



Made available through Montana State University's [ScholarWorks](#)

Understanding phycosomal dynamics to improve industrial microalgae cultivation

Isaac R. Miller, Huyen Bui, Jessica B. Wood,
Matthew W. Fields, Robin Gerlach

© This manuscript version is made available under the CC-BY-NC-ND 4.0 license <https://creativecommons.org/licenses/by-nc-nd/4.0/>

1 **Title: Understanding Phycosomal Dynamics to Improve Industrial Microalgae**
2 **Cultivation**

3 Authors: Isaac R. Miller^{1,2}, Huyen Bui², Jessica B. Wood^{1,2}, Matthew W. Fields^{1,2,3,5*}, and
4 Robin Gerlach^{1,2,4,5}

5
6 Affiliations: ¹Department of Microbiology & Cell Biology, Montana State University,
7 Bozeman, MT

8 ²Center for Biofilm Engineering, Montana State University, Bozeman, MT

9 ³Department of Civil Engineering, Montana State University, Bozeman, MT

10 ⁴Department of Biological and Chemical Engineering, Bozeman, MT

11 ⁵Energy Research Institute, Montana State University, Bozeman, MT

12

13 MW Fields ORCID: 0000-0001-9053-1849

14 IR Miller ORCID: 0000-0001-8528-3182

15 H Bui ORCID: 0000-0001-7369-2878

16 J Wood ORCID: 0000-0002-7174-4838

17 R Gerlach ORCID: 0000-0002-7669-3072

18 IR Miller Twitter: twitter.com/_isaac_miller

19

20 Keywords: microalgae; phycosome; eco-engineering; direct air capture; polycultures

21

22 Correspondence: Matthew W. Fields, PhD
23 Center for Biofilm Engineering
24 366 Barnard Hall
25 Montana State University
26 Bozeman, MT 59717
27 406-994-7340
28 matthew.fields@montana.edu

29

30

31

32

33 Abstract

34 Algal-bacterial interactions are ubiquitous to both natural and industrial systems, and the
35 characterization of these interactions has been re-invigorated with potential applications to bio-
36 system productivity. Different growth conditions can be used for operational functions, such as
37 the use of low-quality water or high pH/alkalinity, and the altered operating conditions likely
38 constrain microbial community structure and function in unique ways. However, research is
39 needed to better understand if consortia can be designed to impact the productivity, processing,
40 and sustainability of industrial-scale cultivations through different controls that can constrain
41 microbial interactions for maximal light-driven outputs. The review will highlight current
42 knowledge and gaps for relevant operating conditions as well as suggestions for near-term and
43 longer-term improvements for large-scale cultivation and polyculture engineering.

44 Motivations and challenges for the microalgal industry

45 Replacing petroleum-based transportation fuels with microalgae-based biofuels will
46 increase energy security and reduce fuel cycle carbon emissions, and microalgae provide several
47 advantages over terrestrial plants as a renewable energy or chemical feedstock. For microalgae,
48 high photosynthetic efficiency and fast growth rates can result in higher productivity per area of
49 marginal land and water relative to terrestrial plants [1]. In addition, microalgae cultivation can
50 be coupled with wastewater treatment and recycling, potentially reducing the need for high-value
51 fertilizer and water [1-5].

52 However, despite years of research and development, production still faces techno-
53 economic challenges. One main challenge is the typically used open ponds are subject to numerous
54 biotic and abiotic stresses that include infestation with grazers and pathogens [6,7]. In addition,
55 fluctuations in light, temperature and nutrients threaten culture stability and consistent yields.

56 While microalgal **monocultures** (See Glossary) are well understood at the laboratory scale,
57 significant perturbations are inherent to the open systems [3,8,9], and the use of closed bioreactors
58 is possible but costly in terms of capital investment as well as light and CO₂ delivery.

59 Industrial scale microalgal cultures are usually seeded with unialgal or co-algal inoculum,
60 and colonization with other microorganisms is considered contamination that needs to be managed.
61 However, not unlike human and plant microbiome research, ‘healthy’ microbial diversity is being
62 appreciated and studied to better understand roles in ecosystem function, where algae, diatoms,
63 and bacteria co-exist to form **commensal** and symbiotic associations [10-16]. In addition,
64 **consortia** consisting of more than one microalgal species can result in higher culture stability and
65 productivity [17,18], likely as a result of niche space occupation towards maximal carrying-
66 capacity. Therefore, creating pond ecosystems with mutually supportive microalgal and microbial
67 species may increase productivity and system resiliency.

68 The basic tenets of community ecology describe uninhabited, open systems as increasing
69 in complexity over time; therefore, monocultures will be invaded from neighboring environments
70 until a stable state is reached that can be resilient to ‘normal’ changes [19,20]. Recent studies have
71 provided evidence for advantages of **polycultures** over monocultures with respect to biomass
72 productivity [21,22], stability [23], resource utilization [24], and resistance to invasive species
73 (*e.g.*, grazers) [25, 26], and these findings coincide with observations for diverse, natural systems
74 (*e.g.*, ocean cyanobacteria) [27]. These studies also highlight potential trade-offs during the
75 cultivation phase and in the downstream processing steps (harvesting, extraction, conversion) [18],
76 and the potential exists for selecting communities based upon maximizing pre- and post-processing
77 for targeted products [28,29]. But as noted by Lian and colleagues [30], studies focused on algal-
78 associated microbial community distribution, structure, and function continue to be needed.

79 Engineering microalgal cultures with **mutualistic** or commensal bacteria has been
80 employed to enhance nutrient removal and bioremediation in wastewater sources [31] but less is
81 understood how engineered polycultures at industrial scales will perform with regards to light-
82 driven productivity and stability. Engineering microalgal cultures with bacteria could augment
83 biofuel production by addressing biomass growth, nutrient recycle, harvesting, and biofuel
84 extraction [3,32] as well as overall stability that could contribute to consistent harvests. This
85 review focuses on the potential of using bacteria to improve algal cultivation and harvesting
86 through comparison with productive natural and/or industrial systems as well as discuss the current
87 understanding on how microalgal and bacterial interactions could alleviate system stress and
88 contribute to overall processing and productivity.

89 **Microalgae and natural systems**

90 While microalgae can have significant impacts on human health and the economy (*e.g.*,
91 harmful microalgal blooms), there are also many beneficial applications of microalgal biomass
92 and metabolism (*e.g.*, biofuels, bioproducts and renewable food sources) [10]. Over 70,000 species
93 of algae have been identified using a combination of morphological- and molecular-based
94 techniques [33] and over 26,000 algal species/strains were recorded in GenBank as of January
95 2020 [34]. While technological advancements in next-generation sequencing have allowed for
96 exponential growth in genome sequencing, the number of publicly available algal genomes (224
97 published genomes) [34] represents less than 0.1% of the known algal species. The gap in genome
98 representation is in part due to the challenge of isolating and cultivating **axenic** algal strains,
99 especially when it is presumed that many algal species rely on symbioses with bacteria and fungi
100 [35,36].

101 Unlike axenic laboratory cultures, natural aquatic habitats are diverse microbial
102 communities that constantly exchange resources and respond to both abiotic and biotic fluctuations
103 [15,37]. In a field study of a microalgal-bacterial community during a diatom-dominated bloom,
104 Teeling and colleagues [38] identified almost 100 microalgal morphologies that co-occurred with
105 bacterial clades. Bell and colleagues [22] surveyed community composition in large wastewater
106 treatment lagoons over a 1-year sampling period and detected extremely diverse communities (*e.g.*,
107 445 eukaryotic OTUs) that included green microalgae and predatory protists. In another survey of
108 wastewater, Hena and coworkers [39] observed signatures of 20 microalgal and diatom species in
109 dairy farm wastewater. In comparison, a 4-year survey of the southern North Sea observed that
110 the overall species composition in plankton is balanced and remains consistent despite interannual
111 variation [40]. It is likely that natural consortia have adapted to certain environments, fluctuations,
112 and extremes, and insights into the role of each microalgal species in a certain habitat could inform
113 strategies to design stable and productive microalgal consortia for industrial cultivation in different
114 locales (*e.g.*, dry/sunny versus humid/cloudy). Microalgae in natural systems are typically
115 observed in diverse communities and understanding relationships of microalgal species with each
116 other and with non-microbial partners could be useful for stable industrial production.

117 Extreme environments can provide novel biochemical capacity relevant to industrial
118 processes and pH is a dominant factor, and many studies of microbial diversity in extremes have
119 focused on low pH, high temperature environments (*e.g.*, hot springs). The ecology of alkaline
120 systems, both natural and artificial, is poorly understood, though it has been observed that
121 communities in alkaline environments are phylogenetically distinct from circumneutral habitats
122 [41,42]. Most phototrophic systems above approximately 45°C become predominated by
123 cyanobacteria up to 75°C, whereas microalgae grow better between 10°C and 40°C [43] depending

124 upon the environment, with the upper limit reported to be approximately 60°C [44]. Alkaline lake
125 systems have some of the highest primary productivity rates on the planet [45] and diverse and
126 unique microalgae have been identified from alkaline systems [46-48]. However, surprisingly, few
127 studies have systematically catalogued the extent of potential diversity and function of microalgae
128 from temperate (20-40°C), alkaline systems. We hypothesize that given the high primary
129 productivity rates and tolerance to high pH, a microalgal cultivation system at alkaline/high pH
130 has the potential to achieve controllable inputs/outputs (*i.e.*, stable productivity with minimized
131 inputs) via **direct air CO₂ capture**, and future work should continue to focus on these and other
132 unique conditions that are relevant to CO₂-processing.

133 **Microalgae in industrial systems**

134 Microalgae require water, sunlight, and nutrients (*e.g.*, N, P, Fe) to grow via
135 photoautotrophy (*i.e.*, light-driven carbon dioxide utilization), and the low-nutrient and low-
136 quality water requirements coupled with rapid growth rates and high biomass yields make
137 microalgal systems a target for biotechnology applications, including biofuel and bioproduct
138 generation [3]. While some microalgae can grow heterotrophically or mixotrophically with higher
139 biomass yields [49,50], the use of additional carbon can increase overall energy demands as well
140 as increase contamination with heterotrophic microorganisms that compete for N and P [51,52].
141 Therefore, the focus of this review is on **photoautotrophic** growth that uses CO₂ as the carbon
142 source and sunlight as the energy-source.

143 Given the open-air nature of large-scale microalgal cultivation under photoautotrophic
144 conditions, maintaining axenic cultures presents many challenges. Invasion or ‘contamination’ is
145 inevitable, resulting in a complicated ecosystem of bacteria, zooplankton, and microalgae as well
146 as fungi and viruses [53-55]. Much research has focused on controlling invading agents with strong

147 chemicals and pesticides, as well as extensive sterilizing methods such as filtration. A more
148 practical approach may be to select for less susceptible or invasion-resistant strains [56], well-
149 structured **polycultures** that are tolerant to invaders, and/or cultivation conditions that limit
150 invasion through selection (*e.g.*, high pH/high alkalinity, salinity). Moreover, naturally high pH
151 and high alkalinity environments (*e.g.*, soda lakes) have some of the highest primary productivities
152 on Earth [57], largely attributed to the increased availability of inorganic carbon in these
153 environments; however, more work is needed that targets these environments. In recent years,
154 high pH/high-alkalinity conditions have been used to increase microalgal biomass and lipid yields
155 in shorter time periods, and these extreme conditions appear to reduce invasion by harmful
156 microorganisms and grazers [45,58-60]. While it is feasible to select and promote stable
157 microalgae-bacterial consortia that can thrive at extremes [45,61], little is known about the possible
158 metabolic and/or ecological interactions specific to dynamic extremes (*e.g.*, pH changes across the
159 diel cycle) nor potential implications for long-term, repeated cultivations.

160 Natural phykosome communities are often more diverse and stable than those observed *ex*
161 *situ* or in industrial settings. The selective forces acting on wild and industrial systems are likely
162 similar, yet the extent to which factors can influence structure and function is likely different. For
163 example, marine microalgal phykosome diversity is correlated with latitudinal temperature
164 gradients [62], but little is known if similar temperature effects could play a role in industrial
165 cultures that can experience large diurnal temperature shifts when maximizing sunlight exposure.
166 In addition, a differentiation between free-living and directly attached bacteria has been observed
167 in natural systems, whereas the distinction is often less clear in *ex situ* cultures [10]. This is likely
168 due to the homogeneity of cultures, relative to natural environments, that is achieved under typical
169 mixing regimes. Industry practices of mixing and nutrient delivery to maximize algal production

170 ultimately minimize ecological niche partitioning and spatial differentiation that are likely
171 important contributors to microbial roles. Influential environmental parameters such as
172 photosynthetically active radiation (PAR), pH, and pO₂ that might change under different
173 industrial growth schemes likely impact both the microalgae and the associated microorganisms.
174 Hence, research is needed to ascertain mechanistic roles that significantly impact algal culture
175 productivity or stability under relevant industrial conditions.

176 [The Phycosphere - the microalgal-bacterial interface](#)

177 The term **phycosphere** refers to the immediate region surrounding a microalgal cell
178 enriched in organic matter (*i.e.*, photosynthate) [63], and the phycosphere is analogous to the
179 rhizosphere in soils that refers to the thin fluid layer (diffusive boundary layer) that surrounds
180 small aquatic microalgae (<100 µm). The size of the phycosphere is determined by the size and
181 shape of the microalgal cell, growth, exudation rates, motility, and the level of mixing in the bulk
182 aqueous phase, with smaller, slower growing cells generally having thinner phycospheres [36, 64].
183 The phycosphere includes the associated microorganisms to microalgal cell surface and/or algal
184 aggregates [10] and includes both direct and indirect metabolic interactions within the effective
185 diffusive boundary layer, while we propose the term **phycosome**, building from the general
186 concept of microbiome [65], as the microbial community occupying a defined habitat (*i.e.*,
187 microalgal growth system) with distinct physio-chemical system-level properties (metabolic
188 potential/function) irrespective of direct attachment or proximity within the diffusive boundary
189 layer [66]. In extreme cases, such as in the interaction between Chlorophyta and Rickettsiales,
190 bacteria are able to develop inside microalgal cells [67]. In natural habitats, there are examples of
191 the following scenarios: (1) a clear partitioning of attached and free-living bacterial taxa [68], and
192 (2) a large overlap of bacterial taxa in the attached and free-living fractions [69]. Interestingly,

193 Eigemann and colleagues [70] reported a shift from scenario (1) to scenario (2) when culturing
194 natural samples in laboratory conditions, and the results suggested that the laboratory conditions
195 that promote high microalgal biomass may also promote an expansion of attached bacteria into the
196 free-living fraction.

197 Although aquatic environments may seem to have a homogeneously low concentration of
198 growth substrates, there is a “sea of gradients” available to planktonic bacteria [71] created through
199 the release of dissolved organic carbon by microalgal cells into the surrounding water. Non-motile
200 bacteria can encounter microalgal cells randomly, but the encounters are relatively rare [36].
201 Beyond random encounters, many marine bacteria may actively gain access to the phycosphere by
202 chemotaxis to gain fitness advantages from the dissolved organic carbon [71-74]. Several
203 microalgal exudates can elicit microbial chemotaxis including glycolate, acrylate, amino acids,
204 and dimethylsulfoniopropionate (DMSP) [63,75-77], and differential DOC exudates have been
205 shown to impact chemotaxis in phylogenetically distinct groups [73]. These results suggest that
206 microorganisms can be attracted to algal cells and that some algae may actively recruit specific
207 microbial populations dependent upon direct or indirect metabolic interactions via different
208 exudates.

209 Once within the phycosphere, bacteria may maintain association via attachment to
210 microalgal cell surface [78], microalgal sheaths [32], or to the polymeric matrix [79] all of which
211 could be potential sources of carbon (Figure 1a-c). In some cases, bacteria colonize transparent
212 exopolymer particles (TEPs), which are made of polysaccharides released by diatoms. Certain
213 bacterial strains can modulate diatoms’ TEP production, thereby promoting the aggregation of
214 diatoms and promoting bacteria-diatom associations [80]. Bacteria themselves can also release

215 exopolysaccharides in response to the presence of microalgae, which might also facilitate
216 interactions [81].

217 [Microalgal-bacterial interactions](#)

218 Microalgal-microbial associations may provide selective advantages for microalgal health
219 [82]; while conversely, axenic cultures of microalgae may be unstable and prone to perturbation
220 [8]. Microalgae can release up to 50% of fixed carbon into the surrounding environment as
221 excreted organic compounds (*e.g.*, carbohydrates) that is thought to directly and indirectly impact
222 associated ecological partners, local photo-inhibition, and/or aggregation [32,83,84]. Microalgal-
223 bacterial interactions are considered species- or even strain-specific, meaning that the phylogenetic
224 composition of the bacterial community can depend on the microalgal host [68,85-87]. However,
225 the level of specificity has been suggested to differ among microalgal genera [88] and the number
226 of algal phycosomes studied for different genera are limited. Establishment of long-term specific
227 interactions likely correlate with metabolic dependencies as well as with long-term co-evolution
228 [89-91]. Less is known about the drivers of short-term interactions (*i.e.*, diel cycle); however,
229 preliminary studies have been conducted in freshwater environments [Papadopoulou, S., 2021].
230 The species-specific nature of microalgal-bacterial interactions could be due to distinct exudates
231 produced by a given microalgal species providing substrates that differentially attract bacteria
232 based on metabolic potential [86,91]. Consistently, natural and industrial cultures containing
233 multiple microalgal species have more diverse bacterial communities than uni-algal [10]. When
234 synthetic phycosome communities were established using a mixed bacterial starting community
235 along with diatom and dinoflagellate host cells, direct relationships between host metabolism and
236 community succession were uncovered based upon host-produced metabolites [92]. These

237 findings suggest that specific host processes could select for specific phycosphere/phycosome
238 compositions.

239 Microalgal cell size could play a role in the interactions with bacteria. For example,
240 microalgae with small cell size (*e.g.*, *Nannochloropsis salina*) may encounter bacteria at lower
241 frequencies given lower surface area (*i.e.*, smaller phycospheres) for bacterial attachment [31].
242 Larger cells might not only provide more surface for attachment but also release more exudates,
243 resulting in wider diffusive boundary layers that can be encountered by chemotactic
244 microorganisms [37,64]. For this reason, the trade-off between costs associated with biomass
245 production and surface area generation is an important consideration in understanding the selective
246 pressures driving morphological diversity in microalgae. Smaller cells, however, may move
247 around more, expanding the impacted area [37].

248 The growth mode or lifestyle of microalgae might also be a determining factor. For
249 example, in cultures enriched for microalgae-associated bacteria, the benthic marine diatom,
250 *Phaeodactylum tricornutum*, appeared to have more bacterial cells attached on the cell surface
251 than *N. salina* [31]. Changes in the environment likely lead to changes in the microalgal
252 metabolism, which changes the chemical “profile” of the phycosphere and might result in
253 microbiome shifts. Ultimately, it is likely that both host morphology and physiology can exert
254 selective control over the composition of the associated microbial community and vice versa.

255 Aggregation in planktonic and biofilm cultures may provide an opportunity to investigate
256 spatial relationships in microalgae-phycosome interaction webs (Figure 2). In addition to algal
257 aggregates and/or the algal cell as the ‘surface’, microalgae can grow attached to physical surfaces
258 (‘macro’-biofilm) in natural and artificial environments, and biofilm growth is an important
259 characteristic to consider in the design of cultivation schemes for larger scale production. Recent

260 comparisons have shown that phototrophic biofilm growth can help overcome challenges with the
261 delivery, mixing, and harvesting of algae [93] and that productive, cyanobacterial-dominated
262 biofilms can have diverse microbial communities [94]. Mixed-species and mixed-domain biofilms
263 can persist over a wide range of conditions given the flexible microalgal metabolisms and the
264 active interplay between heterotrophs and phototrophs [94], but more work is needed to elucidate
265 the occurrence, distribution, and function of microbial populations specifically associated with
266 algal biofilms.

267 The role(s) of bacteria in microalgal cultures

268 Microalgal-bacterial interactions span a broad spectrum from mutualistic to **commensal**,
269 competitive or algicidal [13,85,86,95,96] (Figure 3). For example, for the marine microalgae
270 *Emiliania huxleyi* and the associated *Roseobacter* group, the interactions include distinct
271 mutualistic and algicidal phases. In the first phase, the bacterium provides antibiotics that protect
272 the microalgal cells from bacterial pathogens, and auxins, which promote microalgal growth. As
273 the microalgal population ages, the bacterium switches to the algicidal phase in response to the
274 increased level of *p*-coumaric acid, a breakdown product of dying microalgal cells. In this phase,
275 *Roseobacter* can produce antialgal compounds, the roseobacticides, that kill microalgae [13,97-
276 101-99]. This complex example suggests that at least some interactions will be condition- and
277 time-dependent.

278 Competitive or antagonistic interactions

279 In the relationship with microalgae, bacteria can be benefactors that can utilize dissolved
280 organic matter (DOM) release by microalgae and compete for macro-nutrients (*e.g.*, N, P, Fe),
281 resulting in decreased microalgal productivity [102]. In addition to utilization of microalgal
282 exudates, some bacteria have adapted an algicidal lifestyle, in which bacteria actively attack

283 microalgal cells to obtain nutrients [103]. While algicidal bacteria need to be avoided in microalgal
284 cultivations, they could potentially be used for controlling harmful microalgal blooms [104,105].
285 Directly related to biofuel production, Lenneman and colleagues [106] demonstrated that two
286 algicidal bacteria, *Pseudomonas pseudoalcaligenes* AD6 and *Aeromonas hydrophila* AD9,
287 induced microalgal cell lysis leading to an at least six-fold improvement of lipid extraction from
288 the microalgae *Neochloris oleoabundans* and *Dunaliella tertiolecta* [107]. Bacterial algicides and
289 the potential modes of action were reviewed extensively by Meyer and colleagues [108].

290 Just as bacteria have evolved to prey upon microalgae, microalgae have evolved strategies
291 for self-defense. While released microalgal DOM serve as a food source for bacteria, larger
292 exopolymers may also form a “mucus treadmill” that protects microalgal cells from excessive
293 bacterial colonization [31,109]. Microalgae can also reduce bacterial growth and colonization by
294 interfering with bacterial quorum-sensing systems [110,111]. Diatoms can secrete fatty acids and
295 esters [112] that have been shown to act as signaling molecules to induce bacterial biofilm
296 dispersal [113-114] or provide protection by serving as antibacterial compounds [115].
297 Polyunsaturated aldehydes (PUA) can suppress growth of some marine bacteria, but some bacteria
298 in diatom phycospheres are resistant to PUA and indicate a potential selection mechanism [116].
299 In ways analogous to human gut microbiome roles [117], the presence of a diverse
300 phycosphere/phycosome may help reduce the occurrence/probability of **antagonistic** interactions
301 (*i.e.*, niche exclusion).

302 Beneficial or mutualistic interactions

303 Mutualistic relationships are common between microalgae and bacteria, and bacteria can
304 provide essential nutrients and exert growth effects as strong as those of light and temperature
305 [118]. Capturing and maintaining these relationships in engineered settings is key for highly

306 productive polycultures. There is ample evidence that bacteria can positively influence the
307 productivity of microalgal systems via both direct and indirect routes [21,22,119,120], and similar
308 to other host-microbiome interactions, these routes can include nutrient acquisition, growth
309 effectors, and protection.

310 Nutrient acquisition

311 In photo-aquatic ecosystems, as bacteria process carbon fixed by autotrophs, CO₂ is
312 produced and inorganic nutrients are recycled (*e.g.*, N, P, Fe) [118]. While these nutrients directly
313 benefit the bacteria, the occurrence of bacterial re-mineralization within the
314 phycosphere/phycosome may also provide microalgal cells with elevated nutrient concentrations
315 [37]. Microalgae require iron for photosystem biosynthesis and function, and many bacteria
316 produce siderophores for Fe-acquisition. For example, *Marinobacter* sp. can produce siderophores
317 and contribute to iron chelation and iron uptake by microalgae [121,122]. In addition, bacteria like
318 *Roseobacter* sp. have potential to regenerate iron hemoproteins that are released by lysed and
319 decaying microalgal cells [123].

320 Bacteria also play critical roles in microalgal nitrogen uptake. Diazotrophic organisms such
321 as cyanobacteria and other bacterial genera (*Rhizobium*, *Mesorhizobium* and *Azospirillum*) can fix
322 N₂ into bioavailable forms [12]. Nitrogen fixation may be a more dominant process in the
323 phycosome of microalgal cultures at times when oxygen levels are low (*e.g.*, during dark periods),
324 but more work is needed to ascertain the temporal and spatial distribution of N-cycle functions in
325 algal systems. Indeed, algal systems have been associated with nitrous oxide production [124], and
326 therefore, other functional groups in addition to N-fixation could be important for not only N
327 allocation but also environmental impacts (*e.g.*, mitigation of N₂O emissions).

328 Certain diatoms or prymnesiophytes have been shown to have obligate mutualistic
329 interactions with nitrogen-fixing cyanobacteria [12,125]. Similarly, *Rhizobium* spp. can provide
330 nitrogen for *Chlorella vulgaris* which has been shown to increase microalgal cell levels by ~72%
331 [32,126]. Moreover, bacteria can facilitate microalgal nitrogen uptake by converting nitrogen
332 containing compounds into more bioavailable or preferred forms for microalgal consumption.
333 *Donghicola* spp. have been shown to degrade methylamine and release ammonium that can be
334 utilized by photoautotrophs [127]. In another case, when cultured with the diatom *Pseudo-*
335 *nitzschia multiseriis*, *Sulfitobacter psuedonitzschiae* used nitrate from the medium and released
336 ammonium, which is the preferred nitrogen source for the diatom [128]. Even protists, which are
337 often considered pests in microalgal systems, facilitated nitrogen uptake in a closed system where
338 ammonia released by *Paramecium caudatum* enhanced productivity in *Chlorella* [129].

339 Provision of essential vitamins and growth hormones

340 Stimulation of microalgal growth by bacteria can also occur via the production of vitamins
341 [11,130-133]. Many microalgae are auxotrophic for essential vitamins such as B₁₂, thiamine and
342 biotin [134-136], and these compounds are thought to be exchanged through mutualistic
343 interactions with vitamin-producing microorganisms [11,137,138]. Using metagenomic and
344 metatranscriptomic approaches, Krohn-Molt and coworkers [87] provided strong evidence that α -
345 Proteobacteria (e.g., *Porphyrobacter* and *Blastomonas*) were the main B-vitamin suppliers in
346 *Chlorella* and *Scenedesmus* microbiomes, while *Sphingobacteria* and Bacteroidetes species
347 exhibited high expression of B₁₂ biosynthetic genes in culture with *Micrasterias* [87].

348 Bacteria can also impact microalgal growth via the production and release of growth
349 hormones such as indole-3-acetic acid (IAA) [31,133,139,140]. Although IAA has no clearly
350 documented metabolic role in many bacteria, some bacteria can produce IAA from tryptophan.

351 While the evidence for IAA involvement in microalgal physiology and development remains
352 limited, IAA has been thought to be the driver for a number of intimate microalgal-bacterial
353 interactions [30]. The coccolithophore *Emiliana huxleyi* exudes the amino acid tryptophan, which
354 is utilized by the bacterium *Phaeobacter inhibens* to produce IAA [141]. A similar interaction was
355 reported for a *Sulfitobacter* spp. and *Pseudo-nitzschia multiseriis* symbiont [128]. Interestingly,
356 IAA addition to culture medium resulted in reduced growth of the diatom compared to IAA
357 released by bacteria. One explanation is that bacteria-released IAA had higher local concentration
358 in the phycosphere than the bulk concentration resulting from the addition of IAA to the medium
359 [128]. In a recent comparison between plant-growth promoting and non-plant growth-promoting
360 bacteria, *Escherichia coli* promoted *Chlorella* growth as much as *Azospirillum brasilense* [142],
361 and these results demonstrated the challenge in differentiating the specific and general effects of
362 microbiomes. However, the exogenous addition of the phytohormone, auxin, increased biomass
363 and lipid content in *Scenedesmus* SDEC-8 and *Chlorella sorokiniana* [143]. While difficult to
364 demonstrate definitive cause-and-effect relationships, more research is needed to ascertain the
365 potential for metabolic interactions for phyco-hormone effects at the industrial scale.

366 Protection

367 Bacteria can provide a certain level of protection to microalgae beyond simple niche
368 exclusion. Makridis and colleagues [144] tracked bacterial communities associated with green
369 microalgae grown for aquafeed and observed that the presence of certain populations hindered the
370 growth of pathogenic bacteria. For example, *Roseobacter* sp. produce tropodithietic acid (TDA),
371 a dual sulfur-tropolone compound that inhibits a broad range of marine pathogens and may help
372 to prevent harmful bacteria from colonizing the microalgal phycosphere [145]. Bacteria have also
373 been found to infect and kill microalgal predators, which could result in increased microalgal

374 growth. In particular, the bacterium *Pasteuria ramosa* is a parasite on the crustacean *Daphnia*
375 *magna* [146], while *Holospora undulata* can infect the protozoan *Paramecium caudatum* [147]. In
376 addition, many marine bacteria express antagonistic activity against other bacteria via antibiotics
377 [148]. Finally, phycosphere bacteria can relieve microalgae from oxidative stress induced by
378 reactive oxygen species. The epiphytic bacteria associated with the diatom *Amphiprora kufferathii*
379 were shown to express catalase activity that reduced hydrogen peroxide levels in the local
380 environment [149].

381 Salt stress

382 In addition to nutrients and CO₂, water becomes a major challenge towards sustainable,
383 industrial-scale algal cultivation [3]. Therefore, the replacement of freshwater with low-quality
384 water (*e.g.*, marine water, wastewater) can help improve both environmental and financial burdens
385 on industrial production. Numerous examples exist of halotolerant microalgae that can
386 physiologically adjust to environments with osmotic stresses introduced by saline waters via
387 shifted cell metabolism, osmoprotectants, and/or altered ion exchange [150]. For example,
388 previous work has shown that green algae such as *Desmodesmus*, *Chlorella*, *Dunaliella*,
389 *Scenedesmus* and *Picochlorum*, can tolerate elevated salinity and still produce storage compounds
390 (lipids/starches) [150-153] while more recent work has demonstrated the growth of green algae in
391 actual sea water/sea salts including adaptive evolution for storage compound production in more
392 saline environments [150,154,155]. Moreover, Church and colleagues [156] showed that the salt
393 concentration had more of an effect than the salt type in *Chlorella vulgaris* in terms of both growth
394 and storage compounds.

395 In the context of salinity stress, microalgae can produce osmoprotectants, and
396 dimethylsulfoniopropionate is considered the most abundant and important osmotically active

397 metabolite in phytoplankton although microalgae can use both N- and S-containing osmolytes
398 depending on N levels in the local environment [157,158]. Recently, microalgae species have been
399 shown to utilize either ectoine or proline as alternative osmoprotectants [158,159]. While saline
400 water provides alternative water sourcing as well as limits invasive species during open
401 cultivations, the impacts of resource allocation (C and/or N) to osmoprotectants must be
402 considered when trying to maximize algal biomass and lipids. However, an additional role for the
403 phycosome could be production of osmoprotectants, and recent work has shown microalgal use of
404 bacterially-produced ecotoine [158]. That said, few studies have tracked the structure and function
405 of microbial communities in saline/salt stressed outdoor algal cultivations, and future work is
406 needed to delineate the potential phycosomal roles in salinity tolerance, particularly for high
407 alkalinity conditions (*i.e.*, elevated sodium). Interestingly, when scleractinian corals (and the algal
408 symbiont) were exposed to salinity stress, a functional role of osmolyte production was associated
409 with microbiome re-structuring [160].

410 Temperature stress

411 Temperature stress for photoautotrophic growth can include both climatic- (long-term,
412 seasonal) and weather-related (short-term) changes that must be considered for industrial-scale,
413 algal growth facilities. Inherent to most geographic regions selected for maximizing algal growth
414 (*i.e.*, warm, sunny regions), diurnal temperature swings (in a given day and across seasons) can
415 occur dependent upon extremes in temperature highs and/or lows. Cho and coworkers showed
416 that water temperature throughout a seasonal cycle affected microalgal biomass productivity by
417 approximately 10-fold [161], and small temperature changes 5-10°C can impact biomass
418 allocation (*e.g.*, [162]). Microalgae can modulate phospholipid membrane content in response to
419 temperature changes, and lower temperatures have been shown to increase the production of

420 industrially-relevant metabolites such as EPA (eicosapentaenoic acid) and PUFAs
421 (polyunsaturated fatty acid) [163].

422 Research investigating the comparison between axenic and xenic microalgal cultures and
423 temperature stress is limited, but results from a previous study indicated that a native or non-native
424 microalgal microbiome played a positive role in the microalgal response to heat stress through
425 enhanced chlorophyll fluorescence [164]. Temperature was a dominant factor that affected
426 microbial pre-dominance in xenic microalgal cultures, and predominant microbial species were
427 related to various seasonal temperature changes [161]. Large, sudden increases in temperatures
428 can promote algal cell death [165], and daily temperature fluxes in outdoor microalgal systems
429 were associated with increased species richness [166]. However, it is difficult to delineate direct
430 and indirect temperature effects on the respective algal and microbial populations and how the
431 changes are inter-related; therefore, more work is needed to better understand the potential role of
432 phycosphere/phycosome in algal growth system resiliency to both diurnal temperature changes as
433 well as with different seasonal-regional temperatures.

434 [Learning from nature – ecological engineering for the laboratory and industry](#)

435 It is becoming clear that large scale, outdoor unialgal cultivations are prone to infection,
436 invasion, and contamination that can result in communities of bacteria, zooplankton, fungi, viruses,
437 and other microalgae [8]. Until recently, these contamination events have mostly been addressed
438 through the use of pesticides, as well as extensive sterilizing methods such as filtration [55].
439 However, a more sustainable approach may be to select for stable and resilient strains and the
440 associated communities as microbiome research continues to grow [32,37].

441 However, studies examining inter-organismal interactions in engineered microalgal
442 cultivation systems are still relatively rare, particularly at larger scale, and underscores the nascent

443 understanding of metabolic cooperation within these microbial communities for stable biofuel
444 production [128]. One contributing factor is the typical laboratory practices for enriching and
445 isolating microalgal strains that are not optimal for maintaining the associated
446 phycosome/phycosphere. As isolation protocols generally follow multiple rounds of dilution and
447 enrichment with and without antibiotics, many associated bacteria are likely eliminated. Since
448 prolonged axenic cultivation of microalgal species in industrial scale systems is not practical and
449 realizing that the absence of bacteria can negatively influence microalgal physiology and growth
450 even in supportive media, engineering approaches should be pursued that promote beneficial
451 interactions and minimize detrimental interactions. It is important to note that the term engineering
452 is used to refer to promoting and maintaining mixed consortia of ‘natural’ algae and other
453 microorganisms (*Eukarya*, *Bacteria*, and *Archaea*) with desired outputs and not editing at the
454 genome level.

455 In recent years, there has been increased interest in culturing microalgae with the associated
456 microbial communities [26]. Although such studies have facilitated understanding of the molecular
457 mechanisms of interactions in microalgal-bacterial consortia, laboratory enriched cultures
458 generally have lower diversity compared to *in situ* communities [87]. Analogous to plant-
459 microorganism interactions at plant roots and leaves [167,168], phycosphere/phycosome consortia
460 could be designed/selected to promote beneficial/protective interactions and/or limit negative
461 interactions to improve the stability and resiliency of industrial cultures.

462 The critical questions to address gaps in knowledge between natural and engineered
463 consortia in these settings are (i) whether enriched cultures capture the needed diversity from
464 natural microalgal phycospheres/phycosomes; (ii) can polycultures in managed, open ponds have
465 similar benefits that have been reported in natural habitats; and (iii) is it possible to reconstitute

466 and/or design microbial consortia with predictable and controllable outcomes that can be
467 consistently maintained. While the productivity of natural ecosystems is often measured by
468 biomass production [169], productivity of a microalgal industrial cultivation is likely assessed by
469 biomass quantity, compositional content (*e.g.*, lipids), stability/robustness, the net environmental
470 impact (*e.g.*, water, nutrient, CO₂ requirements), and the overall costs [17]. Therefore, the
471 phycosome and potential impacts should be considered during life cycle and techno-economic
472 analyses [3].

473 Use of algal polycultures to improve productivity and stability

474 In many environments, microorganisms form interactive consortia, in which they are more
475 likely to interact with each other than with outside species [170,171]. Termed ‘small world’
476 networks, these consortia are common in natural and man-made systems in which micro-scale
477 interactions impact overall productivity [172]. In addition, based on modeling clustered food webs
478 [173,174], phycosphere communities can display increased stability compared to randomly
479 assembled food webs and may display increased diversity because extinction rates are more
480 gradual. Sinha and Sinha [174] showed that relationships between species can be independent of
481 both the initial size and connectivity of the network, and that the number of interactive species is
482 a fundamental property of network structure irrespective of biotic or abiotic conditions. The results
483 also suggest that species that interact with too many other species are destabilizing to network
484 persistence [175].

485 Two mechanisms believed to drive diversity-productivity relationships are the selection
486 effect and the complementarity effect [24,28,176]. In the selection effect, the more species in a
487 consortium, the higher the chance of having a species with a specific function. However, simply
488 increasing the number of species does not always lead to increases in yield and stability if species

489 are in the same functional group [18]. To maximize the potential for improving yield, polycultures
490 could be designed based on specific traits/metabolic potential and the functional complementarity
491 between species. Indeed, metabolic dependencies, interactions and exchanges are being
492 increasingly suggested as major drivers of community structure and function in different systems.
493 Metabolic modeling of >800 sub-communities with known species composition indicated that
494 communities with high phylogenetic diversity tend to consist of species with a low degree of
495 metabolic overlap [177]. These models emphasized metabolic dependencies as a key biotic force
496 in determination of microbial communities in nature. Communities with high interaction potential
497 among members are more likely to benefit from complementary biosynthetic capacities and
498 require fewer resources [178]. Therefore, metabolite exchange could be a mechanism that
499 stabilizes phycosphere/phycosome interactions, and communities with metabolic synergy could
500 therefore thrive in nutritionally poor habitats [179]. This principle can guide the design of
501 polycultures through ecological engineering to maximize metabolic capacity and achieve target
502 biomass composition in industrial microalgal cultivation, where the main aim is to maximize
503 outputs (biomass, lipid content) using minimal inputs (*e.g.*, N, P, low quality water). To achieve
504 this goal, it is necessary to identify the mechanisms behind diversity-productivity relationships so
505 that design and control can be attempted.

506 Choosing multiple microalgae strains as “core” biomass producers

507 In the context of managed cultivation, consortia of microalgae with different traits might
508 be more tolerant to changing environmental conditions (light, temperature) and more resistant to
509 invaders [25]. When mono- and poly-algal cultures were evaluated, polycultures exhibited more
510 stable production through time, higher biocrude yields over time, and were more resistant to
511 invasion than monocultures [21,26]. The studies indicated that designing consortia requires

512 characterization and selection of strains based on ecological principles to promote functional
513 diversity [21,26]. A previous study showed that consortia of multiple microalgae resulted in higher
514 biomass production than those of mono-algal cultures when cultivated in wastewater [39].
515 Interestingly, although four “standard” UTEX strains were included in the screen for the best
516 consortia, the optimal consortium contained only native strains isolated from the wastewater
517 sources used for cultivation. These native strains may have developed an optimal interaction
518 network with one another and with other indigenous microorganisms as well as the local
519 environment. Therefore, identifying and promoting these natural relationships respective to a given
520 environment (*e.g.*, water or nutrient source) may be an effective strategy to design stable and
521 productive microalgal consortia.

522 The positive diversity-productivity relationships could be explained by the efficient use of
523 nutrients and light. Microalgae have different light preferences (wavelength, intensity), and
524 microalgal consortia that contained species with non-overlapping optimal wavelengths had higher
525 lipid content and PAR absorbance than polycultures with overlapping light use [24]. In a study
526 about algal nitrogen uptake, algal species differed significantly in capacity to take up ammonium,
527 urea, and nitrate, and as a result, co-cultures that differed in nitrogen preferences showed greater
528 complementarity and higher productivity than compared to monocultures [176]. In addition, mixed
529 microalgal communities were also shown to remove inorganic nutrients more rapidly than mono-
530 algal cultures and exhibited increased growth rates [180].

531 Bacterial consortia as probiotic “amendments”

532 Inoculating microalgal cultures with bacterial communities that confer health benefits
533 could be used as a form of phycosphere engineering [31]. In photoreactor systems, microalgae and
534 the accompanying bacterial flora were strongly positively correlated [120]. In a separate study, the

535 growth of *Navicula veneta* was positively affected by *Halomonas* NC1, and diatom cell levels
536 were 65% less without the bacterium [181]. When *Chlorella vulgaris* was grown in the presence
537 of different bacteria, all important parameters for biofuel production were higher than those
538 measured in corresponding axenic cultures [182]. More recently, Toyama [183] cultured each of
539 three microalgal species *Chlamydomonas reinhardtii*, *Chlorella vulgaris*, and *Euglena gracilis* in
540 the presence and absence of indigenous bacteria in wastewater effluent and for all three species,
541 axenic cultures resulted in higher biomass yields (1.5-2.8-fold) compared to the respective axenic
542 cultures.

543 The improved biomass production in polyculture may be explained in part by the higher
544 rate of nutrient assimilation and uptake by both microalgal and bacterial members. For example,
545 *P. tricornutum* cells in the presence of bacteria fixed 64% more carbon compared to axenic cells,
546 while bacterial cells that attached to microalgal cells consumed more microalgal-fixed carbon than
547 unattached bacteria [31]. In another study assessing the symbiotic relationship between
548 cyanobacteria and diatoms (e.g., *Climacodium*) in bulk seawater, cyanobacterial cells that attached
549 to diatom cells showed higher nitrogen fixation rates (171–420-fold) compared to rates estimated
550 for free-living cells, and the majority of the fixed nitrogen was transferred to the diatom [12].
551 Ortiz-Marquez [184] was able to eliminate the need for providing inorganic nitrogen directly to a
552 microalgal culture by adding *Azotobacter vinelandii*, a nitrogen fixing bacterium, that had been
553 genetically engineered to secrete ammonium into the growth medium. In a recent study,
554 *Janthinobacter* protected *Microchloropsis* from rotifer grazing pressure for short periods of time
555 in outdoor cultures [185]. While these studies demonstrate both direct and indirect, positive
556 impacts from added bacteria, the explicit use in promoting microalgal growth for biofuel
557 production is still limited, owing to the knowledge gaps in interactions of bacteria with microalgae

558 hosts, the potential tradeoff between yield and overall culture health, as well as challenges in
559 maintaining stable healthy consortia for different microalgae and/or polycultures. Similar in the
560 context of human gut microbiomes and person-to-person variability, much more work is needed
561 to discern and define ‘healthy’ phycosomes for different microalgal species under different growth
562 conditions. Different benefits are easier to track than others, and for example, an aggregation
563 phenotype could help with biomass dewatering and harvesting [186, 187].

564 Toward designing microalgal consortia with controllable outputs

565 The enrichment and characterization of microalgal consortia from extreme natural habitats
566 could provide ‘simplified’ communities with higher productivity and consistent stability. Robust,
567 resilient, adaptable and productive communities have been established via simple enrichment of
568 native consortia [10,31], or the assembly of novel synthetic consortia [26,189]. Habitats
569 experiencing extreme environmental conditions hold potential for strains with unique traits, for
570 example alkaline systems, that could contribute to functional parameters (*i.e.*, CO₂ delivery).

571 Alkaline aquatic systems have been shown to be among the most productive natural
572 ecosystems in the world [57,94], and it is hypothesized that distinct but very well-developed
573 metabolic interactions are at least partially responsible for these high productivities. Microalgae
574 of diverse taxa including *Scenedesmus*, *Navicula*, *Chlorella*, and *Neosporangiococcum* can thrive in
575 high pH environments, and they are valuable resources for cultivation with atmospheric CO₂ [47,
576 59, Vadlamani, A. PhD thesis, University of Toledo, 2016; Moll, K., PhD thesis, Montana State
577 University, 2021). For example, *Chlorella sorokiniana* SLA-04, achieved high biomass
578 productivities (>20g/m²/d) in open raceway ponds under high pH/high alkalinity without a culture
579 crash over a two year period [60, 190]. Given the adaptive specialization of such organisms,

580 manipulating cultivation conditions may be a useful approach for selecting stable, beneficial
581 consortia under desired conditions.

582 Consortium design will also benefit from a thorough understanding of the community
583 composition and metabolic interactions between members, which is being facilitated by next-
584 generation sequencing, next-generation physiology and imaging technology [191]. Metagenomic
585 sequencing, for instance, will allow for the estimation of the metabolic potential of the community,
586 and the prediction of possible metabolic relationships between community members. Advanced
587 staining and imaging techniques may make it possible to elucidate how microalgal-bacterial
588 interactions affect C, N, P and energy flow for maximum productivity. At the gene expression
589 level, meta-transcriptomic analyses combined with BONCAT (biorthogonal non-canonical amino
590 acid tagging), isotope-specific Raman confocal microspectroscopy and metabolite analysis can be
591 applied to help reveal how phycosome interactions may influence microalgal physiology and vice
592 versa during both short- and long-term cultivations. These –omics, chemical, and imaging
593 approaches will shed light on the temporal and spatial dynamics to inform consortia design.

594 Furthermore, consortia design can be assisted by mathematical modeling including
595 population-based modeling for prediction of interspecies dynamics [192], as well as metabolic
596 network modeling to predict energy and material flows in a community [193,194]. Ultimately, the
597 performance of consortia needs to be assessed at the industrial scale at point-of-production. While
598 studies so far suggest that engineering microalgal consortia could improve productivity and
599 stability of large-scale cultivation, extensive life cycle and techno-economical assessments (LCAs
600 and TEAs) are required to determine whether these improvements result in more sustainable
601 operation in both economic and environmental terms during anticipated perturbations (*e.g.*,
602 weather fluctuations). From the biofuel processing context, bacteria have potential use in

603 cultivation (provide benefit on growth) [15,25]; reduction in invasion, harvesting (induce
604 aggregation) [186,187]; and extraction (weakening of microalgal cell wall) [98,108].

605 *In silico* design of phycosphere communities

606 Synthetic microbial communities have traditionally been built using either **top-down** or
607 **bottom-up** approaches. Top-down refers to breaking down complex systems into individual parts
608 to simplify and understand function while bottom-up refers to the integration of well-studied
609 systems to form another, more complex system. Both approaches generally require extensive
610 background observation, next-generation sequencing, and physiology work along with intensive
611 experimentation [195,196]. The future of microbiome engineering has been suggested to hinge on
612 the principles of design-build-test-learn (DBTL) [196], and recent advances in microfluidics,
613 modeling, sequence-based technology, and bioinformatics can expedite the process of identifying,
614 culturing, and applying “built” consortia to algal systems.

615 For phycosome applications, designing consortia can be achieved using metabolic
616 predictions of the host algal cell, a native phycosome, a desired microbial community, or a
617 combination. With sufficient metabolic information about the host, exudate composition can be
618 modeled and used to predict how microorganisms may be recruited to the host [74]. Additional
619 approaches are being developed to predict and build systems based upon mathematical models of
620 natural ecosystems [197]. Computational approaches have also been paired with high throughput
621 culturing techniques such as microfluidics to predict host processes that are integral in recruiting
622 microbiomes [198] or how complementarity of host and microbial growth rate and substrate
623 preferences can be used to train phycosome design algorithms [199]. Phycosomes have been
624 designed by “letting the host decide” through swapping complex microbiomes from taxonomically
625 distinct host species and letting the host recruit microbial species to a new microbiome [200]. Used

626 together, *in silico* and *ex situ* tools like these can strengthen or expand the potential of traditional
627 bottom-up or top-down microbiome design.

628 Conclusion and perspective

629 There is evidence that microalgal cultures can increase productivity, stability, and
630 robustness through functional and phylogenetic diversification (both at the algal population(s) and
631 microbial community levels). Therefore, industrial scale microalgal culture productivity and
632 stability might be improved through diversification and thoughtful design of microalgal
633 phycosphere/phycosomes. Multiple microalgal species could provide high culture stability (*e.g.*,
634 temperature and light intensity tolerance) and promote efficient resource utilization while the
635 associated bacterial communities could provide essential nutrients, growth-promoting
636 components, and protections against pathogens, grazers, stresses *etc.*

637 It is evident from the recently published literature that the importance of microalgal
638 microbiomes has been recognized but more research is needed to elucidate mechanistic
639 understanding of phycosphere/phycosome interactions that directly promote productivity and
640 stability at industrial scales (see Outstanding Questions). The challenge can become more complex
641 when polyalgal cultures are considered under different growth conditions, for example high
642 pH/high alkalinity systems that have high but consistent pH with or without higher osmolarity.
643 Yet, understanding these interactions is essential for controlling and optimizing the function of
644 industrial ecosystems for maximal societal benefit in terms of direct air capture of CO₂ and the use
645 of low-quality water and nutrients that can produce different value-added products. In fact, natural
646 systems typically operate with low-quality resources via recycling and a combination of functional
647 redundancy and complementarity to off-set dynamic stresses. An improved understanding of
648 phototrophic biosystems in different environments and geographic locations could inform the

649 operation of biosystems at industrial scale for CO₂ capture by taking advantage of ecology and
650 physiology. Remaining challenges include the completion of in-depth physiological studies with
651 accompanying ecology to understand the potential of combined organismal traits, the relationship
652 between community members, and maintaining the consortia over industrially relevant time- and
653 space-scales.

654

655 **Acknowledgements**

656 The algal work by the Gerlach and Fields research groups is supported by the United States
657 National Science Foundation and the United States Department of Energy. We thank other
658 members of the Algal Biotechnology Group at Montana State University as well as collaborators
659 at University of Toledo and University of North Carolina for helpful discussions.

660

661 **References**

- 662 1 Hussain, J., Rittman, B.E. (2023) Algae as a source of renewable energy: opportunities,
663 challenges, and recent developments. *Sustain Energy and Fuels*, 7, 2515-2544.
664 doi.org/10.1039/D2SE01599D
- 665 2 Aslan, S., Kapdan, I.K. (2006) Batch kinetics of nitrogen and phosphorus removal from
666 synthetic wastewater by algae. *Ecol Eng* 28, 64-70. 10.1016/j.ecoleng.2006.04.003
- 667 3 Fields, M.W. et al. (2014) Sources and resources: importance of nutrients, resource
668 allocation, and ecology in microalgal cultivation for lipid accumulation. *Appl Microbiol*
669 *and Biotechnol* 98, 4805-4816. 10.1007/s00253-014-5694-7
- 670 4 Georgianna, D.R., Mayfield, S.P. (2012) Exploiting diversity and synthetic biology for
671 the production of algal biofuels. *Nature* 488, 329-335. 10.1038/nature11479
- 672 5 Hoffmann, J.P. (1998) Wastewater treatment with suspended and nonsuspended algae. *J*
673 *Phycol* 34, 757-763. 10.1046/j.1529-8817.1998.340757.x

674 6 Widin, S.L. et al. (2022) Biodiversity and disease risk in an algal biofuel system: An
675 experimental test in outdoor ponds using a before-after-control-impact (BACI) design.
676 PLoS One 17, e0267674. 10.1371/journal.pone.0267674

677 7 Fuentes, J. et al. (2016) Impact of microalgae-bacteria interactions on the production of
678 algal biomass and associated compounds. Mar Drugs 14, 100. 10.3390/md14050100

679 8 Kazamia, E. et al. (2014) An Engineered Community Approach for Industrial Cultivation
680 of Microalgae. Ind Biotechnol 10, 184-190. 10.1089/ind.2013.0041

681 9 Smith, V.H., Crews, T. (2014) Applying ecological principles of crop cultivation in large-
682 scale algal biomass production. Algal Res 4, 23-34. 10.1016/j.algal.2013.11.005

683 10 Kimbrel, J.A. et al. (2019) Host selection and stochastic effects influence bacterial
684 community assembly on the microalgal phycosphere. Algal Res 40, 101489.
685 10.1016/j.algal.2019.101489

686 11 Croft, M.T. et al. (2005) Algae acquire vitamin B₁₂ through a symbiotic relationship with
687 bacteria. Nature 438, 90-93. 10.1038/nature04056

688 12 Foster, R.A. et al. (2011) Nitrogen fixation and transfer in open ocean diatom-
689 cyanobacterial symbioses. ISME J 5, 1484-1493. 10.1038/ismej.2011.26

690 13 Geng, H., Belas, R. (2010) Molecular mechanisms underlying roseobacter-phytoplankton
691 symbioses. Curr Opin Biotech 21, 332-338. 10.1016/j.copbio.2010.03.013

692 14 Grant, M.A. et al. (2014) Direct exchange of vitamin B₁₂ is demonstrated by modelling
693 the growth dynamics of algal-bacterial cocultures. ISME J 8, 1418-1427.
694 10.1038/ismej.2014.9

695 15 Kazamia, E. et al. (2012) Mutualistic interactions between vitamin B₁₂-dependent algae
696 and heterotrophic bacteria exhibit regulation: Algal-bacterial interactions for delivery of
697 vitamin B₁₂. Environ Microbiol 14, 1466-1476. 10.1111/j.1462-2920.2012.02733.x

698 16 Zehr, J.P. (2015) How single cells work together. Science 349, 1163-1164.
699 10.1126/science.aac9752

700 17 Beyter, D. et al. (2016) Diversity, productivity, and stability of an industrial microbial
701 ecosystem. Appl Environ Microbiol 82, 2494-2505. 10.1128/AEM.03965-15

702 18 Newby, D.T. et al. (2016) Assessing the potential of polyculture to accelerate algal
703 biofuel production. Algal Res 19, 264-277. 10.1016/j.algal.2016.09.004

704 19 Mallon, C.A. et al. (2015) Microbial invasions: the process, patterns, and mechanisms.
705 Trends Microbiol 23, 719-729. 10.1016/j.tim.2015.07.013

706 20 Wright, E.S., Vestigian, K. H. (2016) Inhibitory interactions promote frequent biostability
707 among competing bacteria. Nat Commun 7, 11274. 10.1038/ncomms11274

708 21 Godwin, C.M. et al. (2018) Biodiversity improves the ecological design of sustainable
709 biofuel systems. GCB Bioenergy 10, 752-765. 10.1111/gcbb.12524

710 22 Bell, T.A.S. et al. (2019) Contributions of the microbial community to algal biomass and
711 biofuel productivity in a wastewater treatment lagoon system. Algal Res 39, 101461.
712 10.1016/j.algal.2019.101461

713 23 Narwani, A. et al. (2016) Power of plankton: effects of algal biodiversity on biocrude
714 production and stability. Environ Sci Technol 50, 13142-13150. 10.1021/acs.est.6b03256

715 24 Stockenreiter, M. et al. (2013) Functional group richness: implications of biodiversity for
716 light use and lipid yield in microalgae. J Phycol, n/a-n/a. 10.1111/jpy.12092

717 25 Corcoran, A.A., Boeing, W.J. (2012) Biodiversity increases the productivity and stability
718 of phytoplankton communities. PLoS ONE 7, e49397. 10.1371/journal.pone.0049397

719 26 Carruthers, D.N. et al. (2019) Biodiversity improves life cycle sustainability metrics in
720 algal biofuel production. Environ Sci Technol 53, 9279-9288. 10.1021/acs.est.9b00909

721 27 Six, C. et al. (2007) Light variability illuminates niche-partitioning among marine
722 Picocyanobacteria. PLoS One 2, e1341. 10.1371/journal.pone.0001341

723 28 Lindemann, S.R. et al. (2016) Engineering microbial consortia for controllable outputs.
724 ISME J 10, 2077-2084. 10.1038/ismej.2016.26

725 29 Thomas, P.K. et al. (2019) A natural algal polyculture outperforms an assembled
726 polyculture in wastewater-based open pond biofuel production. Algal Res 40.
727 10.1016/j.algal.2019.101488

728 30 Lian, J. et al. (2018) The effect of the algal microbiome on industrial production of
729 microalgae. Microb Biotechnol 11, 806-818. 10.1111/1751-7915.13296

730 31 Samo, T.J. et al. (2018) Attachment between heterotrophic bacteria and microalgae
731 influences symbiotic microscale interactions. Environ Microbiol 20, 4385-4400.
732 10.1111/1462-2920.14357

733 32 Ramanan, R. et al. (2016) Algae–bacteria interactions: Evolution, ecology and emerging
734 applications. Biotechnol Adv 34, 14-29. 10.1016/j.biotechadv.2015.12.003

735 33 Guiry, M.D. (2012) How many species of algae are there? *J Phycol* 48, 1057-1063.
736 10.1111/j.1529-8817.2012.01222.x

737 34 Hanschen, E.R., Starckenburg, S.R. (2020) The state of algal genome quality and
738 diversity. *Algal Res* 50, 101968. 10.1016/j.algal.2020.101968

739 35 Goemann, C.L.C. et al. (2023) Genome sequence, phylogenetic analysis, and structure-
740 based annotation reveal metabolic potential of *Chlorella* sp. SLA-04. *Algal Res* 69.
741 10.1016/j.algal.2022.102943

742 36 Pokorny, L. et al. (2022) How to verify non-presence-the challenge of axenic algae
743 cultivation. *Cells* 11. 10.3390/cells11162594

744 37 Seymour, J.R. et al. (2017) Zooming in on the phycosphere: the ecological interface for
745 phytoplankton–bacteria relationships. *Nat Microbiol* 2. 10.1038/nmicrobiol.2017.65

746 38 Teeling, H. et al. (2012) Substrate-controlled succession of marine bacterioplankton
747 populations induced by a phytoplankton bloom. *Science* 336, 608-611.
748 10.1126/science.1218344

749 39 Hena, S. et al. (2015) Cultivation of algae consortium in a dairy farm wastewater for
750 biodiesel production. *Water Res Industry* 10, 1-14. 10.1016/j.wri.2015.02.002

751 40 Teeling, H. et al. (2016) Recurring patterns in bacterioplankton dynamics during coastal
752 spring algae blooms. *eLife* 5. 10.7554/eLife.11888

753 41 Mueller, R.C. et al. (2021) An emerging view of the diversity, ecology and function of
754 Archaea in alkaline hydrothermal environments. *FEMS Microbiol Ecol* 97.
755 10.1093/femsec/fiaa246

756 42 Jones, B.E., Grant, W.D. (2000) Microbial diversity and ecology of alkaline
757 environments. In *Journey to Diverse Microbial Worlds*, pp. 177-190, Springer

758 43 Canale, R.P., Vogel, A.H. (1974) Effects of temperature on phytoplankton growth. *J Env*
759 *Eng Div-ASCE* 100, 229-241. 10.1061/JEEGAV.0000151

760 44 Varshney, P. et al. (2015) Extremophilic micro-algae and their potential contribution in
761 biotechnology. *Bioresour Technol* 184, 363-372. 10.1016/j.biortech.2014.11.040

762 45 Talling, J.F. et al. (1973) The upper limit of photosynthetic productivity by
763 phytoplankton: evidence from Ethiopian soda lakes. *Freshwater Biol* 3, 53-76.
764 10.1111/j.1365-2427.1973.tb00062.x

765 46 Lopez-Archilla, A.I. et al. (2004) Phytoplankton diversity and cyanobacterial dominance
766 in a hypereutrophic shallow lake with biologically produced alkaline pH. *Extremophiles*
767 8, 109-115. 10.1007/s00792-003-0369-9

768 47 Hodgskiss, L.H. et al. (2016) Cultivation of a native alga for biomass and biofuel
769 accumulation in coal bed methane production water. *Algal Res* 19, 63-68.
770 10.1016/j.algal.2016.07.014

771 48 Nega, R. et al. (2018) Spatial and temporal variations in the diversity of microalgae in
772 Lake Hawassa, Ethiopia. *Int J Food Sci Nutr* 4, 16-23. ISSN 2471-7371

773 49 Chojnacka, K., Marquez-Rocha, F.J. (2003) Kinetic and stoichiometric relationships of
774 the energy and carbon metabolism in the culture of microalgae. *Biotechnol* 3, 21-34.
775 10.3923/biotech.2004.21.34

776 50 Liang, Y. et al. (2009) Biomass and lipid productivities of *Chlorella vulgaris* under
777 autotrophic, heterotrophic and mixotrophic growth conditions. *Biotechnol Lett* 31, 1043-
778 1049. 10.1007/s10529-009-9975-7

779 51 Deschênes, J.-S. et al. (2015) Mixotrophic production of microalgae in pilot-scale
780 photobioreactors: Practicability and process considerations. *Algal Res* 10, 80-86.
781 10.1016/j.algal.2015.04.015

782 52 Ruiz, J. et al. (2022) Heterotrophic vs autotrophic production of microalgae: Bringing
783 some light into the everlasting cost controversy. *Algal Res* 64.
784 10.1016/j.algal.2022.102698

785 53 Letcher, P.M. et al. (2013) Characterization of *Amoebophilidium protococcarum*, an
786 algal parasite new to the cryptomycota isolated from an outdoor algal pond used for the
787 production of biofuel. *PLoS One* 8, e56232. 10.1371/journal.pone.0056232

788 54 Yamada, T.O. et al. (2007) *Chlorella* viruses. *Adv Virus Res* 66, 293-336.
789 10.1016/S0065-3527(06)66006-5

790 55 Zhu, Z. et al. (2020) Overcoming the biological contamination in microalgae and
791 cyanobacteria mass cultivations for photosynthetic biofuel production. *Molecules* 25.
792 10.3390/molecules25225220

793 56 Wang, H. et al. (2013) The contamination and control of biological pollutants in mass
794 cultivation of microalgae. *Bioresource Technol* 128, 745-750.
795 10.1016/j.biortech.2012.10.158

796 57 Melack, J.M. (1981) Photosynthetic activity of phytoplankton in tropical African soda
797 lakes. In Salt Lakes (Williams, W.D., ed), pp. 71-85, Springer

798 58 Gardner, R.D. et al. (2012) Use of sodium bicarbonate to stimulate triacylglycerol
799 accumulation in the chlorophyte *Scenedesmus* sp. and the diatom *Phaeodactylum*
800 *tricornutum*. J Appl Phycol 24, 1311-1320. 10.1007/s10811-011-9782-0

801 59 Gardner, R.D. et al. (2013) Comparison of CO₂ and bicarbonate as inorganic carbon
802 sources for triacylglycerol and starch accumulation in *Chlamydomonas reinhardtii*.
803 Biotechnol Bioeng 110, 87-96. 10.1002/bit.24592

804 60 Vadlamani, A. et al. (2017) Cultivation of microalgae at extreme alkaline pH conditions:
805 A novel approach for biofuel production. ACS Sustain Chem Eng 5, 7284-7294.
806 10.1021/acssuschemeng.7b01534

807 61 Baattrup-Pedersen, A. et al. (2022) Alkalinity and diatom assemblages in lowland
808 streams: How to separate alkalinity from inorganic phosphorus in ecological
809 assessments? Sci Total Environ 823, 153829. 10.1016/j.scitotenv.2022.153829

810 62 Martin, K. et al. (2021) The biogeographic differentiation of algal microbiomes in the
811 upper ocean from pole to pole. Nat Commun 12, 5483. 10.1038/s41467-021-25646-9

812 63 Bell, W.H., Mitchell, R. (1972) Chemotactic and growth responses of marine bacteria to
813 algal extracellular products. Biol Bull 143, 265-277. <https://doi.org/10.2307/1540052>

814 64 Amin, S.A. et al. (2012) Interactions between diatoms and bacteria. Microbiol Molec
815 Biol Rev 76, 667-684. 10.1128/MMBR.00007-12

816 65 Whipps, J.M. et al. (1988) Mycoparasitism and plant disease control. Fungi in Biological
817 Control Systems, Manchester University Press

818 66 Berg, G. et al. (2020) Microbiome definition re-visited: old concepts and new challenges.
819 Microbiome 8, 103. 10.1186/s40168-020-00875-0

820 67 Kawafune, K. et al. (2012) Molecular Identification of Rickettsial Endosymbionts in the
821 non-phagotrophic volvocalean green algae. PLoS ONE 7, e31749.
822 10.1371/journal.pone.0031749

823 68 Grossart, H.-P. et al. (2005) Marine diatom species harbour distinct bacterial
824 communities: Marine diatom species harbour distinct bacterial communities. Environ
825 Microbiol 7, 860-873. 10.1111/j.1462-2920.2005.00759.x

826 69 Bižić-Ionescu, M. et al. (2015) Comparison of bacterial communities on limnic versus
827 coastal marine particles reveals profound differences in colonization: Marine and limnic
828 particle-associated bacteria. *Environ Microbiol* 17, 3500-3514. 10.1111/1462-
829 2920.12466

830 70 Eigemann, F. et al. (2013) Bacterial community composition associated with freshwater
831 algae: species specificity vs. dependency on environmental conditions and source
832 community. *FEMS Microbiol Ecol* 83, 650-663. 10.1111/1574-6941.12022

833 71 Stocker, R., Seymour, J.R. (2012) Ecology and physics of bacterial chemotaxis in the
834 ocean. *Microbiol Molec Biol Rev* 76, 792-812. 10.1128/MMBR.00029-12

835 72 Lambert, B.S. et al. (2017) A microfluidics-based in situ chemotaxis assay to study the
836 behaviour of aquatic microbial communities. *Nat Microbiol* 2, 1344-1349.
837 10.1038/s41564-017-0010-9

838 73 Raina, J.B. et al. (2022) Chemotaxis shapes the microscale organization of the ocean's
839 microbiome. *Nature* 605, 132-138. 10.1038/s41586-022-04614-3

840 74 Smriga, S. et al. (2016) Chemotaxis toward phytoplankton drives organic matter
841 partitioning among marine bacteria. *P Natl Acad Sci USA* 113, 1576-1581.
842 10.1073/pnas.1512307113

843 75 Sjoblad, R.D., Mitchell, R. (1979) Chemotactic responses of *Vibrio alginolyticus* to algal
844 extracellular products. *Can J Microbiol* 25, 964-967. 10.1139/m79-147

845 76 Miller, T.R. et al. (2004) Chemotaxis of *Silicibacter* sp. strain TM1040 toward
846 dinoflagellate Products. *Appl Environ Microbiol* 70, 4692-4701.
847 10.1128/AEM.70.8.4692-4701.2004

848 77 Seymour, J.R. et al. (2010) Chemoattraction to Dimethylsulfoniopropionate Throughout
849 the marine microbial food web. *Science* 329, 342-345. 10.1126/science.1188418

850 78 Mayali, X. et al. (2011) Temporal attachment dynamics by distinct bacterial taxa during a
851 dinoflagellate bloom. *Aquat Microb Ecol* 63, 111-122. 10.3354/ame01483

852 79 Staats, N. et al. (2000) Exopolysaccharide production by the epipelagic diatom
853 *Cylindrotheca closterium*: effects of nutrient conditions. *J Exp Mar Biol Ecol* 249, 13-27.
854 10.1016/S0022-0981(00)00166-0

855 80 Passow, U. (2002) Production of transparent exopolymer particles (TEP) by phyto- and
856 bacterioplankton. *Mar Ecol Prog Ser* 236, 1-12. 10.3354/meps236001

857 81 Nissimov, J.I. et al. (2018) Dynamics of transparent exopolymer particle production and
858 aggregation during viral infection of the coccolithophore, *Emiliania huxleyi*. Environ
859 Microbiol 20, 2880-2897. 10.1111/1462-2920.14261

860 82 Martin-Platero, A.M. et al. (2018) High resolution time series reveals cohesive but short-
861 lived communities in coastal plankton. Nat Comm 9, 266. 10.1038/s41467-017-02571-4

862 83 Basu, S., Mackey, K. (2018) Phytoplankton as key mediators of the biological carbon
863 pump: their responses to a changing climate. Sustainability 10. 10.3390/su10030869

864 84 Buesseler, K.O., Boyd, P.W. (2009) Shedding light on processes that control particle
865 export and flux attenuation in the twilight zone of the open ocean. Limnol Oceanogr 54,
866 1210-1232. 10.4319/lo.2009.54.4.1210

867 85 Ashen, J.B., Goff L. J. (2000) Molecular and ecological evidence for species specificity
868 and coevolution in a group of marine algal-bacterial symbioses. Appl Environ Microbiol
869 66, 3024-3030. 10.1128/AEM.66.7.3024-3030.2000

870 86 Sapp, M. et al. (2007) Species-specific bacterial communities in the phycosphere of
871 microalgae? Microb Ecol 53, 683-699. 10.1007/s00248-006-9162-5

872 87 Krohn-Molt, I. et al. (2017) Insights into microalga and bacteria interactions of selected
873 phycosphere biofilms Using metagenomic, transcriptomic, and proteomic approaches.
874 Front Microbiol 8. 10.3389/fmicb.2017.01941

875 88 Behringer, G. et al. (2018) Bacterial communities of diatoms display strong conservation
876 across strains and time. Front Microbiol9. 10.3389/fmicb.2018.00659

877 89 Tada, Y. et al. (2017) Distinct bacterial community and diversity shifts after
878 phytoplankton-derived dissolved organic matter addition in a coastal environment. J Exp
879 Mar Biol Ecol 495, 119-128. 10.1016/j.jembe.2017.06.006

880 90 Landa, M. et al. (2016) Shifts in bacterial community composition associated with
881 increased carbon cycling in a mosaic of phytoplankton blooms. ISME J 10, 39-50.
882 10.1038/ismej.2015.105

883 91 Bell, W.H. (1983) Bacterial utilization of algal extracellular products. 3. The specificity
884 of algal-bacterial interaction: Extracellular products. 3. Limnol Oceanogr 28, 1131-1143.
885 10.4319/lo.1983.28.6.1131

886 92 Fu, H. et al. (2020) Ecological drivers of bacterial community assembly in synthetic
887 phycospheres. P Natl Acad Sci USA 117, 3656-3662. 10.1073/pnas.1917265117

888 93 Morales, M. et al. (2020) Rotating algal biofilm versus planktonic cultivation: LCA
889 perspective. *J Clean Prod* 257. 10.1016/j.jclepro.2020.120547

890 94 Sharp, C.E. et al. (2017) Robust, high-productivity phototrophic carbon capture at high
891 pH and alkalinity using natural microbial communities. *Biotechnol Biofuels* 10.
892 10.1186/s13068-017-0769-1

893 95 Azam, F., Malfatti, F. (2007) Microbial structuring of marine ecosystems. *Nat Rev*
894 *Microbiol* 5, 782-791. 10.1038/nrmicro1747

895 96 Wang, M. and Yuan, W. (2014) Bacterial lysis of microalgal cells. *J Sustain Bioenerg*
896 *Sys* 04, 243-248. 10.4236/jsbs.2014.44022

897 97 Sule, P. and Belas, R. (2013) A novel inducer of *Roseobacter* motility is also a disruptor
898 of algal symbiosis. *J Bacteriol* 195, 637-646. 10.1128/JB.01777-12

899 98 Wagner-Dobler, I., Biebl, H. (2006) Environmental biology of the marine *Roseobacter*
900 lineage. *Annu Rev Microbiol* 60, 255-280. 10.1146/annurev.micro.60.080805.142115

901 99 Seyedsayamdost, M.R. et al. (2011) The Jekyll-and-Hyde chemistry of *Phaeobacter*
902 *gallaeciensis*. *Nat Chem* 3, 331-335. 10.1038/nchem.1002

903 100 Sharifah, E.N., Eguchi, M. (2011) The phytoplankton *Nannochloropsis oculata* enhances
904 the ability of *Roseobacter* clade bacteria to inhibit the growth of fish pathogen *Vibrio*
905 *anguillarum*. *PLoS One* 6, e26756. 10.1371/journal.pone.0026756

906 101 Henriksen, N. et al. (2022) Role is in the eye of the beholder-the multiple functions of the
907 antibacterial compound tropodithietic acid produced by marine Rhodobacteraceae. *FEMS*
908 *Microbiol Rev* 46. 10.1093/femsre/fuac007

909 102 Bratbak, G., Thingstad, T. F. (1985) Phytoplankton-bacteria interactions: an apparent
910 paradox? Analysis of a model system with both competition and commensalism. *Mar*
911 *Ecol Prog Ser* 25, 23-30

912 103 Mayali, X., Azam, F. (2004) Algicidal bacteria in the sea and their impact on algal
913 blooms1. *J Euk Microbiol* 51, 139-144. 10.1111/j.1550-7408.2004.tb00538.x

914 104 Jung, S.W. et al. (2008) *Pseudomonas fluorescens* HYK0210-SK09 offers species-
915 specific biological control of winter algal blooms caused by freshwater diatom
916 *Stephanodiscus hantzschii*. *J Appl Microbiol* 105, 186-195. 10.1111/j.1365-
917 2672.2008.03733.x

918 105 Noh, S.Y. et al. (2017) Algicidal activity of the bacterium, *Pseudomonas fluorescens*
919 SK09, to mitigate *Stephanodiscus hantzschii* (Bacillariophyceae) blooms using field
920 mesocosms. J Freshwat Ecol 32, 477-488. 10.1080/02705060.2017.1323682

921 106 Lenneman, E.M. et al. (2014) Potential application of algicidal bacteria for improved
922 lipid recovery with specific algae. FEMS Microbiol Lett 354, 102-110. 10.1111/1574-
923 6968.12436

924 107 Demuez, M. et al. (2015) Algicidal microorganisms and secreted algicides: New tools to
925 induce microalgal cell disruption. Biotechnol Adv 33, 1615-1625.
926 10.1016/j.biotechadv.2015.08.003

927 108 Meyer, N. et al. (2017) Strategies and ecological roles of algicidal bacteria. FEMS
928 Microbiol Rev 41, 880-899. 10.1093/femsre/fux029

929 109 Azam, F. et al. (1991) The role of the microbial loop in Antarctic pelagic ecosystems.
930 Polar Res 10, 239-244. 10.3402/polar.v10i1.6742

931 110 Fei, C. et al. (2020) Quorum sensing regulates 'swim-or-stick' lifestyle in the
932 phycosphere. Environ Microbiol 22, 4761-4778. 10.1111/1462-2920.15228

933 111 Rajamani, S. et al. (2011) N-ACYL homoserine lactonase inactivation of quorum sensing
934 agonists produced by *Chlamydomonas reinhardtii* and characterization of a transgenic
935 algae. J Phycol 47, 1219-1227. 10.1111/j.1529-8817.2011.01049.x

936 112 Lebeau, T., Robert, J.M. (2003) Diatom cultivation and biotechnologically relevant
937 products. Part II: Current and putative products. Applied Microbiology and
938 Biotechnology 60, 624-632. 10.1007/s00253-002-1177-3

939 113 Davies, D.G., Marques, C.N.H. (2009) A fatty acid messenger is responsible for inducing
940 dispersion in microbial biofilms. J Bacteriol 191, 1393-1403. 10.1128/JB.01214-08

941 114 Sauer, K. et al. (2004) Characterization of nutrient-induced dispersion in *Pseudomonas*
942 *aeruginosa* PAO1 biofilm. J Bacteriol 186, 7312-7326. 10.1128/JB.186.21.7312-
943 7326.2004

944 115 Ozer, E.A. et al. (2005) Human and murine paraoxonase 1 are host modulators of
945 *Pseudomonas aeruginosa* quorum-sensing. FEMS Microbiol Lett 253, 29-37.
946 10.1016/j.femsle.2005.09.023

947 116 Ribalet, F. et al. (2008) Differential effect of three polyunsaturated aldehydes on marine
948 bacterial isolates. Aquat Toxicol 86, 249-255. 10.1016/j.aquatox.2007.11.005

949 117 Hitch, T.C.A. et al. (2022) Microbiome-based interventions to modulate gut ecology and
950 the immune system. *Mucosal Immunol* 15, 1095-1113. 10.1038/s41385-022-00564-1

951 118 Mayali, X. (2018) Editorial: Metabolic interactions between bacteria and phytoplankton.
952 *Front Microbiol* 9, 727. 10.3389/fmicb.2018.00727

953 119 Nikolaev, Y.A. et al. (2011) Effect of bacterial satellites on *Chlamydomonas reinhardtii*
954 growth in an algo-bacterial community. *Microbiology* 77, 78-83.
955 10.1134/s0026261708010116

956 120 Silva-Aciaries, F.R., Riquelme, C.E. (2008) Comparisons of the growth of six diatom
957 species between two configurations of photobioreactors. *Aquacult Eng* 38, 26-35.
958 10.1016/j.aquaeng.2007.10.005

959 121 Amin, S.A. et al. (2007) Boron binding by a siderophore isolated from marine bacteria
960 associated with the toxic dinoflagellate *Gymnodinium catenatum*. *JACS* 129, 478-479.
961 10.1021/ja067369u

962 122 Amin, S.A. et al. (2009) Photolysis of iron-siderophore chelates promotes bacterial-algal
963 mutualism. *P Natl Acad Sci USA* 106, 17071-17076. 10.1073/pnas.0905512106

964 123 Hogle, S.L. et al. (2017) Direct heme uptake by phytoplankton-associated *Roseobacter*
965 bacteria. *mSystems* 2. 10.1128/mSystems.00124-16

966 124 Plouviez, M. et al. (2018) Nitrous oxide emissions from microalgae: potential pathways
967 and significance. *J Appl Phycol* 31, 1-8. 10.1007/s10811-018-1531-1

968 125 Hilton, J.A. et al. (2013) Genomic deletions disrupt nitrogen metabolism pathways of a
969 cyanobacterial diatom symbiont. *Nat Commun* 4, 1767. 10.1038/ncomms2748

970 126 Kim, B.-H. et al. (2014) Role of *Rhizobium*, a plant growth promoting bacterium, in
971 enhancing algal biomass through mutualistic interaction. *Biomass Bioenerg* 69, 95-105.
972 10.1016/j.biombioe.2014.07.015

973 127 Suleiman, M. et al. (2016) Interkingdom cross-feeding of ammonium from marine
974 methylamine-degrading bacteria to the diatom *Phaeodactylum tricorutum*. *Appl Environ*
975 *Microbiol* 82, 7113-7122. 10.1128/AEM.01642-16

976 128 Amin, S.A. et al. (2015) Interaction and signalling between a cosmopolitan
977 phytoplankton and associated bacteria. *Nature* 522, 98-101. 10.1038/nature14488

- 978 129 Pisman, T.I. et al. (2005) Experimental and mathematical modeling of the consumer's
979 influence on productivity of algae in a model aquatic ecosystem. *Adv Space Res* 35,
980 1521-1527. 10.1016/j.asr.2004.12.048
- 981 130 Haines, K.C. L., Guillard, R. (1974) Growth of vitamin B₁₂-requiring marine diatoms in
982 mixed laboratory cultures with vitamin B₁₂-producing bacteria. *J Phycol* 10, 245-252.
983 10.1111/j.1529-8817.1974.tb02709.x
- 984 131 Kurata, A. (1986) Blooms of *Uroglena americana* in relation to concentrations of B
985 group vitamins. In *Chrysophytes aspects and problems* (Kristiansenm J., A., R. A., ed),
986 Cambridge University Press
- 987 132 Kuo, R.C., Lin, S. (2013) Ectobiotic and endobiotic bacteria associated with *Eutreptiella*
988 sp. isolated from Long Island Sound. *Protist* 164, 60-74. 10.1016/j.protis.2012.08.004
- 989 133 Lupette, J. et al. (2016) *Marinobacter* dominates the bacterial community of the
990 *Ostreococcus tauri* phycosphere in culture. *Front Microbiol* 7, 1414.
991 10.3389/fmicb.2016.01414
- 992 134 Warren, M.J. et al. (2002) The biosynthesis of adenosylcobalamin (vitamin B₁₂). *Nat*
993 *Prod Rep* 19, 390-412. 10.1039/b108967f
- 994 135 Helliwell, K.E. et al. (2011) Insights into the evolution of vitamin B₁₂ auxotrophy from
995 sequenced algal genomes. *Mol Biol Evol* 28, 2921-2933. 10.1093/molbev/msr124
- 996 136 Giovannoni, S.J. (2012) Vitamins in the sea. *P Natl Acad Sci USA* 109, 13888-13889.
997 10.1073/pnas.1211722109
- 998 137 Durham, B.P. et al. (2015) Cryptic carbon and sulfur cycling between surface ocean
999 plankton. *P Natl Acad Sci* 112, 453-457. 10.1073/pnas.1413137112
- 1000 138 Wienhausen, G. et al. (2017) The exometabolome of two model strains of the
1001 *Roseobacter* group: a marketplace of microbial metabolites. *Front Microbiol* 8, 1985.
1002 10.3389/fmicb.2017.01985
- 1003 139 de-Bashan, L.E. et al. (2011) Cell-cell interaction in the eukaryote-prokaryote model of
1004 micro algae *Chlorella vulgaris* and the bacterium *Azospirillum brasilense* immobilized
1005 in polymer beads. *J Phycol* 47, 1350-1359. 10.1111/j.1529-8817.2011.01062.x
- 1006 140 Lau, S. et al. (2009) Auxin signaling in algal lineages: fact or myth? *Trends Plant Sci* 14,
1007 182-188. 10.1016/j.tplants.2009.01.004

1008 141 Segev, E. et al. (2016) Dynamic metabolic exchange governs a marine algal-bacterial
1009 interaction. *eLife* 5. 10.7554/eLife.17473

1010 142 Peng, H. et al. (2021) Comparison of algae growth and symbiotic mechanisms in the
1011 presence of plant growth promoting bacteria and non-plant growth promoting bacteria.
1012 *Algal Res* 53. 10.1016/j.algal.2020.102156

1013 143 Yu, Z. et al. (2017) The effects of combined agricultural phytohormones on the growth,
1014 carbon partitioning and cell morphology of two screened algae. *Bioresource Technol* 239,
1015 87-96. 10.1016/j.biortech.2017.04.120

1016 144 Makridis, P. et al. (2006) Microbial conditions and antimicrobial activity in cultures of
1017 two microalgae species, *Tetraselmis chuii* and *Chlorella minutissima*, and effect on
1018 bacterial load of enriched *Artemia metanauplii*. *Aquaculture* 255, 76-81.
1019 10.1016/j.aquaculture.2005.12.010

1020 145 Geng, H. et al. (2008) Genetic dissection of tropodithietic acid biosynthesis by marine
1021 Roseobacters. *Appl and Environ Microbiol* 74, 1535-1545. 10.1128/AEM.02339-07

1022 146 Mitchell, S.E. et al. (2005) Host-parasite and genotype-by-environment interactions:
1023 temperature modifies potential for selection by a sterilizing pathogen. *Evolution* 59.
1024 10.1554/04-526

1025 147 Fels, D., Kaltz, O. (2006) Temperature-dependent transmission and latency of *Holospora*
1026 *undulata*, a micronucleus-specific parasite of the ciliate *Paramecium caudatum*. *P R Soc*
1027 *B* 273, 1031-1038. 10.1098/rspb.2005.3404

1028 148 Long, R.A., Azam, F. (2001) Antagonistic interactions among marine pelagic bacteria.
1029 *Appl Environ Microbiol* 67, 4975-4983. 10.1128/AEM.67.11.4975-4983.2001

1030 149 Hunken, M. et al. (2008) Epiphytic bacteria on the Antarctic ice diatom *Amphiprora*
1031 *kufferathii* Manguin cleave hydrogen peroxide produced during algal photosynthesis.
1032 *Plant Biol (Stuttg)* 10, 519-526. 10.1111/j.1438-8677.2008.00040.x

1033 150 Arora, N. et al. (2019) Elucidating the unique physiological responses of halotolerant
1034 *Scenedesmus* sp. cultivated in sea water for biofuel production. *Algal Res* 37, 260-268.
1035 10.1016/j.algal.2018.12.003

1036 151 Xia, L. et al. (2014) NaCl as an effective inducer for lipid accumulation in freshwater
1037 microalgae *Desmodesmus abundans*. *Bioresource Technol* 161, 402-409.
1038 10.1016/j.biortech.2014.03.063

1039 152 Wang, T. et al. (2016) Salt stress induced lipid accumulation in heterotrophic culture cells
1040 of *Chlorella protothecoides*: Mechanisms based on the multi-level analysis of oxidative
1041 response, key enzyme activity and biochemical alteration. *J Biotechnol* 228, 18-27.
1042 10.1016/j.jbiotec.2016.04.025

1043 153 Foflanker, F. et al. (2016) The unexpected extremophile: Tolerance to fluctuating salinity
1044 in the green alga *Picochlorum*. *Algal Res* 16, 465-472. 10.1016/j.algal.2016.04.003

1045 154 Kim, Z.H. et al. (2021) Enhanced fatty acid productivity by *Parachlorella* sp., a
1046 freshwater microalga, via adaptive laboratory evolution under salt stress. *Biotechnol*
1047 *Bioproc E* 26, 223-231. 10.1007/s12257-020-0001-1

1048 155 Zhao, Y. et al. (2022) Stimulating biolipid production from the novel alga
1049 *Ankistrodesmus* sp. by coupling salt stress and chemical induction. *Renewable Energy*
1050 183, 480-490. 10.1016/j.renene.2021.11.034

1051 156 Church, J. et al. (2017) Effect of salt type and concentration on the growth and lipid
1052 content of *Chlorella vulgaris* in synthetic saline wastewater for biofuel production.
1053 *Bioresource Technol* 243, 147-153. 10.1016/j.biortech.2017.06.081

1054 157 Moran, M.A. and Durham, B.P. (2019) Sulfur metabolites in the pelagic ocean. *Nat Rev*
1055 *Microbiol* 17, 665-678. 10.1038/s41579-019-0250-1

1056 158 Fenizia, S. et al. (2020) Ectoine from bacterial and algal origin is a compatible solute in
1057 microalgae. *Mar Drugs* 18. 10.3390/md18010042

1058 159 Barera, S. and Forlani, G. (2023) The role of proline in the adaptation of eukaryotic
1059 microalgae to environmental stress: An underestimated tool for the optimization of algal
1060 growth. *J Appl Phycol* 35, 1635-1648. 10.1007/s10811-023-03017-9

1061 160 Rothig, T. et al. (2016) Long-term salinity tolerance is accompanied by major
1062 restructuring of the coral bacterial microbiome. *Mol Ecol* 25, 1308-1323.
1063 10.1111/mec.13567

1064 161 Cho, D.-H. et al. (2015) Organic carbon, influent microbial diversity and temperature
1065 strongly influence algal diversity and biomass in raceway ponds treating raw municipal
1066 wastewater. *Bioresource Technol* 191, 481-487. 10.1016/j.biortech.2015.02.013

1067 162 Corredor, L. et al. (2021) Effect of temperature, nitrate concentration, pH and bicarbonate
1068 addition on biomass and lipid accumulation in the sporulating green alga PW95. *Algal*
1069 *Res* 53. 10.1016/j.algal.2020.102148

1070 163 Jiang, H. and Gao, K. (2004) Effects of lowering temperature during culture on the
1071 production of polyunsaturated fatty acids in the marine diatom *Phaeodactylum*
1072 *tricornutum* (Bacillariophyceae)1. J Phycol 40, 651-654. 10.1111/j.1529-
1073 8817.2004.03112.x

1074 164 Samo, T.J. et al. (2023) The algal microbiome protects *Desmodesmus intermedius* from
1075 high light and temperature stress. Algal Res 75. 10.1016/j.algal.2023.103245

1076 165 Bechet, Q. et al. (2017) Modeling the impact of high temperatures on microalgal viability
1077 and photosynthetic activity. Biotechnol Biofuels 10, 136. 10.1186/s13068-017-0823-z

1078 166 Schabhüttl, S. et al. (2013) Temperature and species richness effects in phytoplankton
1079 communities. Oecologia 171, 527-536. 10.1007/s00442-012-2419-4

1080 167 Utami, Y.D. et al. (2022) Investigating plant-microbe interactions within the root. Arch
1081 Microbiol 204, 639. 10.1007/s00203-022-03257-2

1082 168 Chaudhry, V. et al. (2021) Shaping the leaf microbiota: plant-microbe-microbe
1083 interactions. J Exp Bot 72, 36-56. 10.1093/jxb/eraa417

1084 169 Yachi, S., Loreau, M. (1999) Biodiversity and ecosystem productivity in a fluctuating
1085 environment: The insurance hypothesis. P Natl Acad Sci USA 96, 1463-1468.
1086 10.1073/pnas.96.4.1463

1087 170 D'Souza, G. et al. (2018) Ecology and evolution of metabolic cross-feeding interactions
1088 in bacteria. NatProd Rep 35, 455-488. 10.1039/C8NP00009C

1089 171 Montoya, J.M., Sol, R.V. (2002) Small world patterns in food webs. J. Theor. Biol. 214,
1090 405-412. 10.1006/jtbi.2001.2460

1091 172 Watts, D.J. and Strogatz, S.H. (1998) Collective dynamics of 'small-world' networks.
1092 Nature 393, 440-442. 10.1038/30918

1093 173 Sinha, S. (2005) Complexity vs. stability in small-world networks. Physica A 346, 147-
1094 153. 10.1016/j.physa.2004.08.062

1095 174 Sinha, S., Sinha, S. (2005) Evidence of universality for the May-Wigner stability theorem
1096 for random networks with local dynamics. Phys Rev E 71. 10.1103/PhysRevE.71.020902

1097 175 Wilmers, C.C. (2007) Understanding ecosystem robustness. Trend Ecol Evol 22, 504-
1098 506. 10.1016/j.tree.2007.08.008

1099 176 Mandal, S. et al. (2018) Functional divergence in nitrogen uptake rates explains
1100 diversity–productivity relationship in microalgal communities. *Ecosphere* 9.
1101 10.1002/ecs2.2228

1102 177 Zelezniak, A. et al. (2015) Metabolic dependencies drive species co-occurrence in diverse
1103 microbial communities. *P Natl Acad Sci USA* 112, 6449-6454.
1104 10.1073/pnas.1421834112

1105 178 Klitgord, N., Segre, D. (2010) Environments that induce synthetic microbial ecosystems.
1106 *PLoS Comput Biol* 6, e1001002. 10.1371/journal.pcbi.1001002

1107 179 Turner, P.E., et al. (1996) Tests of ecological mechanisms promoting the stable
1108 coexistence of two bacterial genotypes. *Ecology* 77, 2119-2129. 10.2307/2265706

1109 180 Stockenreiter, M. et al. (2016) Nutrient uptake and lipid yield in diverse microalgal
1110 communities grown in wastewater. *Algal Res* 15, 77-82. 10.1016/j.algal.2016.02.013

1111 181 Avendano-Herrera, R.E., Riquelme, C.E. (2007) Production of a diatom-bacteria biofilm
1112 in a photobioreactor for aquaculture applications. *Aquacult Eng* 36, 97-104.
1113 10.1016/j.aquaeng.2006.08.001

1114 182 Cho, D.-H. et al. (2015) Enhancing microalgal biomass productivity by engineering a
1115 microalgal–bacterial community. *Bioresource Technol* 175, 578-585.
1116 10.1016/j.biortech.2014.10.159

1117 183 Toyama, T. et al. (2018) Growth promotion of three microalgae, *Chlamydomonas*
1118 *reinhardtii*, *Chlorella vulgaris* and *Euglena gracilis*, by in situ indigenous bacteria in
1119 wastewater effluent. *Biotechnol Biofuels* 11. 10.1186/s13068-018-1174-0

1120 184 Ortiz-Marquez, J.C.F. et al. (2012) Association with an ammonium-excreting bacterium
1121 allows diazotrophic culture of oil-rich eukaryotic microalgae. *Appl Environ Microbiol*
1122 78, 2345-2352. 10.1128/AEM.06260-11

1123 185 Ward, C.S. et al. (2021) Janthinobacter additions reduce rotifer grazing of microalga
1124 *Microchloropsis salina* in biotically complex communities. *Algal Res* 58, 102400.
1125 10.1016/j.algal.2021.102400

1126 186 Gardes, A. et al. (2011) Diatom-associated bacteria are required for aggregation of
1127 *Thalassiosira weissflogii*. *ISME J* 5, 436-445. 10.1038/ismej.2010.145

1128 187 Wang, H. et al. (2012) Novel bacterial isolate from Permian groundwater, capable of
1129 aggregating potential biofuel-producing microalga *Nannochloropsis oceanica* IMET1.
1130 Appl Environ Microbiol 78, 1445-1453. 10.1128/AEM.06474-11

1131 188 Kazamia, E. et al. (2012) Synthetic ecology – A way forward for sustainable algal biofuel
1132 production? J Biotechnol 162, 163-169. 10.1016/j.jbiotec.2012.03.022

1133 189 Godwin, C.M. et al. (2017) Ecological stoichiometry meets ecological engineering: using
1134 polycultures to enhance the multifunctionality of algal biocrude systems. Environ Sci
1135 Technol 51, 11450-11458. 10.1021/acs.est.7b02137

1136 190 Vadlamani, A. et al. (2019) High productivity cultivation of microalgae without
1137 concentrated CO₂ input. ACS Sustain Chem Eng 7, 1933-1943.
1138 10.1021/acssuschemeng.8b04094

1139 191 Schaible, G.A. et al. (2022) Correlative SIP-FISH-Raman-SEM-NanoSIMS links
1140 identity, morphology, biochemistry, and physiology of environmental microbes. ISME
1141 Comm 2. 10.1038/s43705-022-00134-3

1142 192 Bouskill, N.J. et al. (2012) Trait-based representation of biological nitrification: model
1143 development, testing, and predicted community composition. Front Microbiol 3, 364.
1144 10.3389/fmicb.2012.00364

1145 193 Stolyar, S. et al. (2007) Metabolic modeling of a mutualistic microbial community. Mol
1146 Syst Biol 3, 92. 10.1038/msb4100131

1147 194 Taffs, R. et al. (2009) In silico approaches to study mass and energy flows in microbial
1148 consortia: a syntrophic case study. BMC Syst Biol 3, 114. 10.1186/1752-0509-3-114

1149 195 Briones, A., Raskin, L. (2003) Diversity and dynamics of microbial communities in
1150 engineered environments and their implications for process stability. Curr Opin Biotech
1151 14, 270-276. 10.1016/s0958-1669(03)00065-x

1152 196 Lawson, C.E. et al. (2019) Common principles and best practices for engineering
1153 microbiomes. Nat Rev Microbiol 17, 725-741. 10.1038/s41579-019-0255-9

1154 197 Succurro, A. et al. (2017) A diverse community to study communities: Integration of
1155 experiments and mathematical models to study microbial consortia. J Bacteriol 199.
1156 10.1128/JB.00865-16

1157 198 Raina, J.B. et al. (2023) Chemotaxis increases metabolic exchanges between marine
1158 picophytoplankton and heterotrophic bacteria. *Nat Microbiol* 8, 510-521.
1159 10.1038/s41564-023-01327-9
1160 199 Liu, F. et al. (2021) Microfluidic and mathematical modeling of aquatic microbial
1161 communities. *Anal Bioanal Chem* 413, 2331-2344. 10.1007/s00216-020-03085-7
1162 200 Duran, P. et al. (2022) Shared features and reciprocal complementation of the
1163 *Chlamydomonas* and *Arabidopsis* microbiota. *Nat Commun* 13, 406. 10.1038/s41467-
1164 022-28055-8
1165

1166 **FIGURE LEGENDS:**

1167 **Figure 1.** High resolution depictions of interactions between the alkalitolerant green microalga
1168 *Chlorella* sp. SLA-04 and associated microorganisms. Scanning helium ion microscopy image (a)
1169 and transmission electron microscopy images (b) and (c) depicting attachment of microorganisms
1170 to SLA-04 cells. Credit: Alice Dohnalkova and Shuttha Shutthanandan (Environmental Molecular
1171 Science Laboratory, Pacific Northwest National Laboratory) via a Facilities Integrating
1172 Collaborations for User Science (FICUS) grant. The scale bars for a, b and c represent 1 um. The
1173 images are meant as representative observations of algal-bacterial direct contact.

1174

1175 **Figure 2.** Aggregate of *Chlorella* sp. SLA-04 and associated phycosome from a xenic, high pH,
1176 high alkalinity laboratory culture. Chlorophyll autofluorescence is colored in red, DAPI (4',6-
1177 diamidino-2-phenylindole) (ThermoFisher) stained DNA in cyan, and Bodipy 505/515 (4,4-
1178 Difluoro-1,3,5,7-Tetramethyl-4-Bora-3a,4a-Diaza-s-Indacene) (ThermoFisher) stained lipids in
1179 yellow. The scale bar at bottom left represents 30 um. The image represents general observation
1180 of an algal-bacterial co-culture.

1181

1182 **Figure 3.** Mutualistic (left) and algicidal (right) interactions that occur in the phycosphere. A
1183 generic microalgal cell is portrayed to represent multiple species. Shading around microalgal
1184 cells represents gradients of metabolites that, through diffusion away from cells, comprise the
1185 phycosphere. Metabolite exchanges highlighted in this summary are ammonia (NH_4^+), iron (Fe),
1186 dissolved organic carbon (DOC), B-vitamins, indole-3-acetic acid (IAA), tryptophan (Trp),
1187 antibiotics, p-coumaric acid, enzymes, orfamide A, and roseobacticides.

1188

1189 **GLOSSARY:**

1190

1191 **Antagonism:** an association between organisms in which one benefits at the expense of
1192 the other

1193 **Axenic:** a culture that consists of a single microalgal or bacterial species and is free
1194 from contamination

1195 **Bioprocessing:** a process that uses living organisms or part of organisms to create useful
1196 products

1197 **Bottom-up design:** designing a community with a desired phenotype based on a sum of
1198 individual parts

1199 **Commensalism:** an association between organisms in which one organism benefits and the
1200 other neither benefits or is harmed

1201 **Consortia:** a group of organisms that work together to function at a higher level than
1202 they could individually

1203 **Direct air CO₂ capture:** capacity of alkaline systems to take advantage of enhanced
1204 dissolution of CO₂ into aqueous solution at high pH

1205 **High pH/high alkalinity:** cultivation conditions with pH > 10 and alkalinity > 50 mEq/L that
1206 are designed to mimic some of the most productive natural ecosystems on
1207 earth

1208 **Monoculture:** the cultivation of a single species, often resulting from active disclusion of
1209 other species

1210 **Mutualism:** an association between organisms in which both species benefit

1211 **Photoautotroph:** an organism that uses energy from light, namely to fix CO₂ in organic
1212 molecules via photosynthesis

1213 **Phycosome:** the microbial community, or microbiome, that is associated with algal
1214 cells

1215 **Phycosphere:** the region immediately surrounding algal cells that is comprised of
1216 chemical gradients that result from algal metabolism

1217 **Polyculture:** the simultaneous cultivation of two or more compatible species such as
1218 microalgae

1219 **Top-down design:** designing a community with a desired phenotype based upon the directed
1220 selection from more to less complex