and Kuhn, 2014). Whether nodule growth proceeds purely
549 abiotically, or is influenced by microbial activity or seeding is not currently known, although
550 microbial communities have been detected in nodules (Tully and Heidelberg, 2013; Lindh et al.,
551 2017). Recent studies have also documented novel animal communities that are supported by
552 nodule fields (Bluhm et al., 1995; Purser et al., 2016; Vanreusel et al., 2016; Peukert et al.,
553 2018).

554

555 Several studies have correlated water depth with nodule coverage to extrapolate and predict
556 nodule occurrences over a wider area (Park et al., 1997; Jung et al., 2001; Kim et al., 2012;
557 Peukert et al., 2018). Invariably, the occurrence of nodules coincides with areas of low

558 sedimentation; for example, the sedimentation rate in the Clarion Clipperton Zone is estimated to
559 be 0.3-15 mm per 1000 yrs (Jeong et al., 1994). These low sedimentation rates, which are typical
560 of ocean gyres, are due to extremely low productivity of the overlying ocean, which exports low
561 amounts of particles and organic matter to the deeper ocean. Thus, mining of nodules would
562 disrupt deep-sea sediment environments that have evolved over millennia and would likely take
563 just as long to recover to pre-disturbance conditions.

564
565 Manganese nodules harbor active microbial communities with cell densities three orders of
566 magnitude higher than in surrounding sediment (Shiraishi et al., 2016). However, the specific
567 organism(s) responsible for manganese oxidation and precipitation in those environments remain
568 unidentified, despite some recent studies suggesting different chemical processes and structures
569 occurring in the interiors versus exteriors of nodules, and nodule microbial communities that are
570 distinct from the surrounding sediment (Tully and Heidelberg, 2013; Blöthe et al., 2015; Shulse
571 et al., 2017). The interplay between sediment geochemistry and nodule microbial community
572 structure remains poorly understood. It is therefore difficult to predict what the microbial and
573 biogeochemical response and recovery would be to disturbance caused by deep sea mining
574 (Figure 3).

576 **2.3.1. Limited impacts to organic carbon sequestration from mining ferromanganese** 577 **nodules**

578
579 Marine sediments are a major sink of organic matter over geological timescales and an important
580 part of the global carbon and oxygen cycles (Berner, 2003). Sinking particles settling on the
581 ocean floor are buried, effectively protecting and preserving their organic matter contents, and
582 impeding it from microbial “remineralization” to carbon dioxide. The burial of organic carbon in
583 the deep ocean is an important component of the global carbon cycle, thus regulating
584 atmospheric CO₂ and global climate through the sequestration of carbon, and allowing the build-
585 up of oxygen in the atmosphere (Arndt et al., 2013; Hülse et al., 2017). Deep-sea mining of
586 ferromanganese nodules will cause the re-suspension of sediments (Thiel and Schriever, 1990),
587 potentially altering the ecosystem service of carbon sequestration that occurs in this habitat.

588
589 We estimate (see [Supplemental Materials](#) for calculations), however, that proposed mining of
590 these nodule-bearing sediments and resulting re-suspension of particles and organic matter will
591 have a trivial impact on the ecosystem service of carbon sequestration for two reasons (Figure 3).
592 First, these sediments contain extremely low quantities of organic matter (<0.5% percent
593 (Khrpounoff et al., 2006). This is typical for deep-sea sediment (Seiter et al., 2004), since the
594 particles delivering organic carbon to the ocean floor must sink over long distances to reach the
595 ocean floor, during which the majority of organic matter is remineralized by microbes in the
596 water column (Marsay et al., 2015; Cavan et al., 2017). Thus, only a relatively small mass of
597 carbon might be re-suspended, compared to the much higher carbon loads in nearshore sediment
598 environments. Second, the organic matter contained in these deep-sea sediments is likely to be
599 highly processed and thus not particularly bioavailable to microbial remineralization, so most of
600 the organic carbon would be redeposited on the seafloor and sequestered. Furthermore, as
601 stimulation of organic carbon remineralization in the overlying water column is likely to be low,
602 there would be inconsequential changes in dissolved oxygen concentration in bottom seawater
603 (<0.5%).

604

605 **2.3.2. Other microbial ecosystem service impacts from mining ferromanganese nodules**

606

607 Although the carbon sequestration ecosystem service of nodule fields would not be impacted,
608 other microbial ecosystem services in nodule fields are expected to be impacted by mining
609 activity (Figure 3). For example, as part of the European JPI Oceans Mining Impact project (Paul
610 et al., 2018), the DISturbance and reCOLonization (DISCOL) area was recently revisited to
611 study the long-term impact of nodule mining. The DISCOL experiment was carried out in 1989
612 in the Peru Basin in which the deep seafloor was plowed in an area of ~ 11 km² to mimic nodule
613 mining (Thiel et al., 2001). Clear geochemical differences, including metal distributions, in the
614 upper 20 cm of disturbed and undisturbed sediments could be observed even 26 years after
615 plowing (Paul et al., 2018). Based on their observations, the authors noted that nodule mining
616 will likely have long-lasting impacts on the geochemistry of the underlying sediment (Paul et al.,
617 2018). Specifically, solid-phase manganese concentrations were lower in disturbed areas
618 compared to reference areas. This finding suggests that the capacity for metal sequestration via
619 scavenging onto nodules will be substantially limited during the recovery period. The absence of
620 nodules in the disturbed area increases metal flux out of sediment, although it is argued that these
621 flux rates do not reach rates that are potentially toxic to animals (Paul et al., 2018).

622

623 Nodule regrowth may also be limited by both the geochemical and microbiological changes
624 following mining-related disturbances. For example, thermodynamic and kinetic constraints limit
625 the oxidation of reduced manganese to oxidized manganese by oxygen (Luther, 2010). Microbes
626 can catalyze this reaction via direct and indirect pathways; thus, the formation of most
627 manganese oxide minerals in the environment is microbially mediated (Hansel and Learman,
628 2015). A broad diversity of organisms are capable of manganese oxidation, from bacteria to
629 fungi (Hansel, 2017), although microbial manganese oxidation does not provide an energetic
630 benefit to the organism and the physiological purpose is unclear.

631

632 Mining activities will cause a decrease in the ecosystem service of this habitat through the
633 destruction of paleoscientific records, a valuable education aspect of this environment. Marine
634 sediment cores are an immensely valuable resource for reconstructing climate conditions over
635 Earth's history (Figure 3). Plant and animal fossils found in sediments are frequently used to
636 reconstruct and understand the past chemistry and temperature of the ocean. For example, the
637 calcium carbonate shells of microorganisms such as foraminifera or coccoliths can be analyzed
638 using oxygen isotopes to determine the temperature and chemistry of ancient seawater and how
639 cold the ocean was at the time the shell formed (Spero et al., 1997; Ornella Amore et al., 2004;
640 Maeda et al., 2017). Moreover, diatom microfossils can be used to understand upwelling currents
641 and reconstruct past wind and weather patterns (Abrantes, 1991; Schrader and Sorknes, 1991;
642 Zúñiga et al., 2017). In addition, dust layers found in sediment cores can be analyzed to
643 determine its origin to understand the direction and strength of winds and how dry the climate
644 may have been at that particular time (Rea, 1994; Middleton et al., 2018). Nodules themselves
645 record paleoclimate and seawater conditions. For example, rare earth element ratios in nodules
646 serve as a proxy for bottom water redox state and changes in deep currents over time (Glasby et
647 al., 1987; Kasten et al., 1998), and some rare earth element isotopes in ferromanganese nodules
648 also reveal patterns in ocean circulation throughout time (Albarède et al., 1997; Frank et al.,
649 1999; van de Flierdt et al., 2004). Marine sediment and nodules thus serve as a valuable resource

650 for reconstructing past climate conditions as well as understanding and predicting future climate
651 change. Sediment in nodule-rich regions is particularly valuable due to a low sedimentation rate
652 that allows for piston coring techniques to readily access extremely old sediment. Widespread
653 sediment disturbances from nodule mining would result in the loss of this record and educational
654 ecosystem service (Figure 3).

655
656 Overall, mining activities in nodule fields will have varied impacts on microbial ecosystem
657 services (Figure 3). Some services, such as carbon sequestration potential, will be minimally
658 impacted. Other services, such as research and educational value from paleoscientific records
659 contained within sediment layers, would be severely perturbed and not recoverable. Decades-long
660 studies have identified that microbial processes within the sediments underlying nodules remain
661 impacted for quite some time (Paul et al., 2018), but the corresponding impact this has to
662 biogeochemical cycling and ecological functioning is not constrained and requires further
663 investigation, despite this resource type having been the most studied for these kinds of impacts
664 (Figure 4).

665
666

667 **2.4. COBALT CRUSTS ON BASALTIC SEAMOUNTS**

668

669 Cobalt-rich crusts (also called polymetallic crusts) occur on sediment-free rock surfaces in all
670 oceans of the world (Figures 1, 2), ranging in thickness from <1 mm to ~260 mm. They are most
671 common in the Pacific Ocean where there are estimated to be over 50,000 seamounts and knolls
672 and many more seamounts likely exist in uncharted waters (Wessel et al., 2010; Levin et al.,
673 2016c). In addition to cobalt, other rare and trace metals of high economic value – including
674 copper, nickel, platinum and tellurium (used in the solar cell industry) – are adsorbed to the crust
675 from seawater. In the central Pacific, ~7,500 million dry tons of crusts are estimated, containing
676 4 times more cobalt, 9 times more tellurium, and a third of the manganese that makes up the
677 entire land based reserve of these metals (Hein et al., 2013b). Polymetallic crusts are formed
678 slowly (1-5 mm per million years), and biomineralization by microorganisms plays a role in
679 initiation of crust accretion, serving as a biological nuclei (Wang and Müller, 2009).
680 Microorganisms may also play a role promoting the enrichment of cobalt in the crust through
681 sorption/immobilization processes (Krishnan et al., 2006; Sujith et al., 2017).

682

683 **2.4.1. Possible impacts to biomass, primary production and microbial diversity in cobalt 684 crusts**

685

686 The alteration rinds that form on seafloor exposed basalts at seamounts and outcrops (i.e. cobalt-
687 rich crusts) provide a habitat suitable for sessile animals like corals and sponges that require a
688 hard substrate to attach to (Etnoyer et al., 2010; Shank, 2010), as well as for brooding animals
689 like octopus (Hartwell et al., 2018). However, the role microorganisms play in faunal
690 colonization and presence in these regions remains unknown, as does the relative role of
691 microbial chemosynthesis and heterotrophy in this ecosystem. Some studies suggest persistent
692 patterns in microbial community composition on highly altered seafloor-exposed basalts that
693 have ferromanganese crusts (Lee et al., 2015). Surveys of indigenous microorganisms from
694 sediments associated with cobalt-rich crusts (Liao et al., 2011; Huo et al., 2015) and manganese
695 rich crust (Nitahara et al., 2011) have detected the potential for microbial chemosynthetic

696 primary production supported by ammonia oxidation. Similarly, the amount of primary
697 production supported by microbial communities on altered seafloor basalts could be significant
698 for carbon cycling in the deep sea (Orcutt 2015).

699
700 Removal of alteration crusts from seamounts and outcrops through mining/dredging is expected
701 to physically alter the seafloor substantially. The overall slope of the seamount may be flattened,
702 and the amount of soft sediment increased through disturbance and release of waste during the
703 mining process (Levin, 2013). This mining activity would dramatically impact sessile animal
704 communities, although recovery rates from these disturbances are unknown. Mining activity
705 would expose fresh surfaces of underlying basalt rocks, which would eventually be altered
706 through seawater exposure, although this process would be very slow (1-5 mm per million
707 years). Due to the slow growth of both the alteration crust as well as the fauna that live on them,
708 the recovery time for physically disturbed crusts on seamounts caused by mining is predicted to
709 be long (Schlacher et al., 2014). For example, recovery and recolonization of seamounts by
710 animals was found to not have occurred after 10 years of closing bottom-trawling activities in
711 coastal New Zealand and Australia (Williams et al., 2010). Dredging activities for recovering the
712 crusted rocks underneath, and not just animals, is expected to have even slower recovery rates.

713
714 Moreover, mining/dredging activities that change the physical structure of the seamount/outcrop
715 would potentially impact fluid circulation pathways through basaltic crust, especially on ridge
716 flanks. This outcrop-to-outcrop fluid circulation away from the ridge axis ventilates the majority
717 of heat from the oceans (Fisher et al., 2003; Fisher and Wheat, 2010) and is an important
718 component of global geochemical cycles (Wheat et al., 2017; Wheat et al., submitted). For
719 example, a recent study indicated that at least 5% of the global ocean dissolved organic pool is
720 removed via microbial oxidation within the subsurface ocean crust during ridge flank fluid
721 circulation (Shah Walter et al., 2018). Mining activities could change the permeability, porosity
722 and locations of fluid discharge, which would impact fluid circulation and could have
723 consequences on the nature of microbial communities resident in these environments that are
724 influenced by fluid conditions (Figure 3) (Zinke et al., 2018). However, very little is known
725 about fluid circulation far away from ridge axes where many seamounts occur, so it is difficult to
726 know how widespread this disruption could be.

727

728 **2.4.2. Other possible impacts to microbial ecosystem services in cobalt crusts**

729

730 Microorganisms in cobalt crusts can likely use metals as an energy source and carry adaptations
731 to tolerate the high heavy metal concentrations that occur in polymetallic crusts, potentially
732 playing a role in metal cycling in oceans. Crustal microorganisms have demonstrated the ability
733 to immobilize cobalt from seawater, release trace metals like nickel, and may also be capable of
734 scavenging other metals (Krishnan et al., 2006; Antony et al., 2011). These traits are of interest
735 for biotechnological applications, or applications that involve metal/microbe interactions such as
736 bioremediation of polluted sites, bioleaching, and metal recovery (Figure 3). However, the
737 financial considerations for dredging cobalt crusts from the seafloor limit the viability of this
738 natural product discovery track.

739

740

741 **3. RECOMMENDATIONS FOR BASELINE AND MONITORING DATA TO**
742 **EVALUATE IMPACTS TO MICROBIAL ECOSYSTEM SERVICES**

743
744 Recommendations for baseline measurements and monitoring of mining impacts have been
745 published elsewhere (Gjerde et al., 2016; Henocque, 2017; Boetius and Haeckel, 2018; Cuyvers
746 et al., 2018; Durden et al., 2018; Jones et al., 2018). These recommendations include
747 measurements of animal biodiversity and deep-sea ecosystem structure and function. We propose
748 that including microorganisms in these biodiversity and ecosystem measurements is critical for
749 effective monitoring of mining impacts. In high energy environments like hydrothermal vents,
750 where many microorganisms have short generation times, measurements of microbial diversity
751 are likely to be highly sensitive and quickly responsive to environmental impacts. Alternatively,
752 impacts in lower energy environments like deep-sea sediments hosting ferromanganese nodules
753 may be harder to discern. Furthermore, the responses of microbial communities to mining
754 impacts will be more complex than a simple "good" or "bad", as microbial species will respond
755 in many different ways. Changes in microbial community composition are likely to convey a
756 wealth of information about changes to the environment, if we are able to detect and decipher
757 these signals. With enough research, monitoring of microbial communities could become
758 sufficiently sensitive and specific to enable adjustments of ongoing mining activities before
759 impacts to animal communities reach a dangerous threshold.

760
761 Predicting and assessing the environmental impacts of mining in the deep sea is fundamentally
762 more challenging than on land because so little of the deep sea has been explored in any detail.
763 In many areas under consideration for mining, we lack any knowledge of how the resident
764 microbial communities contribute to primary production and element cycling in their habitats
765 and how these local activities relate to regional- and global-scale chemical cycles. Therefore, any
766 assessment or monitoring of mining impacts should consider the potential unexpected
767 consequences associated with undiscovered microbial organisms and activities stimulated,
768 directly or indirectly, by mining activities.

769
770 One pragmatic approach to the monitoring of mining impacts is the creation of protected areas
771 and reserves, as recommended by others. Protected areas, such as Preservation Reference Zones
772 where no impacts occur within mining sites (International Seabed Authority, 2018), and reserves
773 would be particularly useful as reference points for the monitoring of microbial communities,
774 since there is no way to assess the status of a microbial community *a priori* without reference
775 points. Samples for detailed microbial community analysis through DNA sequencing approaches
776 should be collected from both protected and impacted sites, to evaluate change. It is worth
777 noting, though, that the technology for measuring microbial diversity is advancing so quickly
778 that baseline measurements collected prior to mining activities are likely to be rendered obsolete
779 shortly thereafter, therefore appropriate samples should be archived for re-analysis with new
780 techniques as they become available. We also recommend the use of in situ and lab-based
781 activity-oriented experiments to evaluate changes in metabolic activities that could alter element
782 and nutrient distributions due to anticipated disruptions.

783
784 In conclusion, while some ecosystem services provided by microbial life in deep-sea habitats
785 may be minimally impacted by mining activities, others are expected to be severely impacted
786 (Figure 3). Active vent environments are expected to suffer the most extreme impacts from

787 mining activity, which will be hard to avoid even with protected offsets (Dunn et al., 2018).
788 There are several critical knowledge gaps that remain, and these are not evenly distributed across
789 habitat type (Figure 4). For example, the long-term impacts of mining inactive sulfide deposits
790 are poorly known, but could be dramatic in comparison to open-pit mines on land. When
791 considering that the total estimated copper and zinc potential of these deposits are only slightly
792 larger than the annual production on land (Hannington et al., 2011), it is important to weigh the
793 consequences of these activities in environmental impact assessments. Moreover, it is unclear
794 how extensively the seabed and overlying water column can be disturbed before tipping points
795 are reached and some ecosystem services become negatively and/or critically impacted on local
796 and regional scales. We highly recommend that baseline assessments of microbial diversity,
797 biomass, and rates of chemical processes be included in environmental impact assessment
798 planning, as they are currently lacking in policy recommendations (International Seabed
799 Authority, 2012).

800

801

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818

819 **AUTHOR CONTRIBUTIONS STATEMENT:**

820 RMJ constructed the maps in Figure 1, JJM developed the dataset for Figure 5, JAH contributed
821 to Figure 2, and BNO created the remaining figures. BNO wrote the manuscript with input from
822 all authors.

823

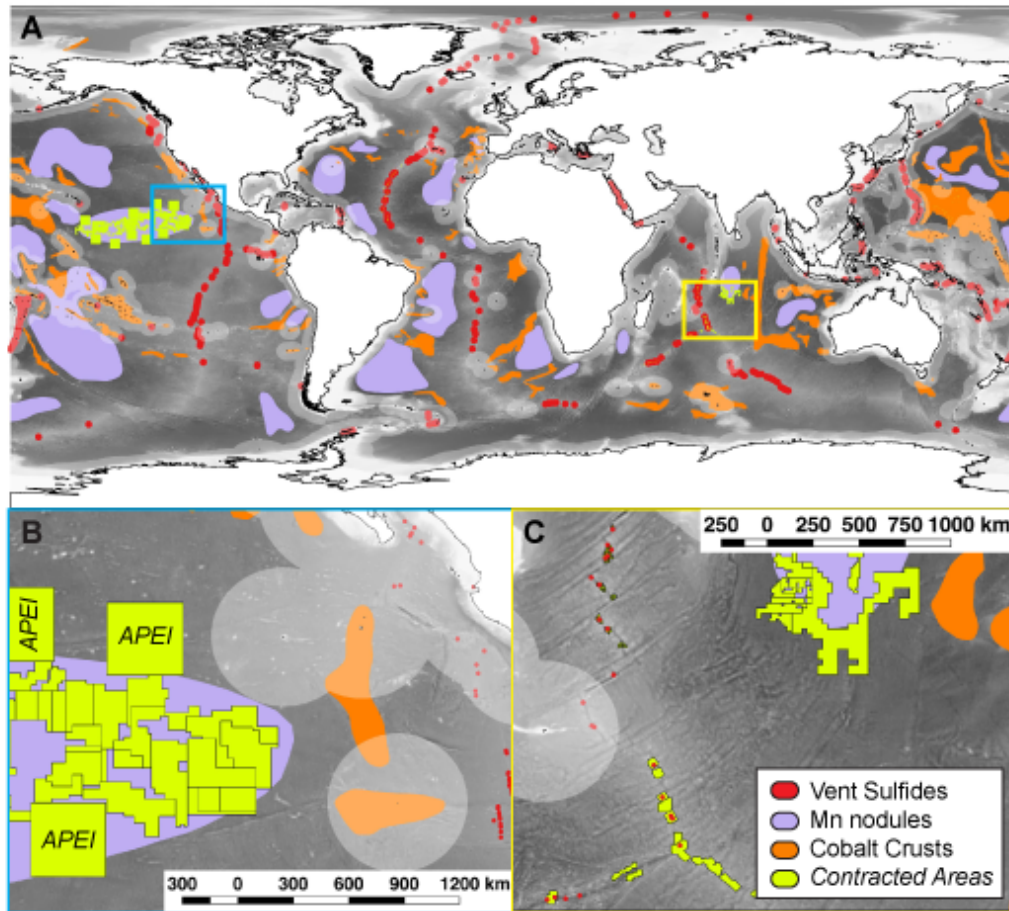
824 **CONFLICT OF INTEREST STATEMENT:**

825 The authors declare no conflicts of interest.

826 **FIGURES**

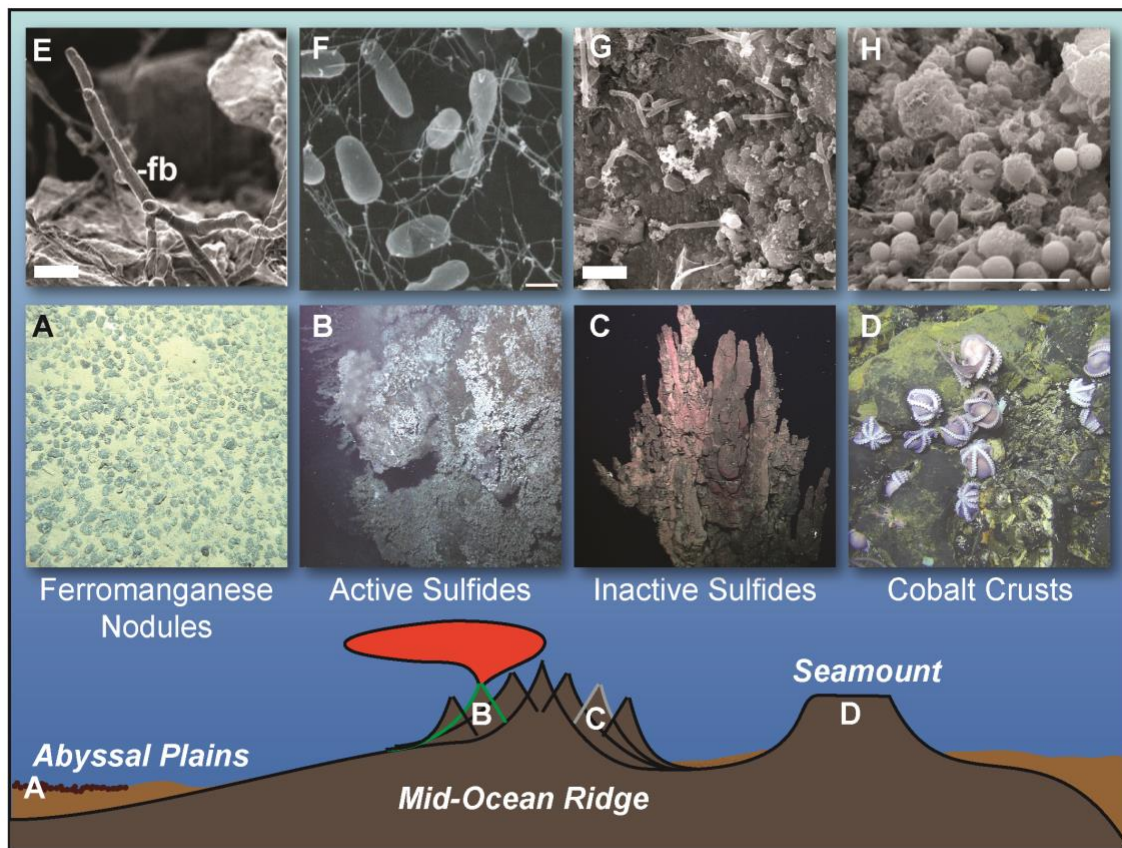
827

828 **Figure 1.** Locations of deep-sea mineral resources and current exploration contract zones. In all
829 panels, areas of the seafloor within nations' Exclusive Economic Zones (EEZ) highlighted in
830 light grey boundaries along coastlines, whereas remaining seafloor within "the Area" not shaded.
831 Panel A – Global seafloor distribution of hydrothermal vent (active and inactive) polymetallic
832 sulfide deposits (red), ferromanganese nodules (purple), and cobalt crusts on seamounts (orange)
833 overlain by current exploration contract zones (green) issued by the International Seabed
834 Authority (ISA). Panel B – Highlight of exploration contracts and Areas of Particular Ecological
835 Interest (APEI) in the eastern region of the Clarion Clipperton Zone and the East Pacific Rise
836 vent locations on the western edge of Mexico, as shown by the blue bounding box in A. Panel C
837 – Highlight of vent sites and contracted zones along part of the Southwest Indian Ridge and
838 nodule exploration contracts in the Indian Ocean, as shown by the yellow bounding box in A.
839 Underlying maps generated with data from the U.S. National Oceanographic and Atmospheric
840 Administration (NOAA; coastlines), the General Bathymetric Chart of the Oceans (GEBCO)
841 hosted by the British Oceanographic Data Centre (gridded bathymetry data), and the
842 marineregions.org database (EEZ). Shape file information for nodules and cobalt crusts from
843 (Hein et al., 2013a), for polymetallic sulfides InterRidge Vents Database version 3.3 hosted by
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846 exploration contract areas from the Deep Sea Mining Watch project version 1.2 hosted by the
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848

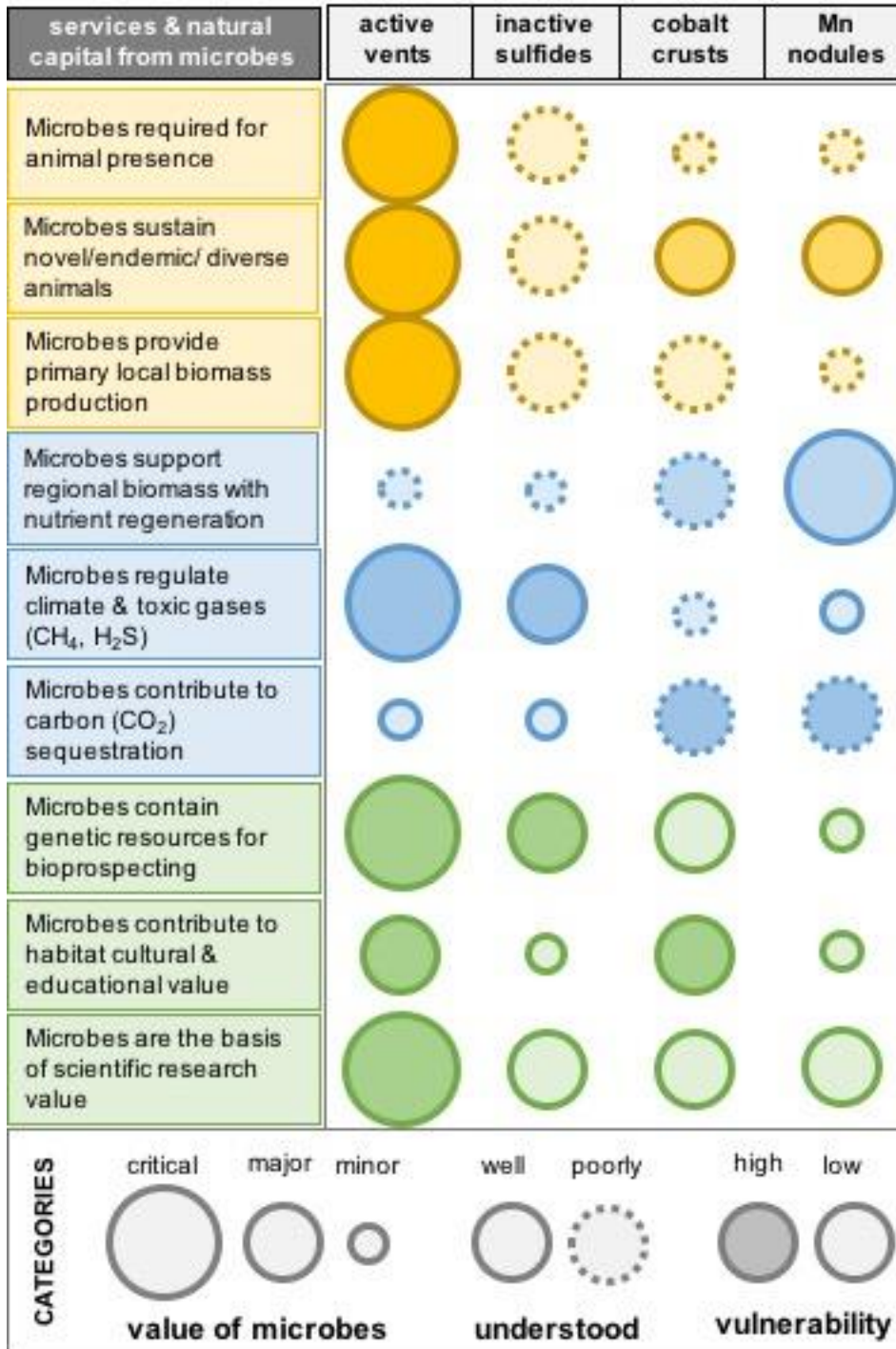


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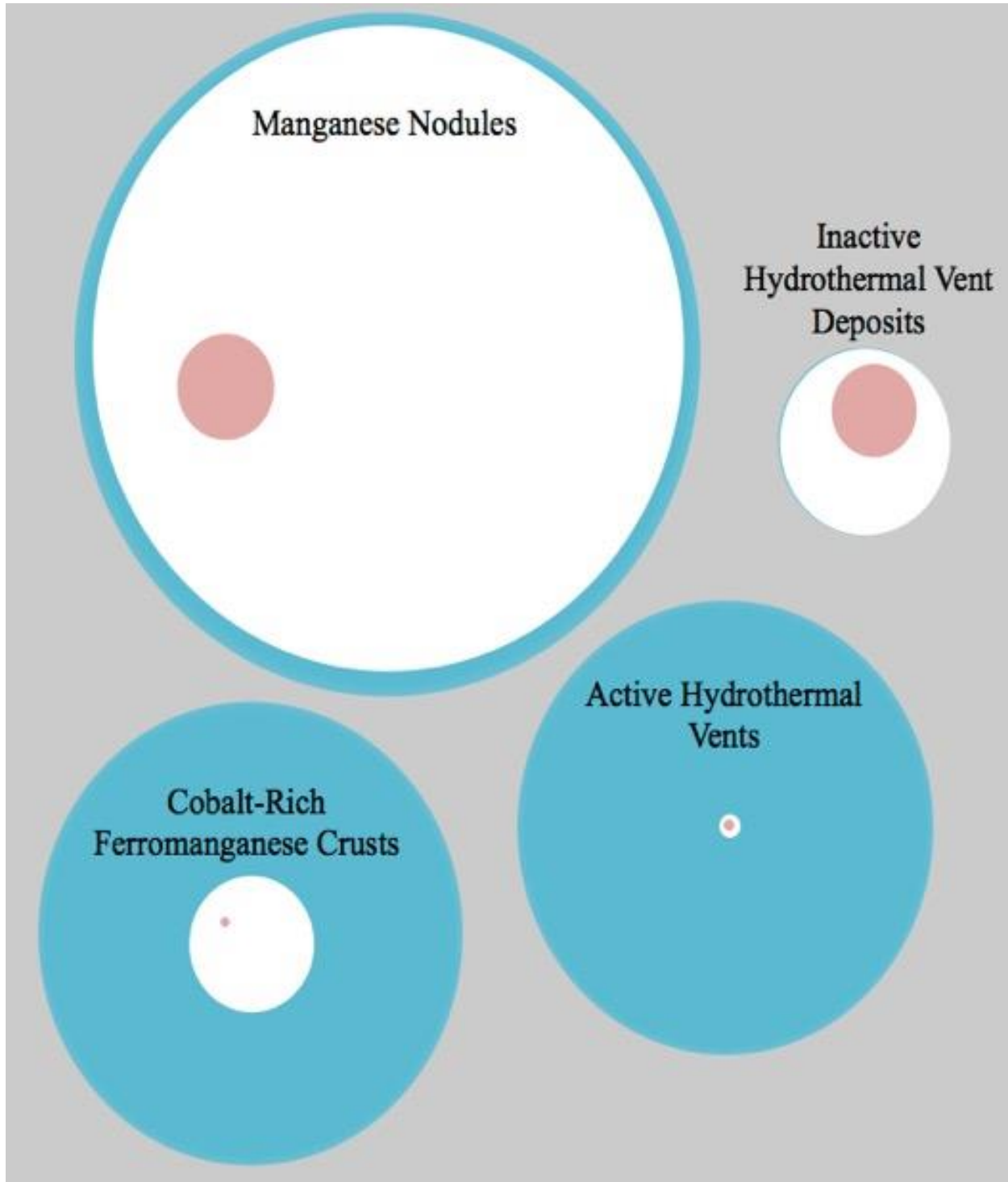
852 **Figure 2.** Types of deep-sea habitats with mineable resources. A – Ferromanganese nodules on
853 the sediment of abyssal plains, image modified from Wikipedia courtesy Abramax. B – Active
854 hydrothermal vent sulfide deposits from the Juan de Fuca Ridge, with chemosynthetic animals
855 colonizing the sulfide surface around areas of fluid venting. Photo courtesy of AT15-34 cruise
856 chief scientist Ray Lee, Western Washington University, U.S. National Science Foundation,
857 HOV *Alvin* dive 4420, 2008, © Woods Hole Oceanographic Institution. C – Inactive
858 hydrothermal vent sulfide deposits from the Galapagos Rift. Image by ROV *Hercules* courtesy of
859 NOAA Okeanos Explorer Program 2011 Galapagos Rift Expedition. D – Cobalt-rich crusts that
860 form on seafloor basalts on seamounts, which can be areas of diffusive hydrothermal fluid flow
861 and sites of deep-sea animal brooding. Photo courtesy of AT26-24 Chief Scientist Geoff Wheat,
862 Univ. of Alaska Fairbanks, U.S. National Science Foundation, HOV *Alvin*, 2014, © Woods Hole
863 Oceanographic Institution. Panels E-H show examples of microscopic organisms living on these
864 resources. E – Microbial filaments on a manganese nodule, modified from (Wang et al., 2009);
865 scale bar 1 μm . F – *Desulfurobacterium* bacteria isolated from a deep-sea hydrothermal vent at
866 Axial volcano, courtesy of Julie A. Huber; scale bar 1 μm . G – Rod-shaped cells on inactive
867 sulfides from the East Pacific Rise, modified from (Toner et al., 2013) and courtesy of Brandy
868 Toner; scale bar 2 μm . H – Ferromanganese crust on seafloor basalt from the East Pacific Rise,
869 modified from (Santelli et al., 2008) and courtesy of Cara Santelli; scale bar 10 μm . Cartoon
870 schematic modified from (Schrenk et al., 2009) with permission.



873 **Figure 3.** A qualitative assessment of the ecosystem services from microorganisms in deep sea
 874 habitats with mineable resources. The size, outline, and shading of symbols reflects the value
 875 that microbes support in each system, how well microbial aspects of the ecosystem are
 876 understood, and the vulnerability of microbial aspects to mining impacts, respectively, per the
 877 legend.
 878



881 **Figure 4.** A schematic depicting the relative state of knowledge of deep-sea habitats with
882 mineral resources versus the areal extent of the resources. White circles represent the relative
883 area of the potentially exploitable resource (Petersen et al., 2016), and red circles signify the
884 fraction of that area that is subject to current or pending exploration licenses (Hein et al., 2013a).
885 Blue halos indicate the relative number of peer-reviewed publications with relevant key words.
886 See Supplementary Materials Dataset 1 for more details.
887



888

889 **REFERENCES**

- 890 Abrantes, F. (1991). Increased upwelling off Portugal during the last glaciation: Diatom
891 evidence. *Marine Micropaleontology* 17(3-4), 285-310.
- 892 Albarède, F., Goldstein, S.L., and Dautel, D. (1997). The neodymium isotopic composition of
893 manganese nodules from the Southern and Indian oceans, the global oceanic neodymium
894 budget, and their bearing on deep ocean circulation. *Geochimica et Cosmochimica Acta*
895 61(6), 1277-1291.
- 896 Almeida, A., Cunha, Â., Fernandes, S., Sobral, P., and Alcântara, F. (2007). Copper effects on
897 bacterial activity of estuarine silty sediments. *Estuarine Coastal Shelf Science* 73, 743.
898 doi: 10.1016/j.ecss.2007.03.013.
- 899 Anantharaman, K., Breier, J.A., Sheik, C.S., and Dick, G.J. (2013). Evidence for hydrogen
900 oxidation and metabolic plasticity in widespread deep-sea sulfur-oxidizing bacteria.
901 *Proceedings of the National Academy of Science U.S.A.* 110, 330-335. doi:
902 10.1073/pnas.1215340110.
- 903 Anderson, R.E., Brazelton, W.J., and Baross, J.A. (2011). Is the genetic landscape of the deep
904 subsurface biosphere affected by viruses? *Frontiers in Microbiology* 2, 219.
- 905 Antony, R., Sujith, P., Fernandes, S.O., Verma, P., Khedekar, V., and Bharathi, P.L. (2011).
906 Cobalt immobilization by manganese oxidizing bacteria from the Indian Ridge System.
907 *Current microbiology* 62(3), 840-849.
- 908 Arndt, S., Jørgensen, B.B., LaRowe, D.E., Middelburg, J.L., Pancost, R.D., and Regnier, P.
909 (2013). Quantifying the degradation of organic matter in marine sediments: A review and
910 synthesis. *Earth-Science Reviews* 123, 53-86. doi: 10.1016/j.earscirev.2013.02.008.
- 911 Baaske, P., Weinert, F.M., Duhr, S., Lemke, K.H., Russell, M.J., and Braun, D. (2007). Extreme
912 accumulation of nucleotides in simulated hydrothermal pore systems. *Proceedings of the*
913 *National Academy of Sciences* 104(22), 9346-9351.
- 914 Baross, J.A., and Hoffman, S.E. (1985). Submarine hydrothermal vents and associated gradient
915 environments as sites for the origin and evolution of life. *Origins of Life and Evolution of*
916 *the Biosphere* 15(4), 327-345.
- 917 Bender, M.L., Ku, T.-L., and Broecker, W.S. (1966). Manganese nodules: their evolution.
918 *Science* 151(3708), 325-328.
- 919 Bender, M.L., Ku, T.-L., and Broecker, W.S. (1970). Accumulation rates of manganese in
920 pelagic sediments and nodules. *Earth and Planetary Science Letters* 8(2), 143-148.
- 921 Berner, R.A. (2003). The long-term carbon cycle, fossil fuels and atmospheric composition.
922 *Nature* 426, 323-326.
- 923 Bethke, C.M., Sanford, R.A., Kirk, M.F., Jin, Q., and Flynn, T.M. (2011). The thermodynamic
924 ladder in geomicrobiology. *Am. J. Sci.* 311, 183-210.
- 925 Bird, G. (2016). The influence of the scale of mining activity and mine site remediation on the
926 contamination legacy of historical metal mining activity. *Environmental Science and*
927 *Pollution Research* 23, 23456–23466. doi: 10.1007/s11356-016-7400-z.
- 928 Blöthe, M., Węgorzewski, A., Müller, C., Simon, F., Kuhn, T., and Schippers, A. (2015).
929 Manganese-cycling microbial communities inside deep-sea manganese nodules.
930 *Environmental science & technology* 49(13), 7692-7700.
- 931 Bluhm, H., Schriever, G., and Thiel, H. (1995). Megabenthic recolonization in an experimentall
932 disturbed abyssal manganese area. *Marine Georesources and Geotechnology* 13, 393-
933 416. doi: 10.1080/10641199509388295.
- 934 Boetius, A., and Haeckel, M. (2018). Mind the seafloor. *Science* 359(6371), 34-36.

- 935 Boltenkov, B. (2012). Mechanisms of formation of deep-sea ferromanganese nodules:
936 mathematical modeling and experimental results. *Geochemistry International* 50(2), 125-
937 132.
- 938 Boschen, R.E., Rowden, A.A., Clark, M.R., and Gardner, J.P.A. (2013). Mining of deep-sea
939 seafloor massive sulfides: A review of the deposits, their benthic communities, impacts
940 from mining, regulatory frameworks and management strategies. *Ocean Coastal*
941 *Management* 84, 54-67. doi: 10.1016/j.ocecoaman.2013.07.005.
- 942 Bourbonnais, A., Juniper, S.K., Butterfield, D.A., Anderson, R.E., and Lehmann, M.F. (2014).
943 Diversity and abundance of Bacteria and nirS-encoding denitrifiers associated with the
944 Juan de Fuca Ridge hydrothermal system. *Annals of Microbiology* 64(4), 1691-1705. doi:
945 10.1007/s13213-014-0813-3.
- 946 Campbell, B.J., Summers Engel, A., Porter, M.L., and Takai, K. (2006). The versatile ϵ -
947 proteobacteria: key players in sulphidic habitats. *Nature Reviews Microbiology* 4, 458-
948 468.
- 949 Cavan, E.L., Trimmer, M., Shelley, F., and Sanders, R. (2017). Remineralization of particulate
950 organic carbon in an ocean oxygen minimum zone. *Nature Communications* 8, 14847.
951 doi: 10.1038/ncomms14847.
- 952 Coffey Natural Systems (2008). "Environmental Impact Statement: Nautilus Minerals Miugini
953 Limited, Solwara 1").
- 954 Corliss, J., Dymond, J., Gordon, L.I., Edmond, J., von Herzen, R., Ballard, R., et al. (1979).
955 Submarine thermal springs on the Galapagos Rift. *Science* 203, 1073-1083.
- 956 Cuyvers, L., Berry, W., Gjerde, K., Thiele, T., and Wilhelm, C. (2018). "Deep seabed mining: a
957 rising environmental challenge". (Gland, Switzerland: IUCN and Gallifrey Foundation).
- 958 Deming, J.W., and Baross, J.A. (1993). Deep-sea smokers: Windows to a subsurface biosphere?
959 *Geochimica et Cosmochimica Acta* 57(14), 3219-3230.
- 960 Dick, G.J., Anantharaman, K., Baker, B.J., Li, M., Reed, D.C., and Sheik, C.S. (2013). The
961 microbiology of deep-sea hydrothermal vent plumes: Ecological and biogeographic
962 linkages to seafloor and water column habitats. *Frontiers in Microbiology* 4, 124. doi:
963 10.3389/fmicb.2013.00124.
- 964 Dold, B. (2014). Submarine Tailings Disposal (STD) - A review. *Minerals* 4, 642-666. doi:
965 10.3390/min4030642.
- 966 Donohue, I., and al., e. (2016). Navigating the complexity of ecological stability. *Ecology Letters*
967 19(9), 1172-1185.
- 968 Dubilier, N., Bergin, C., and Lott, C. (2008). Symbiotic diversity in marine animals: the art of
969 harnessing chemosynthesis. *Nature Reviews Microbiology* 6(10), 725.
- 970 Dunn, D.C., Van Dover, C.L., Etter, R.J., Smith, C.R., Levin, L.A., Morato, T., et al. (2018). A
971 strategy for the conservation of biodiversity on mid-ocean ridges from deep-sea mining.
972 *Science Advances* 4, eaar4313. doi: 10.1126/sciadv.aar4313.
- 973 Durden, J.M., Lallier, L.E., Murphy, K., Jaeckel, A., Gjerde, K., and Jones, D.O.B. (2018).
974 Environmental Impact Assessment process for deep-sea mining in 'the Area'. *Marine*
975 *Policy* 87, 194-202. doi: 10.1016/j.marpol.2017.10.013.
- 976 Edgcomb, V.P., Kysela, D.T., Teske, A., de Vera Gomez, A., and Sogin, M.L. (2002). Benthic
977 eukaryotic diversity in the Guaymas Basin hydrothermal vent environment. *Proceedings*
978 *of the National Academy of Science U.S.A.* 99, 7658-7662.

- 979 Erickson, K.L., Macko, S.A., and Van Dover, C.L. (2009). Evidence for chemoautotrophically
980 based food web at inactive hydrothermal vents (Manus Basin). *Deep Sea Research II* 56,
981 1577-1585. doi: 10.1016/j.dsr2.2009.05.002.
- 982 Etnoyer, P.J., Wood, J., and Shirley, T.C. (2010). How Large is the Seamount Biome.
983 *Oceanography* 23(1), 206-209.
- 984 Fallon, E.K., Niehorster, E., Brooker, R.A., and Scott, T.B. (2018). Experimental leaching of
985 massive sulphide from TAG active hydrothermal mound and implications for seafloor
986 mining. *Marine Pollution Bulletin* 126, 501-515. doi: 10.1016/j.marpolbul.2017.10.079.
- 987 Fallon, E.K., Petersen, S., Brooker, R.A., and Scott, T.B. (2017). Oxidative dissolution of
988 hydrothermal mixed-sulphide ore: An assessment of current knowledge in relation to
989 seafloor massive sulphide mining. *Ore Geology Reviews* 86, 309-337. doi:
990 10.1016/j.oregeorev.2017.02.028.
- 991 Fisher, A.T., Davis, E.E., Hutnak, M., Spless, V., Zühlsdorff, L., Cherkaoul, A., et al. (2003).
992 Hydrothermal recharge and discharge across 50 km guided by seamounts on a young
993 ridge flank. *Nature* 421, 618-621.
- 994 Fisher, A.T., and Wheat, C.G. (2010). Seamounts as conduits for massive fluid, heat, and solute
995 fluxes on ridge flanks. *Oceanography* 23, 74-87.
- 996 Fisher, C.R., Takai, K., and le Bris, N. (2007). Hydrothermal vent ecosystems. *Oceanography*
997 20(1), 14-23.
- 998 Fortunato, C.S., and Huber, J.A. (2016). Coupled RNA-SIP and metatranscriptomics of active
999 chemolithoautotrophic communities at a deep-sea hydrothermal vent. *The ISME journal*
1000 10(8), 1925.
- 1001 Fortunato, C.S., Larson, B., Butterfield, D.A., and Huber, J.A. (2018). Spatially distinct,
1002 temporally stable microbial populations mediate biogeochemical cycling at and below the
1003 seafloor in hydrothermal vent fluids. *Environmental microbiology* 20(2), 769-784.
- 1004 Fouquet, Y., Cambon, P., Etoubleau, J., Charlou, J.L., Ondreas, H., Barriga, F., et al. (2010).
1005 "Geodiversity of hydrothermal processes along the Mid-Atlantic Ridge and ultramafic-
1006 hosted mineralization: A new type of oceanic Cu-Zn-Co-Au volcanogenic massive
1007 sulfide deposit," in *Diversity of hydrothermal system on slow spreading ocean ridges.*,
1008 321-367.
- 1009 Frank, M., O'nions, R., Hein, J., and Banakar, V. (1999). 60 Myr records of major elements and
1010 Pb-Nd isotopes from hydrogenous ferromanganese crusts: reconstruction of seawater
1011 paleochemistry. *Geochimica et Cosmochimica Acta* 63(11-12), 1689-1708.
- 1012 German, C.R., Petersen, S., and Hannington, M.D. (2016). Hydrothermal exploration of mid-
1013 ocean ridges: Where might the largest sulfide deposits be forming? *Chemical Geology*
1014 420, 114-126.
- 1015 Gjerde, K.M., Weaver, P., Billet, D.S.M., Paterson, G., Colaco, A., Dale, A.W., et al. (2016).
1016 "Implications of MIDAS results for policy makers: Recommendation for future
1017 regulations".).
- 1018 Glasby, G., Gwozdz, R., Kunzendorf, H., Friedrich, G., and Thijssen, T. (1987). The distribution
1019 of rare earth and minor elements in manganese nodules and sediments from the equatorial
1020 and SW Pacific. *Lithos* 20(2), 97-113.
- 1021 Gollner, S., Govenar, B., Arbizu, P.M., Mills, S., le Bris, N., Weinbauer, M., et al. (2015).
1022 Differences in recovery between deep-sea hydrothermal vent and vent-proximate
1023 communities after a volcanic eruption. *Deep-Sea Res. Part I - Oceanogr. Res. Pap.* 106,
1024 167-182.

- 1025 Gollner, S., Reimer, B., Arbizu, R.P., le Bris, N., and Bright, M. (2010). Diversity of meiofauna
1026 from the 9°50'N East Pacific Rise across a gradient of hydrothermal fluid emissions.
1027 *PLoS ONE* 8, e12321. doi: 10.1371/journal.pone.0012321.
- 1028 Han, Y., Gonnella, G., Adam, N., Schippers, A., Burkhardt, L., Kurtz, S., et al. (2018).
1029 Hydrothermal chimneys host habitat-specific microbial communities: analogues for
1030 studying the possible impact of mining seafloor massive sulfide deposits. *Scientific*
1031 *Reports* 8, 10386. doi: 10.1038/s41598-018-28613-5.
- 1032 Hannington, M.D., Jamieson, J., Monecke, T., Petersen, S., and Beaulieu, S. (2011). The
1033 abundance of seafloor massive sulfide deposits. *Geology* 39, 1155-1158. doi:
1034 10.1130/G32468.1.
- 1035 Hansel, C., and Learman, D. (2015). The geomicrobiology of manganese. *Erlich's*
1036 *Geomicrobiology*, 347-420.
- 1037 Hansel, C.M. (2017). "Manganese in marine microbiology," in *Advances in microbial*
1038 *physiology*. Elsevier), 37-83.
- 1039 Hartwell, A.M., Voight, J.R., and Wheat, C.G. (2018). Clusters of deep-sea egg-brooding
1040 octopods associated with warm fluid discharge: An ill-fated fragment of a larger, discrete
1041 population? *Deep Sea Research Part I: Oceanographic Research Papers* 135, 1-8. doi:
1042 10.1016/j.dsr.2018.03.011.
- 1043 Hauton, C., Brown, A., Thatje, S., Mestre, N.C., Bebianno, M.J., Martins, I., et al. (2017).
1044 Identifying toxic impacts of metals potentially released during deep-sea mining - A
1045 synthesis of the challenges to quantifying risk. *Frontiers in Marine Science* 4, 368. doi:
1046 10.3389/fmars.2017.00368.
- 1047 He, T., Li, H., and Zhang, X. (2017). Deep-Sea Hydrothermal Vent Viruses Compensate for
1048 Microbial Metabolism in Virus-Host Interactions. *mBio* 8(4). doi: 10.1128/mBio.00893-
1049 17.
- 1050 Hein, J.R., Mizell, K., Koschinsky, A., and Conrad, T.A. (2013a). Deep-ocean mineral deposits
1051 as a source of critical metals for high- and green-technology applications: Comparison
1052 with land-based resources. *Ore Geology Reviews* 51, 1-14. doi:
1053 10.1016/j.oregeorev.2012.12.001.
- 1054 Hein, J.R., Mizell, K., Koschinsky, A., and Conrad, T.A. (2013b). Deep-ocean mineral deposits
1055 as a source of critical metals for high-and green-technology applications: Comparison
1056 with land-based resources. *Ore Geology Reviews* 51, 1-14.
- 1057 Henocque, Y. (2017). "The Crafting of Seabed Mining Ecosystem-Based Management," in
1058 *Deep-Sea Mining*. Springer), 507-526.
- 1059 Hoagland, P., Beaulieu, S., Tivey, M.A., Eggert, R.G., German, C.R., Glowka, L., et al. (2010).
1060 Deep-sea mining of seafloor massive sulfides. *Marine Policy* 34, 728-732. doi:
1061 10.1016/j.marpol.2009.12.001.
- 1062 Holden, J.F., Breier, J.A., Rogers, K.L., Schulte, M.D., and Toner, B.M. (2012). Biogeochemical
1063 processes at hydrothermal vents: microbes and minerals, bioenergetics, and carbon
1064 fluxes. *Oceanography* 25(1), 196-208.
- 1065 Huber, J.A., Butterfield, D.A., and Baross, J.A. (2002). Temporal changes in archaeal diversity
1066 and chemistry in a mid-ocean ridge seafloor habitat. *Applied and Environmental*
1067 *Microbiology* 68(4), 1585-1594. doi: 10.1128/aem.68.4.1585-1594.2002.
- 1068 Huber, J.A., Cantin, H.V., Huse, S.M., Mark Welch, D.B., Sogin, M.L., and Butterfield, D.A.
1069 (2010). Isolated communities of Epsilonproteobacteria in hydrothermal vent fluids of the
1070 Mariana Arc seamounts. *FEMS microbiology ecology* 73(3), 538-549.

- 1071 Huber, J.A., and Holden, J.F. (2008). "Modeling the impact of diffuse vent microorganisms
1072 along mid-ocean ridges and flanks," in *Magma to Microbe: Modeling Hydrothermal*
1073 *Processes at Oceanic Spreading Ridges*, eds. R.P. Lowell, J.S. Seewald, A. Metaxas &
1074 M.R. Perfit. (Washington, D.C.: American Geophysical Union Press), 215-231.
- 1075 Hughes, D.J., Shimmield, T.M., Black, K.D., and Howe, J.A. (2015). Ecological impacts of
1076 large-scale disposal of mining waste in the deep sea. *Scientific Reports* 5, 9985. doi:
1077 10.1038/srep09985.
- 1078 Hülse, D., Arndt, S., Wilson, J.D., Munhoven, G., and Ridgwell, A. (2017). Understanding the
1079 causes and consequences of past marine carbon cycling variability through models.
1080 *Earth-Science Reviews* 171, 349-382. doi: 10.1016/j.earscirev.2017.06.004.
- 1081 Huo, Y., Cheng, H., Post, A.F., Wang, C., Jiang, X., Pan, J., et al. (2015). Ecological functions of
1082 uncultured microorganisms in the cobalt-rich ferromanganese crust of a seamount in the
1083 central Pacific are elucidated by fosmid sequencing. *Acta Oceanologica Sinica* 34(4), 92-
1084 113.
- 1085 International Seabed Authority (2012). "Environmental management needs for exploration and
1086 exploitation of deep sea minerals: report of a workshop held by the International Seabed
1087 Authority in collaboration with the Government of Fiji and the SOPAC Division of the
1088 Secretariat of the Pacific Community in Nadi, Fiji, from 29 November to 2 December,
1089 2011". (Kingston, Jamaica: International Seabed Authority).
- 1090 International Seabed Authority (Year). "Towards the development of a regulatory framework for
1091 polymetallic nodule exploitation in the Area. Technical Study: No. 11": International
1092 Seabed Authority, Kingston, Jamaica), 90.
- 1093 International Seabed Authority (2018). "Design of IRZs and PRZs in Deep-Sea Mining Contract
1094 Areas. Briefing Paper 02/2018". (Kingston, Jamaica: International Seabed Authority).
- 1095 IUCN (2016). "Policy on Biodiversity Offsets".
- 1096 Jamieson, J.W., Hannington, M.D., Clague, D.A., Kelley, D.S., Delaney, J.R., Holden, J.F., et al.
1097 (2013). Sulfide geochronology along the Endeavour Segment of the Juan de Fuca Ridge.
1098 *Geochemistry Geophysics Geosystems* 14, 2084-2099.
- 1099 Jeong, K., Kang, J., and Chough, S. (1994). Sedimentary processes and manganese nodule
1100 formation in the Korea Deep Ocean Study (KODOS) area, western part of Clarion-
1101 Clipperton fracture zones, northeast equatorial Pacific. *Marine Geology* 122(1-2), 125-
1102 150.
- 1103 Jin, Q., and Kirk, M.F. (2018). pH as a primary control in environmental microbiology: 1.
1104 Thermodynamic perspective. *Frontiers in Environmental Science* 6, 21. doi:
1105 10.3389/FENV.2018.00021.
- 1106 Jones, D.O.B., Amon, D.J., and Chapman, A.S.A. (2018). Mining deep-ocean mineral deposits:
1107 What are the ecological risks? *Elements* 14, 325-330. doi: 10.2138/gselements.14.5.325.
- 1108 Jones, D.O.B., Kaiser, S., Sweetman, A.K., Smith, C.R., Menot, L., Vink, A., et al. (2017).
1109 Biological responses to disturbance from simulated deep-sea polymetallic nodule
1110 mining. *PLoS ONE* 12(2), e0171750. doi: 10.1371/journal.pone.0171750.
- 1111 Jones, S.G., and Ellis, D.V. (1995). Deep water std at the Misima gold and silver mine, Papua,
1112 New Guinea. *Marine Georesources and Geotechnology* 13, 183-200. doi:
1113 10.1080/10641199509388283.
- 1114 Jung, H.-S., Ko, Y.-T., Chi, S.-B., and Moon, J.-W. (2001). Characteristics of seafloor
1115 morphology and ferromanganese module occurrence in the Korea Deep-sea

- 1116 Environmental Study (KODES) Area, NE Equatorial Pacific. *Marine Georesources and*
1117 *Geotechnology* 19, 167-180. doi: 10.1080/10641190109353811.
- 1118 Karl, D.M., Wirsén, C.O., and Jannasch, H.W. (1980). Deep-Sea Primary Production at the
1119 Galapagos Hydrothermal Vents. *Science* 207(4437), 1345-1347.
- 1120 Kasten, S., Glasby, G., Schulz, H., Friedrich, G., and Andreev, S. (1998). Rare earth elements in
1121 manganese nodules from the South Atlantic Ocean as indicators of oceanic bottom water
1122 flow. *Marine Geology* 146(1), 33-52.
- 1123 Kato, S., Takano, Y., Kakegawa, T., Oba, H., Inoue, K., and Kobayashi, C. (2010).
1124 Biogeography and biodiversity in sulfide structures of active and inactive vents at deep-
1125 sea hydrothermal fields of the southern Mariana Trough. *Applied and Environmental*
1126 *Microbiology* 76, 2968-2979. doi: 10.1128/AEM.00478-10.
- 1127 Khripounoff, A., Caprais, J.-C., Crassous, P., and Etoubleau, J. (2006). Geochemical and
1128 biological recovery of the disturbed seafloor in polymetallic nodule fields of the
1129 Clipperton-Clarion Fracture Zone (CCFZ) at 5,000-m depth. *Limnology and*
1130 *Oceanography* 51(5), 2033-2041. doi: 10.4319/lo.2006.51.5.2033.
- 1131 Kim, J., Hyeong, K., Lee, H.-B., and Ko, Y.-T. (2012). Relationship between polymetallic
1132 nodule genesis and sediment distribution in the KODOS (Korea Deep Ocean Study)
1133 Area, Northeastern Pacific. *Ocean Science Journal* 47, 197-207. doi: 10.1007/s12601-
1134 012-0020-8.
- 1135 Kline, E.R., and Stekoll, M.S. (2001). Colonization of mine tailings by marine invertebrates.
1136 *Marine Environmental Research* 51, 301-325. doi: 10.1016/S0141-1136(00)00105-7.
- 1137 Krishnan, K., Fernandes, C.E., Fernandes, S.O., and Bharathi, P.L. (2006). Tolerance and
1138 immobilization of cobalt by some bacteria from ferromanganese crusts of the Afanasiy
1139 Nikitin Seamounts. *Geomicrobiology Journal* 23(1), 31-36.
- 1140 Ku, T.-L., and Broecker, W.S. (1965). Rates of sedimentation in the Arctic Ocean. *Progress in*
1141 *oceanography* 4, 95-104.
- 1142 Lee, M.D., Walworth, N.G., Sylvan, J.B., Edwards, K.J., and Orcutt, B.N. (2015). Microbial
1143 communities on seafloor basalts at Dorado Outcrop reflect level of alteration and
1144 highlight global lithic clades. *Frontiers in Microbiology* 6, 1470. doi:
1145 10.3389/fmicb.2015.01470.
- 1146 Levin, L.A., Baco, A.R., Bowden, D.A., Colaco, A., Cordes, E.E., and Cunha, M.R. (2016a).
1147 Hydrothermal vents and methane seeps: Rethinking the sphere of influence. *Frontiers in*
1148 *Marine Science* 3, 72. doi: 10.3389/fmars.2016.00072.
- 1149 Levin, L.A., Mengerink, K., Gjerde, K., Rowden, A.A., Van Dover, C.L., and Clark, M.R.
1150 (2016b). Defining “serious harm” to the marine environment in the context of deep
1151 seabed mining. *Marine Policy* 74, 245-259. doi: 10.1016/j.marpol.2016.09.032.
- 1152 Levin, L.A., Mengerink, K., Gjerde, K.M., Rowden, A.A., Van Dover, C.L., Clark, M.R., et al.
1153 (2016c). Defining “serious harm” to the marine environment in the context of deep-
1154 seabed mining. *Marine Policy* 74, 245-259. doi:
1155 <https://doi.org/10.1016/j.marpol.2016.09.032>.
- 1156 Liao, L., Xu, X.-W., Jiang, X.-W., Wang, C.-S., Zhang, D.-S., Ni, J.-Y., et al. (2011). Microbial
1157 diversity in deep-sea sediment from the cobalt-rich crust deposit region in the Pacific
1158 Ocean. *FEMS Microbiology Ecology* 78(3), 565-585. doi: 10.1111/j.1574-
1159 6941.2011.01186.x.
- 1160 Lindh, M.V., Maillot, B.M., Shulse, C.N., Gooday, A.J., Amon, D.J., Smith, C.R., et al. (2017).
1161 From the Surface to the Deep-Sea: Bacterial Distributions across Polymetallic Nodule

- 1162 Fields in the Clarion-Clipperton Zone of the Pacific Ocean. *Frontiers in Microbiology*
1163 8(1696). doi: 10.3389/fmicb.2017.01696.
- 1164 López-García, P., Vereshchaka, A., and Moreira, D. (2007). Eukaryotic diversity associated with
1165 carbonates and fluid-seawater interface in Lost City Hydrothermal Field. *Environmental*
1166 *Microbiology* 9, 546-554.
- 1167 Lowell, R.P., Houghton, J.L., Farough, A., Craft, K.L., Larson, B.I., and Meile, C. (2015).
1168 Mathematical modeling of diffuse flow in seafloor hydrothermal systems: The potential
1169 extent of the subsurface biosphere at mid-ocean ridges. *Earth and Planetary Science*
1170 *Letters* 425, 145-153.
- 1171 Luther, G.W. (2010). The role of one- and two-electron transfer reactions in forming
1172 thermodynamically unstable intermediates as barriers in multi-electron redox reactions.
1173 *Aquatic Geochemistry* 16(3), 395-420.
- 1174 Ma, W., Schott, D., and Lodewijks, G. (2017). A new procedure for deep sea mining tailings
1175 disposal. *Minerals* 7, 47. doi: 10.3390/min7040047.
- 1176 Maeda, A., Fujita, K., Horikawa, K., Suzuki, A., Yoshimura, T., Tamemori, Y., et al. (2017).
1177 Evaluation of oxygen isotope and Mg/Ca ratios in high-magnesium calcite from benthic
1178 foraminifera as a proxy for water temperature. *Journal of Geophysical Research-*
1179 *Biogeosciences* 122, 185-199. doi: 10.1002/2016jg003587.
- 1180 Marsay, C.M., Sanders, R.J., Henson, S.A., Pabortsava, K., Achterberg, E.P., and Lampitt, R.S.
1181 (2015). Attenuation of sinking particulate organic carbon flux through the mesopelagic
1182 ocean. *Proceedings of the National Academy of Science U.S.A.* 112(4), 1089-1094. doi:
1183 10.1073/pnas.1415311112.
- 1184 Martin, W., Baross, J., Kelley, D., and Russell, M.J. (2008). Hydrothermal vents and the origin
1185 of life. *Nature Reviews Microbiology* 6(11), 805.
- 1186 Martins, A., Viera, H., Gaspar, H., and Santos, S. (2014). Marketed marine natural products in
1187 the pharmaceutical and cosmeceutical industries: Tips for success. *Marine Drugs* 12,
1188 1066-1101.
- 1189 McNichol, J., Stryhanyuk, H., Sylva, S.P., Thomas, F., Musat, N., Seewald, J.S., et al. (2018).
1190 Primary productivity below the seafloor at deep-sea hot springs. *Proceedings of the*
1191 *National Academy of Sciences*, 201804351.
- 1192 Mehta, M.P., and Baross, J.A. (2006). Nitrogen fixation at 92 C by a hydrothermal vent
1193 archaeon. *Science* 314(5806), 1783-1786.
- 1194 Meier, D.V., Pjevac, P., Bach, W., Hourdez, S., Girguis, P.R., Vidoudez, C., et al. (2017). Niche
1195 partitioning of diverse sulfur-oxidizing bacteria at hydrothermal vents. *The ISME Journal*
1196 11(7), 1545.
- 1197 Mestre, N.C., Rocha, T.L., Canals, M., Cardoso, C., Danovaro, R., Dell'Anno, A., et al. (2017).
1198 Environmental hazard assessment of a marine mine tailings deposit site and potential
1199 implication for deep-sea mining. *Environmental Pollution* 228, 169-178. doi:
1200 10.1016/j.envpol.2017.05.027.
- 1201 Mewes, K., Mogollón, J.M., Picard, A., Rühlemann, C., Kuhn, T., Nöthen, K., et al. (2014).
1202 Impact of depositional and biogeochemical processes on small scale variations in nodule
1203 abundance in the Clarion-Clipperton Fracture Zone. *Deep-Sea Research I* 91, 125-141.
- 1204 Meyer, J.L., Akerman, N.H., Proskurowski, G., and Huber, J.A. (2013). Microbiological
1205 characterization of post-eruption "snowblower" vents at Axial Seamount, Juan de Fuca
1206 Ridge. *Frontiers in Microbiology* 4, article 153. doi: 10.3389/fmicb.2013.00153.

- 1207 Middleton, J.L., Mukhopadhyay, S., Langmuir, C.H., McManus, J.F., and Huybers, P.J. (2018).
1208 Millennial-scale variations in dustiness recorded in Mid-Atlantic sediments from 0 to 70
1209 ka. *Earth and Planetary Science Letters* 482, 12-22. doi: 10.1016/j.epsl.2017.10.034.
- 1210 Miller, K.A., Thompson, K.F., Johnston, P., and Santillo, D. (2018). An overview of seabed
1211 mining including the current state of development, environmental impacts, and
1212 knowledge gaps. *Frontiers in Marine Science* 4, 418. doi: 10.3389/fmars.2017.00418.
- 1213 Murali Mohan, A., Hartsock, A., Bibby, K.J., Hammack, R.W., Vidic, R.D., and Gregory, K.B.
1214 (2013). Microbial community changes in hydraulic fracturing fluids and produced water
1215 from shale gas extraction. *Environmental science & technology* 47(22), 13141-13150.
- 1216 Nasir, A., Kim, K.M., and Caetano-Anollés, G. (2015). Lokiarchaeota: eukaryote-like missing
1217 links from microbial dark matter? *TRENDS in Microbiology* 23(8), 448-450.
- 1218 Nath, B.N., Khadge, N.H., Nabar, S., Raghukumar, C., Ingole, B.S., Valsangkar, A.B., et al.
1219 (2012). Monitoring the sedimentary carbon in an artificially disturbed deep-sea
1220 sedimentary environment. *Environmental Monitoring Assessment* 184, 2829-2844. doi:
1221 10.1007/s10661-011-2154-z.
- 1222 Navarri, M., Jégou, C., Meslet-Cladière, L., Brillet, B., Barbier, G., Bergaud, G., et al. (2016).
1223 Deep seafloor fungi as an untapped reservoir of amphipathic antimicrobial
1224 compounds. *Marine Drugs* 14, 50. doi: 10.3390/md14030050.
- 1225 Niner, H.J., Ardron, J.A., Escobar, E.G., Gianni, M., Jaeckel, A.L., Jones, D.O.B., et al. (2018).
1226 Deep-sea mining with no net loss of biodiversity - An impossible aim. *Frontiers in*
1227 *Marine Science* 5, 53. doi: 10.3389/fmars.2018.00053.
- 1228 Nisbet, E., and Sleep, N.H. (2001). The habitat and nature of early life. *Nature* 409(6823), 1083-
1229 1091.
- 1230 Nitahara, S., Kato, S., Urabe, T., Usui, A., and Yamagishi, A. (2011). Molecular characterization
1231 of the microbial community in hydrogenetic ferromanganese crusts of the Takuyo-Daigo
1232 Seamount, northwest Pacific. *FEMS Microbiology Letters* 321(2), 121-129. doi:
1233 10.1111/j.1574-6968.2011.02323.x.
- 1234 Nordstrom, D.K. (2011). Mine waters: Acidic to circumneutral. *Elements* 7, 393-398. doi:
1235 10.2113/gselements.7.6.393.
- 1236 O'Brien, C.E., Giovannelli, D., Govenar, B., Luther, G.W., Lutz, R.A., Shank, T.M., et al.
1237 (2015). Microbial biofilms associated with fluid chemistry and megafaunal colonization
1238 at post-eruptive deep-sea hydrothermal vents. *Deep Sea Research Part II: Topical Studies*
1239 *in Oceanography* 121, 31-40.
- 1240 OECD (2016). "Biodiversity Offsets Effective Design and Implementation".).
- 1241 Olins, H., Rogers, D., Frank, K., Vidoudez, C., and Girguis, P. (2013). Assessing the influence of
1242 physical, geochemical and biological factors on anaerobic microbial primary productivity
1243 within hydrothermal vent chimneys. *Geobiology* 11(3), 279-293.
- 1244 Opatkiewicz, A.D., Butterfield, D.A., and Baross, J.A. (2009). Individual hydrothermal vents at
1245 Axial Seamount harbor distinct seafloor microbial communities. *FEMS Microbiology*
1246 *Ecology* 70(3), 413-424.
- 1247 Orcutt, B.N., Sylvan, J.B., Rogers, D.R., Delaney, J., Lee, R.W., and Girguis, P.R. (2015).
1248 Carbon fixation by basalt-hosted microbial communities. *Frontiers in Microbiology* 6,
1249 904. doi: 10.3389/fmicb.2015.00904.
- 1250 Ornella Amore, F., Caffau, M., Massa, B., and Morabito, S. (2004). Late Pleistocene–Holocene
1251 paleoclimate and related paleoenvironmental changes as recorded by calcareous
1252 nannofossils and planktonic foraminifera assemblages in the southern Tyrrhenian Sea

- 1253 (Cape Palinuro, Italy). *Marine Micropaleontology* 52(1-4), 255-276. doi:
1254 10.1016/j.marmicro.2004.05.004.
- 1255 Ortmann, A.C., and Suttle, C.A. (2005). High abundances of viruses in a deep-sea hydrothermal
1256 vent system indicates viral mediated microbial mortality. *Deep-Sea Research Part I-*
1257 *Oceanographic Research Papers* 52(8), 1515-1527. doi: 10.1016/j.dsr.2005.04.002.
- 1258 Park, S.H., Kim, D.H., Kim, C.W., Park, C.Y., and Kang, J.K. (1997). "Estimation of coverage
1259 and size distribution on manganese nodules based on image processing techniques", in:
1260 *Second ISOPE Ocean Mining Symposium, International Society of Offshore and Polar*
1261 *Engineers.*).
- 1262 Paul, S.A.L., Gaye, B., Haeckel, M., Kasten, S., and Koschinsky, A. (2018). Biogeochemical
1263 Regeneration of a Nodule Mining Disturbance Site: Trace Metals, DOC and Amino
1264 Acids in Deep-Sea Sediments and Pore Waters. *Frontiers in Marine Science* 5(117). doi:
1265 10.3389/fmars.2018.00117.
- 1266 Pedersen, T.F. (1984). Interstitial water metabolite chemistry in a marine mine tailings deposit,
1267 Rupert Inlet, B. C. *Canadian Journal of Earth Science* 21, 1-9. doi: 10.1139/e84-001.
- 1268 Pedersen, T.F., and Losher, A.J. (1988). "Diagenetic processes in aquatic mine tailings deposits
1269 in British Columbia," in *Chemistry and Biology of Solid Waste*. (Berlin, Heidelberg:
1270 Springer Berlin Heidelberg), 238-258.
- 1271 Penesyanyan, A., Kjelleberg, S., and Egan, S. (2010). Development of novel drugs from marine
1272 surface associated microorganisms. *Marine Drugs* 8, 438-459. doi: 10.3390/md8030438.
- 1273 Petersen, S., Krättschell, A., Augustin, N., Jamieson, J., Hein, J.R., and Hannington, M.D. (2016).
1274 News from the seabed - Geological characteristics and resource potential of deep-sea
1275 mineral resources. *Marine Policy* 70, 175-187. doi: 10.1016/j.marpol.2016.03.012.
- 1276 Peukert, A., Schoening, T., Alevizos, E., Köser, K., Kwasnitschka, T., and Greinert, J. (2018).
1277 Understanding Mn-nodule distribution and evaluation of related deep-sea mining impacts
1278 using AUV-based hydroacoustic and optical data. *Biogeosciences* 15, 2525-2549. doi:
1279 10.5194/bg-15-2525-2018.
- 1280 Piel, J. (2009). Metabolites from symbiotic bacteria. *Natural Products Reports* 26, 338-362. doi:
1281 10.1039/b703499g.
- 1282 Purser, A., Marcon, Y., Hoving, H.J.T., Vecchione, M., Piatkowski, U., Eason, D., et al. (2016).
1283 Association of deep-sea incirrate octopods with manganese crusts and nodule fields in the
1284 Pacific Ocean. *Current Biology* 26(24), 1268-1269. doi: 10.1016/j.cub.2016.10.052.
- 1285 Ramirez-Llodra, E., Trannum, H.C., Evenset, A., Levin, L.A., Andersson, M., Finner, T.E., et al.
1286 (2015). Submarine and deep-sea mine tailing placement: A review of current practices,
1287 environmental issues, natural analogs and knowledge gaps in Norway and internationally.
1288 *Marine Pollution Bulletin* 97, 13-35. doi: 10.1016/j.marpolbul.2015.05.062.
- 1289 Rea, D.K. (1994). The paleoclimatic record provided by eolian deposition in the deep sea: The
1290 geologic history of wind. *Reviews of Geophysics* 32(2), 159-195.
- 1291 Russell, M.J., and Martin, W. (2004). The rocky roots of the acetyl-CoA pathway. *Trends in*
1292 *biochemical sciences* 29(7), 358-363.
- 1293 Santelli, C.M., Orcutt, B.N., Banning, E., Bach, W., Moyer, C.L., Sogin, M.L., et al. (2008).
1294 Abundance and diversity of microbial life in ocean crust. *Nature* 453, 653-656. doi:
1295 10.1038/nature06899.
- 1296 Sapsford, D., De Leeuw, L., Phillips, J., and Brabham, P. (2015). "The feasibility of treatment of
1297 acid mine drainage with seawater", in: *10th International Conference on Acid Rock*
1298 *Drainage IWMA Annual Conference*. (Santiago, Chile).

- 1299 Schippers, A., Breuker, A., Blazejak, A., Bosecker, K., Kock, D., and Wright, T.L. (2010). The
1300 biogeochemistry and microbiology of sulfidic mine waste and bioleaching dumps and
1301 heaps, and novel Fe(II)-oxidizing bacteria. *Hydrometallurgy* 104, 342-350. doi:
1302 10.1016/j.hydromet.2010.01.012.
- 1303 Schlacher, T.A., Baco, A.R., Rowden, A.A., O'Hara, T.D., Clark, M.R., Kelley, C., et al. (2014).
1304 Seamount benthos in a cobalt-rich crust region of the central Pacific: conservation
1305 challenges for future seabed mining. *Diversity and distributions* 20(5), 491-502.
- 1306 Schrader, H., and Sorknes, R. (1991). Peruvian coastal upwelling: Late Quaternary productivity
1307 changes revealed by diatoms. *Marine Geology* 97(3-4), 233-249.
- 1308 Schrenk, M.O., Huber, J.A., and Edwards, K.J. (2009). Microbial provinces in the seafloor.
1309 *Annual Reviews of Marine Sciences* 2, 85-110.
- 1310 Schrenk, M.O., Kelley, D.S., Delaney, J.R., and Baross, J.A. (2003). Incidence and diversity of
1311 microorganisms within the walls of an active deep-sea sulfide chimney. *Applied and
1312 Environmental Microbiology* 69(6), 3580-3592.
- 1313 Schriever, G., and Thiel, H. (Year). "Tailings and their disposal in deep-sea mining", in: *ISOPE
1314 Ocean Minerals Symposium*.
- 1315 Seiter, K., Hensen, C., Schröter, J., and Zabel, M. (2004). Organic carbon content in surface
1316 sediments—defining regional provinces. *Deep Sea Research Part I: Oceanographic
1317 Research Papers* 51(12), 2001-2026.
- 1318 Shah Walter, S.R., Jaekel, U., Osterholz, H., Fisher, A.T., Huber, J.A., Pearson, A., et al. (2018).
1319 Microbial decomposition of marine dissolved organic matter in cool oceanic crust.
1320 *Nature Geoscience* 11(5), 334-339. doi: 10.1038/s41561-018-0109-5.
- 1321 Shank, T.M. (2010). Seamounts: Deep-ocean Laboratories of Faunal Connectivity, Evolution,
1322 and Endemism. *Oceanography* 23(1), 108-122. doi: 10.5670/oceanog.2010.65.
- 1323 Shi, Y., Pan, C., Auckloo, B.N., Chen, X., Chen, C.-T.A., Wang, K., et al. (2017). Stress-driven
1324 discovery of a cryptic antibiotic produced by *Streptomyces* sp. WU20 from Kueishantao
1325 hydrothermal vent with an integrated metabolomics strategy. *Applied microbiology and
1326 biotechnology* 101(4), 1395-1408.
- 1327 Shimmiel, T.M., Black, K.D., Howe, J.A., Hughes, D.J., and Sherwin, T. (2007). "Independent
1328 evaluation of deep-sea mine tailings placement (DSTP) in PNG".
- 1329 Shiraishi, F., Mitsunobu, S., Suzuki, K., Hoshino, T., Morono, Y., and Inagaki, F. (2016). Dense
1330 microbial community on a ferromanganese nodule from the ultra-oligotrophic South
1331 Pacific Gyre: implications for biogeochemical cycles. *Earth and Planetary Science
1332 Letters* 447, 10-20.
- 1333 Shulse, C.N., Maillot, B., Smith, C.R., and Church, M.J. (2017). Polymetallic nodules,
1334 sediments, and deep waters in the equatorial North Pacific exhibit highly diverse and
1335 distinct bacterial, archaeal, and microeukaryotic communities. *MicrobiologyOpen* 6(2),
1336 e00428.
- 1337 Sievert, S.M., and Vetriani, C. (2012). Chemoautotrophy at deep-sea vents: past, present, and
1338 future. *Oceanography* 25(1), 218-233.
- 1339 Spero, H.J., Bijma, J., Lea, D.W., and Bemis, B.E. (1997). Effect of seawater carbonate
1340 concentration on foraminiferal carbon and oxygen isotopes. *Nature* 390(6659), 497.
- 1341 Sujith, P., Ramanan, D., Gonsalves, M.J.B., and LokaBharathi, P. (2017). Microbial activity
1342 promotes the enrichment of cobalt over nickel on hydrogenetic ferromanganese crusts.
1343 *Marine Georesources & Geotechnology* 35(8), 1158-1167.

- 1344 Suzuki, K., Yoshida, K., Watanabe, H., and Yamamoto, H. (2018). Mapping the resilience of
1345 chemosynthetic communities in hydrothermal vent fields. *Nature* 8, 9364. doi:
1346 10.1038/s41598-018-27596-7.
- 1347 Suzuki, Y., Inagaki, F., Takai, K., Nealson, K.H., and Horikoshi, K. (2004). Microbial diversity
1348 in inactive chimney structures from deep-sea hydrothermal systems. *Microbial Ecology*
1349 47, 186-196.
- 1350 Sylvan, J.B., Toner, B.M., and Edwards, K. (2012). Life and death of deep-sea vents: Bacterial
1351 diversity and ecosystem succession on inactive hydrothermal sulfides. *mBio* 3(1), doi:
1352 10.1128/mBio.00279-00211.
- 1353 Takai, K., Nakamura, K., Toki, T., Tsunogai, U., Miyazaki, M., Miyazaki, J., et al. (2008). Cell
1354 proliferation at 122 degrees C and isotopically heavy CH₄ production by a
1355 hyperthermophilic methanogen under high-pressure cultivation. *Proceedings of the*
1356 *National Academy of Sciences of the United States of America* 105(31), 10949-10954.
1357 doi: 10.1073/pnas.0712334105.
- 1358 Thiel, H., and Schriever, G. (1990). Deep-sea mining, environmental impact and the DISCOL
1359 project. *Ambio. Stockholm* 19(5), 245-250.
- 1360 Thiel, H., Schriever, G., Ahnert, A., Bluhm, H., Borowski, C., and Vopel, K. (2001). The large-
1361 scale environmental impact experiment DISCOL—reflection and foresight. *Deep Sea*
1362 *Research Part II: Topical Studies in Oceanography* 48(17-18), 3869-3882.
- 1363 Thornburg, C.C., Zabriskie, T.M., and McPhail, K.L. (2010). Deep-Sea Hydrothermal Vents:
1364 Potential Hot Spots for Natural Products Discovery? *Journal of Natural Products* 73(3),
1365 489-499. doi: 10.1021/np900662k.
- 1366 Toner, B.M., Lesnewski, R.A., Marlow, J.J., Briscoe, L.J., Santelli, C.M., Bach, W., et al.
1367 (2013). Mineralogy drives bacterial biogeography at hydrothermally-inactive seafloor
1368 sulfide deposits. *Geomicrobiology Journal* 30(4), 313-326.
- 1369 Tully, B.J., and Heidelberg, J.F. (2013). Microbial communities associated with ferromanganese
1370 nodules and the surrounding sediments. *Frontiers in Microbiology* 4, 161. doi:
1371 10.3389/fmicb.2013.00161.
- 1372 van de Fliedert, T., Frank, M., Halliday, A.N., Hein, J.R., Hattendorf, B., Günther, D., et al.
1373 (2004). Deep and bottom water export from the Southern Ocean to the Pacific over the
1374 past 38 million years. *Paleoceanography and Paleoclimatology* 19(1).
- 1375 Van Dover, C.L. (2000). *The ecology of deep-sea hydrothermal vents*. Princeton: Princeton
1376 University Press.
- 1377 Van Dover, C.L., Arnaud-Haond, S., Gianni, M., Helmreich, S., Huber, J.A., Jaekel, A.L., et al.
1378 (2018). Scientific rationale and international obligations for protection of active
1379 hydrothermal vent ecosystems from deep-sea mining. *Marine Policy* 90, 20-28. doi:
1380 10.1016/j.marpol.2018.01.020.
- 1381 Vanreusel, A., Hilario, A., Ribeiro, A., Menot, L., and Arbizu, P.M. (2016). Threatened by
1382 mining, polymetallic nodules are required to preserve abyssal epifauna. *Scientific Reports*
1383 6, 26808. doi: 10.1038/srep26808.
- 1384 Vare, L.L., Baker, M.C., Howe, J.A., Levin, L.A., Neira, C., Ramirez-Llodra, E., et al. (2018).
1385 Scientific considerations for the assessment and management of mine tailings disposal in
1386 the deep sea. *Frontiers in Marine Science* 5, 17. doi: 10.3389/fmars.2018.00017.
- 1387 Wang, X., and Müller, W.E.G. (2009). Marine biominerals: perspectives and challenges for
1388 polymetallic nodules and crusts. *Trends in Biotechnology* 27(6), 375-383. doi:
1389 <https://doi.org/10.1016/j.tibtech.2009.03.004>.

- 1390 Wang, X., Schröder, H.C., Wiens, M., Schloßmacher, U., and Müller, W.E.G. (2009).
1391 Manganese/polymetallic nodules: Micro-structural characterization of exolithobiontic-
1392 and endolithobiontic microbial biofilms by scanning electron microscopy. *Micron* 40,
1393 350-358. doi: 10.1026/j.micron.2008.10.005.
- 1394 Wankel, S.D., Adams, M.M., Johnston, D.T., Hansel, C.M., Joye, S.B., and Girguis, P.R. (2012).
1395 Anaerobic methane oxidation in metalliferous hydrothermal sediments: influence on
1396 carbon flux and decoupling from sulfate reduction. *Environmental Microbiology* 14(10),
1397 2726-2740. doi: 10.1111/j.1462-2920.2012.02825.x.
- 1398 Wankel, S.D., Germanovitch, L.N., Lilley, M.D., Gence, G., DiPerna, C.J., Bradley, A.S., et al.
1399 (2011). Influence of subsurface biosphere on geochemical fluxes from diffuse
1400 hydrothermal vents. *Nature Geoscience* 4, 461-468.
- 1401 Wedding, L.M., Reiter, S.M., Smith, C.R., Gjerde, K.M., Kittinger, J.N., Friedlander, A.M., et al.
1402 (2015). Managing mining of the deep seabed. *Science* 349(6244), 144-145. doi:
1403 10.1126/science.aac6647.
- 1404 Wegorzewski, A.V., and Kuhn, T. (2014). The influence of suboxic diagenesis on the formation
1405 of manganese nodules in the Clarion Clipperton nodule belt of the Pacific Ocean. *Marine*
1406 *Geology* 357, 123-138.
- 1407 Wessel, P., Sandwell, D.T., and Kim, S.-S. (2010). The global seamount census. *Oceanography*
1408 23(1), 24-33.
- 1409 Wheat, C.G., Fisher, A.T., McManus, J., Hulme, S.M., and Orcutt, B.N. (2017). Cool seafloor
1410 hydrothermal springs reveal global geochemical fluxes. *Earth and Planetary Science*
1411 *Letters* 476, 179-188. doi: 10.1016/j.epsl.2017.07.049.
- 1412 Wheat, C.G., Hartwell, A.M., McManus, J., Fisher, A.T., Orcutt, B.N., Schlicht, L.E.M., et al.
1413 (submitted). Geologic constraints and geochemical consequences of fluid discharge from
1414 Dorado Outcrop, A Cool Ridge Flank Hydrothermal System. *Geochemistry Geophysics*
1415 *Geosystems*.
- 1416 Williams, A., Schlacher, T.A., Rowden, A.A., Althaus, F., Clark, M.R., Bowden, D.A., et al.
1417 (2010). Seamount megabenthic assemblages fail to recover from trawling impacts.
1418 *Marine Ecology* 31, 183-199.
- 1419 Williamson, S.J., Cary, S.C., Williamson, K.E., Helton, R.R., Bench, S.R., Winget, D., et al.
1420 (2008). Lysogenic virus-host interactions predominate at deep-sea diffuse-flow
1421 hydrothermal vents. *The ISME Journal* 2(11), 1112-1121.
- 1422 Xie, W., Wang, F., Guo, L., Chen, Z., Sievert, S.M., Meng, J., et al. (2011). Comparative
1423 metagenomics of microbial communities inhabiting deep-sea hydrothermal vent
1424 chimneys with contrasting chemistries. *The ISME journal* 5(3), 414.
- 1425 Xu, C., Sun, X., Jin, M., and Zhang, X. (2017). A Novel Benzoquinone Compound Isolated from
1426 Deep-Sea Hydrothermal Vent Triggers Apoptosis of Tumor Cells. *Marine drugs* 15(7),
1427 200.
- 1428 Zhang, X., Li, S.-J., Li, J.-J., Liang, Z.-Z., and Chao, C.-Q. (2018). Novel natural products from
1429 extremophilic fungi. *Marine Drugs* 16, 194. doi: 10.3390/md16060194.
- 1430 Zinke, L.A., Kiel Reese, B., McManus, J., Wheat, C.G., Orcutt, B.N., and Amend, J.P. (2018).
1431 Sediment microbial communities influenced by cool hydrothermal fluid migration.
1432 *Frontiers in Microbiology* 9, 1249. doi: 10/3389/fmicb.2018.01249.
- 1433 Zúñiga, D., Santos, C., Froján, M., Salgueiro, E., Rufino, M.M., De la Granda, F., et al. (2017).
1434 Diatoms as a paleoproductivity proxy in the NW Iberian coastal upwelling system (NE
1435 Atlantic). *Biogeosciences* 14(5), 1165-1179. doi: 10.5194/bg-14-1165-2017.

