CellPress

Trends in Biotechnology

Feature Review

Understanding phycosomal dynamics to improve industrial microalgae cultivation

Isaac R. Miller [1,2], Huyen Bui [2], Jessica B. Wood [1,2], Matthew W. Fields [1,2,3,4,*], and Robin Gerlach [1,2,4,5]

Algal–bacterial interactions are ubiquitous in both natural and industrial systems, and the characterization of these interactions has been reinvigorated by potential applications in biosystem productivity. Different growth conditions can be used for operational functions, such as the use of low-quality water or high pH/alkalinity, and the altered operating conditions likely constrain microbial community structure and function in unique ways. However, research is necessary to better understand whether consortia can be designed to improve the productivity, processing, and sustainability of industrial-scale cultivations through different controls that can constrain microbial interactions for maximal light-driven outputs. The review highlights current knowledge and gaps for relevant operating conditions, as well as suggestions for near-term and longer-term improvements for large-scale cultivation and polyculture engineering.

Highlights

The phycosome – the algal microbiome – can impact microalgal cultivation productivity and stability through metabolic interaction networks.

Polycultures can be designed based upon complementary traits and metabolic potential to maximize yields.

Stable and resilient consortia are a sustainable approach to contamination challenges compared to the traditional use of pesticides and extensive sterilizing methods.

High-pH/high-alkalinity microalgal cultivation can better control inputs and outputs via direct air CO_2 capture.

Less is known about the ecology of extreme and productive systems, with implications for long-term, repeated cultivations.

Lessons learned from natural and industrial systems with varying pH, alkalinity, temperature, and salinity can inform input and output control of algal growth systems.

Motivations and challenges for the microalgal industry

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

However, despite years of research and development, production still faces techno-economic challenges. One main challenge is that the typically used open ponds are subject to numerous biotic and abiotic stresses that include infestation with grazers and pathogens [6,7]. In addition, fluctuations in light, temperature, and nutrients threaten culture stability and consistent yields. Although microalgal **monocultures** (see Glossary) are well understood at the laboratory scale, significant perturbations are inherent to the open systems [3,8,9], and the use of closed bioreactors is possible but costly in terms of capital investment as well as light and CO_2 delivery.

Industrial scale microalgal cultures are usually seeded with a monoalgal or coalgal inoculum, and colonization by other microorganisms is considered to be contamination that needs to be managed. However, not unlike human and plant microbiome research, 'healthy' microbial diversity is being appreciated and studied to better understand its roles in ecosystem function, where algae, diatoms, and bacteria coexist to form **commensal** and symbiotic associations [10–16]. In addition, **consortia** consisting of more than one microalgal species can result in higher culture stability and productivity [17,18], likely as a result of niche space occupation towards maximal carrying capacity. Therefore, creating pond ecosystems with mutually supportive microalgal and microbial species may increase productivity and system resilience.

[1]Department of Microbiology and Cell Biology, Montana State University, Bozeman, MT, USA
[2]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA
[3]Department of Civil Engineering, Montana State University, Bozeman, MT, USA
[4]Energy Research Institute, Montana State University, Bozeman, MT, USA
[5]Department of Biological and Chemical Engineering, Bozeman, MT, USA

*Correspondence:
matthew.fields@montana.edu
(M.W. Fields).

© 2023 Elsevier Ltd. All rights reserved.

Check for updates

Downloaded for Sally Bauer (sbauer2@billingsclinic.org) at BILLINGS CLINIC from ClinicalKey.com by Elsevier on July 18, 2024. For personal use only. No other uses without permission. Copyright ©2024. Elsevier Inc. All rights reserved.

A basic tenet of community ecology is that uninhabited, open systems increase in complexity over time; therefore, monocultures will be invaded from neighboring environments until a stable state is reached that can be resilient to 'normal' changes [19,20]. Recent studies have provided evidence that **polycultures** have advantages over monocultures with respect to biomass productivity [21,22], stability [23], resource utilization [24], and resistance to invasive species (e.g., grazers) [25,26], and these findings coincide with observations for diverse, natural systems (e.g., ocean cyanobacteria) [27]. These studies also highlight potential tradeoffs during the cultivation phase and in the downstream processing steps (harvesting, extraction, conversion) [18], and there is a clear potential to select communities based upon maximizing pre- and post-processing for targeted products [28,29]. However, as noted by Lian and colleagues [30], studies focused on the distribution, structure, and function of algae-associated microbial communities continue to be needed.

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

Microalgae and natural systems

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

Unlike axenic laboratory cultures, natural aquatic habitats are diverse microbial communities that constantly exchange resources and respond to both abiotic and biotic fluctuations [15,37]. In a field study of a microalgal–bacterial community during a diatom-dominated bloom, Teeling and colleagues [38] identified almost 100 microalgal morphologies that co-occurred with bacterial clades. Bell and colleagues [22] surveyed community composition in large wastewater treatment lagoons over a 1 year sampling period and detected extremely diverse communities (e.g., 445 eukaryotic operational taxonomic units, OTUs) that included green microalgae and predatory protists. In another survey of wastewater, Hena and coworkers [39] observed signatures of 20 microalgal and diatom species in dairy farm wastewater. By comparison, a 4 year survey of the southern North Sea found that the overall species composition in plankton is balanced and remains consistent despite interannual variation [40]. It is likely that natural consortia have adapted to particular environments, fluctuations, and extremes, and insights into the role of each microalgal species in a given habitat could inform strategies to design stable and productive microalgal consortia for industrial cultivation in different locales (e.g., dry/sunny versus humid/

Glossary

Antagonism: an association between organisms in which one benefits at the expense of the other.
Axenic: a culture that consists of a single microalgal or bacterial species and is free from contamination.
Bottom-up design: designing a community with a desired phenotype based on the sum of the individual parts.
Commensalism: an association between organisms in which one organism benefits and the other neither benefits nor is harmed.
Consortium: a group of organisms that work together to function at a higher level than they could individually.
Direct air CO_2 capture: the capacity of alkaline systems to take advantage of enhanced dissolution of CO_2 into aqueous solution at high pH.
High pH/high alkalinity: cultivation conditions with pH >10 and alkalinity >50 mEq/l that are designed to mimic some of the most productive natural ecosystems on Earth.
Monoculture: the cultivation of a single species often resulting from active exclusion of other species.
Mutualism: an association between organisms in which both species benefit.
Photoautotroph: an organism that uses energy from light to fix CO_2 in organic molecules via photosynthesis.
Phycosome: the microbial community, or microbiome, that is associated with algal cells.
Phycosphere: the region immediately surrounding algal cells that is composed of chemical gradients that result from algal metabolism.
Polyculture: the simultaneous cultivation of two or more compatible species such as microalgae.
Top-down design: designing a community with a desired phenotype based upon directed selection from more to less complex.

Downloaded for Sally Bauer (sbauer2@billingsclinic.org) at BILLINGS CLINIC from ClinicalKey.com by Elsevier on July 18, 2024. For personal use only. No other uses without permission. Copyright ©2024. Elsevier Inc. All rights reserved.

cloudy). Microalgae in natural systems are typically observed in diverse communities, and understanding relationships of microalgal species with each other and with non-microalgal partners could be useful for stable industrial production.

Extreme environments can provide novel biochemical capacity relevant to industrial processes. pH is a dominant factor, and many studies of microbial diversity in extremes have focused on low-pH and high-temperature environments (e.g., hot springs). The ecology of alkaline systems, both natural and artificial, is poorly understood, although it has been observed that communities in alkaline environments are phylogenetically distinct from circumneutral habitats [41,42]. Most phototrophic systems above ~45°C become dominated by cyanobacteria up to 75°C, whereas microalgae grow better between 10°C and 40°C [43] depending upon the environment, with the upper limit reported to be ~60°C [44]. Alkaline lake systems have some of the highest primary productivity rates on the planet [45], and diverse and unique microalgae have been identified from alkaline systems [46–48]. However, surprisingly, few studies have systematically catalogued the extent of potential diversity and function of microalgae from temperate (20–40°C) alkaline systems. We hypothesize that, given the high primary productivity rates and tolerance to high pH, a microalgal cultivation system at alkaline/high pH has the potential to achieve controllable inputs/outputs (i.e., stable productivity with minimized inputs) via **direct air CO_2 capture**, and future work should continue to focus on these and other unique conditions that are relevant to CO_2 processing.

Microalgae in industrial systems

Microalgae require water, sunlight, and nutrients (e.g., N, P, Fe) to grow via **photoautotrophy** (i.e., light-driven CO_2 utilization), and their low-nutrient and low-quality water requirements coupled with rapid growth rates and high biomass yields make microalgal systems a target for biotechnology applications, including biofuel and bioproduct generation [3]. Although some microalgae can grow heterotrophically or mixotrophically with higher biomass yields [49,50], the use of additional carbon can increase overall energy demands as well as increase contamination with heterotrophic microorganisms that compete for nitrogen and phosphorus [51,52]. Therefore, the focus of this review is on photoautotrophic growth that uses CO_2 as the carbon source and sunlight as the energy source.

Given the open-air nature of large-scale microalgal cultivation under photoautotrophic conditions, maintaining axenic cultures presents many challenges. Invasion or 'contamination' is inevitable, resulting in a complex ecosystem of bacteria, zooplankton, and microalgae as well as fungi and viruses [53–55]. Much research has focused on controlling invading agents with strong chemicals and pesticides, as well as on extensive sterilizing methods such as filtration. A more practical approach may be to select for less susceptible or invasion-resistant strains [56], well-structured polycultures that are tolerant to invaders, and/or cultivation conditions that limit invasion through selection (e.g., **high pH/high alkalinity**, salinity). Moreover, naturally high-pH and high-alkalinity environments (e.g., soda lakes) have some of the highest primary productivities on Earth [57], largely attributed to the increased availability of inorganic carbon in these environments; however, more work is needed that targets these environments. In recent years, high-pH/high-alkalinity conditions have been used to increase microalgal biomass and lipid yields in shorter time-periods, and these extreme conditions appear to reduce invasion by harmful microorganisms and grazers [45,58–60]. Although it is feasible to select and promote stable microalgae–bacterial consortia that can thrive at extremes [45,61], little is known about the possible metabolic and/or ecological interactions specific to dynamic extremes (e.g., pH changes across the diel cycle) and about their potential implications for long-term, repeated cultivations.

Downloaded for Sally Bauer (sbauer2@billingsclinic.org) at BILLINGS CLINIC from ClinicalKey.com by Elsevier on July 18, 2024. For personal use only. No other uses without permission. Copyright ©2024. Elsevier Inc. All rights reserved.

Natural **phycosome** communities are often more diverse and stable than those observed *ex situ* or in industrial settings. The selective forces acting on wild and industrial systems are likely similar, but the extent to which factors can influence structure and function is likely different. For example, marine microalgal phycosome diversity correlates with latitudinal temperature gradients [62], but little is known about whether similar temperature effects could play a role in industrial cultures that can experience large diurnal temperature shifts when maximizing sunlight exposure. In addition, a differentiation between free-living and directly attached bacteria has been observed in natural systems, whereas the distinction is often less clear in *ex situ* cultures [10]. This is likely due to the homogeneity of cultures, relative to natural environments, that is achieved under typical mixing regimes. Industry practices of mixing and nutrient delivery to maximize algal production ultimately minimize ecological niche partitioning and spatial differentiation that are likely important contributors to microbial roles. Influential environmental parameters such as photosynthetically active radiation (PAR), pH, and pO_2 that might change under different industrial growth schemes likely impact both the microalgae and the associated microorganisms. Hence, research will be necessary to ascertain the mechanistic roles that significantly impact algal culture productivity or stability under relevant industrial conditions.

The phycosphere: the microalgal–bacterial interface

The term **phycosphere** refers to the immediate region surrounding a microalgal cell that is enriched in organic matter (i.e., photosynthate) [63], and the phycosphere is analogous to the soil rhizosphere that refers to the thin fluid layer (diffusive boundary layer) that surrounds small aquatic microalgae (<100 μm). The size of the phycosphere is determined by the size and shape of the microalgal cell, growth, exudation rates, motility, and the level of mixing in the bulk aqueous phase, and smaller, slower-growing cells generally have thinner phycospheres [36,64]. The phycosphere includes microorganisms associated with the microalgal cell surface and/or algal aggregates [10], and includes both direct and indirect metabolic interactions within the effective diffusive boundary layer. We propose the term 'phycosome', building from the general concept of microbiome [65], as the microbial community occupying a defined habitat (i.e., microalgal growth system) with distinct physiochemical system-level properties (metabolic potential/function), irrespective of direct attachment or proximity within the diffusive boundary layer [66]. In extreme cases, such as in the interaction between Chlorophyta and Rickettsiales, bacteria can develop inside microalgal cells [67]. In natural habitats, there are examples of the following scenarios: (i) a clear partitioning of attached and free-living bacterial taxa [68], and (ii) a large overlap of bacterial taxa in the attached and free-living fractions [69]. Interestingly, Eigemann and colleagues [70] reported a shift from scenario (i) to scenario (ii) when culturing natural samples in laboratory conditions, and the results suggested that the laboratory conditions that promote high microalgal biomass may also promote an expansion of attached bacteria into the free-living fraction.

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

Downloaded for Sally Bauer (sbauer2@billingsclinic.org) at BILLINGS CLINIC from ClinicalKey.com by Elsevier on July 18, 2024. For personal use only. No other uses without permission. Copyright ©2024. Elsevier Inc. All rights reserved.

Once within the phycosphere, bacteria may maintain an association via attachment to microalgal cell surface [78], microalgal sheaths [32], or to the polymeric matrix [79], all of which could be potential sources of carbon (Figure 1A–C). In some cases bacteria colonize transparent exopolymer particles (TEPs), which are made of polysaccharides released by diatoms. Some bacterial strains can modulate diatom TEP production, thereby promoting the aggregation of diatoms and promoting bacteria-diatom associations [80]. Bacteria themselves can also release exopolysaccharides in response to the presence of microalgae, which might also facilitate interactions [81].

Microalgal–bacterial interactions

Microalgal–microbial associations may provide selective advantages for microalgal health [82]; conversely, axenic cultures of microalgae may be unstable and prone to perturbation [8]. Microalgae can release up to 50% of fixed carbon into the surrounding environment as excreted organic compounds (e.g., carbohydrates) that are thought to directly and indirectly impact on associated ecological partners, local photoinhibition, and/or aggregation [32,83,84]. Microalgal–bacterial interactions are considered to be species- or even strain-specific, meaning that the phylogenetic composition of the bacterial community can depend on the microalgal host [68,85–87]. However, the level of specificity has been suggested to differ between microalgal genera [88], but only a limited number of algal phycosomes have been studied for different genera. The establishment of long-term specific interactions is probably determined by metabolic dependencies as well as by long-term coevolution [89–91]. Less is known about the drivers of short-term interactions (i.e., diel cycle); however, preliminary studies have been conducted in freshwater environments (S. Papadopoulou, MS Thesis, Uppsala University, 2021). The species-specific nature of microalgal–bacterial interactions could be due to distinct exudates produced by a given microalgal species which provide substrates that differentially attract bacteria based on their metabolic potential [86,91]. Consistently, natural and industrial cultures containing multiple microalgal species have more diverse bacterial communities than monoalgal cultures [10]. When synthetic phycosome communities were established using a mixed bacterial starting community with diatom and dinoflagellate host cells, direct relationships between host metabolism and community succession were uncovered based upon host-produced metabolites [92]. These findings suggest that specific host processes could select for specific phycosphere/phycosome compositions.

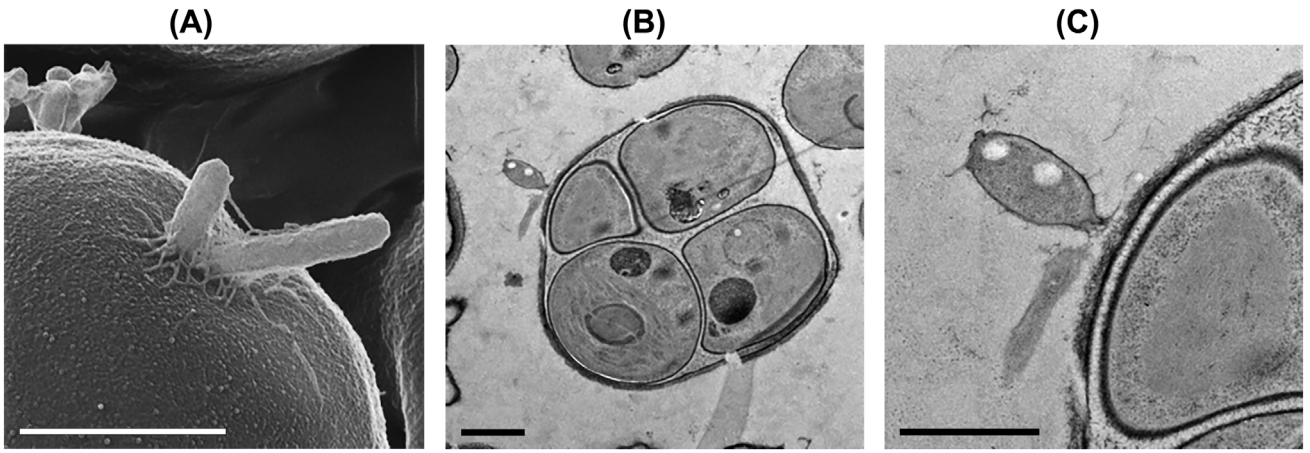

Figure 1. High-resolution depictions of interactions between the alkali-tolerant green microalga *Chlorella* sp. SLA-04 and associated microorganisms. Scanning helium ion microscopy image (A) and transmission electron microscopy images (B,C) depicting attachment of microorganisms to SLA-04 cells. Credit: Alice Dohnalkova and Shuttha Shutthanandan (Environmental Molecular Science Laboratory, Pacific Northwest National Laboratory) via a Facilities Integrating Collaborations for User Science (FICUS) grant. The scale bars in A–C represent 1 μm. The images are representative observations of algal–bacterial direct contact.

Downloaded for Sally Bauer (sbauer2@billingsclinic.org) at BILLINGS CLINIC from ClinicalKey.com by Elsevier on July 18, 2024. For personal use only. No other uses without permission. Copyright ©2024. Elsevier Inc. All rights reserved.

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

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

Aggregation in planktonic and biofilm cultures may provide an opportunity to investigate spatial relationships in microalgae–phycosome interaction webs (Figure 2). In addition to algal aggregates and/or the algal cell as the 'surface', microalgae can grow attached to physical surfaces ('macro'-biofilm) in natural and artificial environments, and biofilm growth is an important characteristic to consider in the design of cultivation schemes for larger-scale production. Recent comparisons have shown that phototrophic biofilm growth can help to overcome challenges with the delivery, mixing, and harvesting of algae [93], and that productive, cyanobacteria-dominated biofilms can have diverse microbial communities [94]. Mixed-species and mixed-domain biofilms can persist over a wide range of conditions given the flexible nature of microalgal metabolism and the active interplay between heterotrophs and phototrophs [94], but more work will be necessary to elucidate the occurrence, distribution, and function of the microbial populations specifically associated with algal biofilms.

The roles of bacteria in microalgal cultures

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

Competitive or antagonistic interactions

In the relationship with microalgae, bacteria can be benefactors that can utilize dissolved organic matter (DOM) release by microalgae and compete for macronutrients (e.g., N, P, Fe), resulting in decreased microalgal productivity [102]. In addition to utilizing microalgal exudates, some bacteria have adapted an algicidal lifestyle in which bacteria actively attack microalgal cells to obtain nutrients [103]. Although algicidal bacteria need to be avoided in microalgal cultivations, they could potentially be used for controlling harmful microalgal blooms [104,105]. Directly related to biofuel production, Lenneman and colleagues [106] demonstrated that two algicidal bacteria, *Pseudomonas*

Downloaded for Sally Bauer (sbauer2@billingsclinic.org) at BILLINGS CLINIC from ClinicalKey.com by Elsevier on July 18, 2024. For personal use only. No other uses without permission. Copyright ©2024. Elsevier Inc. All rights reserved.

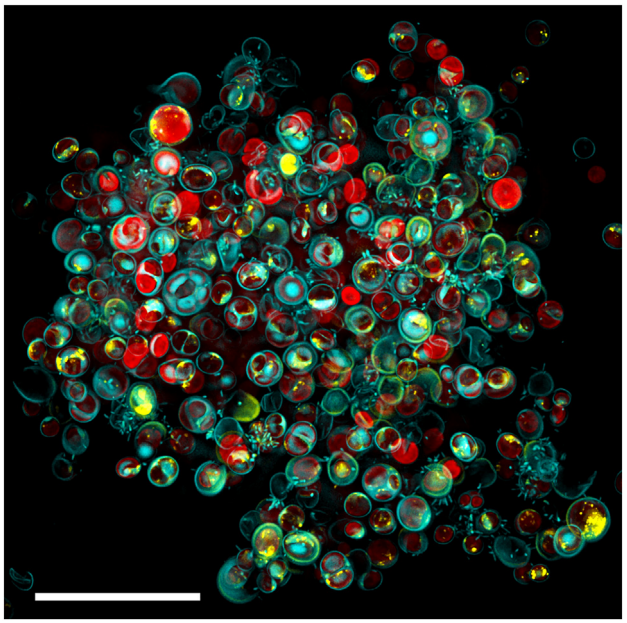

Figure 2. An aggregate of *Chlorella* sp. SLA-04 and its associated phycosome from a xenic, high-pH, high-alkalinity laboratory culture. Chlorophyll autofluorescence is colored in red, DAPI (4′,6-diamidino-2-phenylindole)-stained DNA is in cyan, and BODIPY 505/515 (4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene)-stained lipids are in yellow. The scale bar at bottom left represents 30 μm. The image represents a general observation of an algal–bacterial coculture.

pseudoalcaligenes AD6 and *Aeromonas hydrophila* AD9, induced microalgal cell lysis leading to at least a sixfold improvement of lipid extraction from the microalgae *Neochloris oleoabundans* and *Dunaliella tertiolecta* [107]. Bacterial algicides and the potential modes of action were reviewed extensively by Meyer and colleagues [108].

In the same way as bacteria have evolved to prey upon microalgae, microalgae have evolved strategies for self-defense. Although released microalgal DOM serves as a food source for bacteria, larger

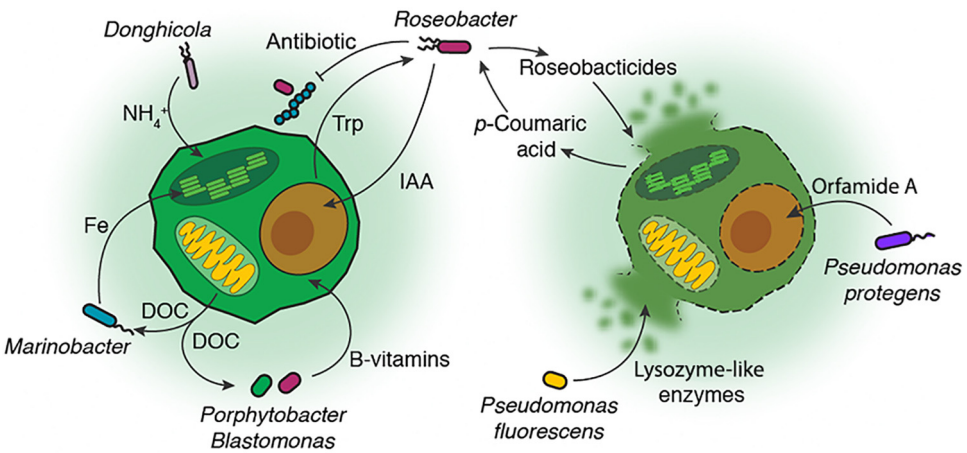

Figure 3. Mutualistic (left) and algicidal (right) interactions that occur in the phycosphere. A generic microalgal cell is portrayed to represent multiple species. The shading around the microalgal cells represents gradients of metabolites that, through diffusion away from cells, comprise the phycosphere.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

Downloaded for Sally Bauer (sbauer2@billingsclinic.org) at BILLINGS CLINIC from ClinicalKey.com by Elsevier on July 18, 2024. For personal use only. No other uses without permission. Copyright ©2024. Elsevier Inc. All rights reserved.

exopolymers may also form a 'mucus treadmill' that protects microalgal cells from excessive bacterial colonization [31,109]. Microalgae can also reduce bacterial growth and colonization by interfering with bacterial quorum-sensing systems [110,111]. Diatoms can secrete fatty acids and esters [112] that have been shown to act as signaling molecules to induce bacterial biofilm dispersal [113,114] or provide protection by serving as antibacterial compounds [115]. Polyunsaturated aldehydes (PUAs) can suppress growth of some marine bacteria, but some bacteria in diatom phycospheres are resistant to PUAs, indicative of a potential selection mechanism [116]. In ways analogous to the role of the human gut microbiome [117], the presence of a diverse phycosphere/phycosome may help to reduce the occurrence/probability of **antagonistic** interactions (i.e., niche exclusion).

Beneficial or mutualistic interactions

Mutualistic relationships are common between microalgae and bacteria, and bacteria can provide essential nutrients and exert growth effects as strong as those of light and temperature [118]. Capturing and maintaining these relationships in engineered settings is crucial for highly productive polycultures. There is ample evidence that bacteria can positively influence the productivity of microalgal systems via both direct and indirect routes [21,22,119,120]; similar to other host–microbiome interactions, these routes can include nutrient acquisition, growth effectors, and protection.

Nutrient acquisition

In photo-aquatic ecosystems, as bacteria process carbon fixed by autotrophs, CO_2 is produced and inorganic nutrients are recycled (e.g., N, P, Fe) [118]. Although these nutrients directly benefit the bacteria, the occurrence of bacterial remineralization within the phycosphere/phycosome may also provide microalgal cells with elevated nutrient concentrations [37]. Microalgae require iron for photosystem biosynthesis and function, and many bacteria produce siderophores for iron acquisition. For example, *Marinobacter* spp. can produce siderophores that contribute to iron chelation and iron uptake by microalgae [121,122]. In addition, bacteria such as *Roseobacter* spp. have the potential to regenerate iron hemoproteins that are released by lyzed and decaying microalgal cells [123].

Bacteria also play critical roles in microalgal nitrogen uptake. Diazotrophic organisms such as cyanobacteria and other bacterial genera (*Rhizobium*, *Mesorhizobium*, and *Azospirillum)* can fix N_2 into bioavailable forms [12]. Nitrogen fixation may be a more dominant process in the phycosome of microalgal cultures at times when oxygen levels are low (e.g., during dark periods), but more work will be necessary to ascertain the temporal and spatial distribution of nitrogen-cycle functions in algal systems. Indeed, algal systems have been associated with nitrous oxide production [124], and therefore other functional groups in addition to nitrogen fixation could be important for not only nitrogen allocation but also environmental impacts (e.g., mitigation of N_2O emissions).

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

Downloaded for Sally Bauer (sbauer2@billingsclinic.org) at BILLINGS CLINIC from ClinicalKey.com by Elsevier on July 18, 2024. For personal use only. No other uses without permission. Copyright ©2024. Elsevier Inc. All rights reserved.

Provision of essential vitamins and growth hormones

Stimulation of microalgal growth by bacteria can also occur via the production of vitamins [11,130–133]. Many microalgae are auxotrophic for essential vitamins such as B_{12}, thiamine, and biotin [134–136], and these compounds are thought to be exchanged through mutualistic interactions with vitamin-producing microorganisms [11,137,138]. Using metagenomic and metatranscriptomic approaches, Krohn-Molt and coworkers [87] provided strong evidence that α-Proteobacteria (e.g., *Porphytobacter* and *Blastomonas* spp.) are the main B-vitamin suppliers in *Chlorella* and *Scenedesmus* microbiomes, whereas Sphingobacteria and Bacteroidetes species exhibited high expression of B_{12} biosynthetic genes in culture with *Micrasterias* [87].

Bacteria can also impact microalgal growth via the production and release of growth hormones such as indole-3-acetic acid (IAA) [31,133,139,140]. Although IAA has no clearly documented metabolic role in many bacteria, some bacteria can produce IAA from tryptophan. Although the evidence for IAA involvement in microalgal physiology and development remains limited, IAA has been thought to be the driver for several intimate microalgal–bacterial interactions [30]. The coccolithophore *Emiliania huxleyi* exudes the amino acid tryptophan which is utilized by the bacterium *Phaeobacter inhibens* to produce IAA [141]. A similar interaction was reported for a *Sulfitobacter* spp. and *Pseudo-nitzschia multiseries* symbiont [128]. Interestingly, IAA addition to the culture medium resulted in reduced growth of the diatom compared to IAA released by bacteria. One explanation is that bacteria-released IAA achieved higher local concentration in the phycosphere than the bulk concentration resulting from IAA addition to the medium [128]. In a recent comparison between plant growth-promoting and non-plant growth-promoting bacteria, *Escherichia coli* promoted *Chlorella* sp. growth as much as *Azospirillum brasilense* [142], and these results demonstrated the challenge in differentiating between the specific and general effects of microbiomes. However, exogenous addition of the phytohormone, auxin, increased biomass and lipid content in *Scenedesmus* SDEC-8 and *Chlorella sorokiniana* [143]. Because it is difficult to demonstrate definitive cause-and-effect relationships, more research will be necessary to ascertain the potential for phycohormone effects at the industrial scale.

Protection

Bacteria can provide a degree of protection to microalgae beyond simple niche exclusion. Makridis and colleagues [144] tracked bacterial communities associated with green microalgae grown for aquafeed, and observed that the presence of particular populations hindered the growth of pathogenic bacteria. For example, *Roseobacter* spp. produce tropodithietic acid (TDA), a dual sulfur-tropolone compound that inhibits a broad range of marine pathogens and may help to prevent harmful bacteria from colonizing the microalgal phycosphere [145]. Bacteria have also been found to infect and kill microalgal predators, which could result in increased microalgal growth. In particular, the bacterium *Pasteuria ramosa* is a parasite of the crustacean *Daphnia magna* [146], and *Holospora undulata* can infect the protozoan *Paramecium caudatum* [147]. In addition, many marine bacteria express antagonistic activity against other bacteria via antibiotics [148]. Finally, phycosphere bacteria can relieve microalgae from oxidative stress induced by reactive oxygen species. Epiphytic bacteria associated with the diatom *Amphiprora kufferathii* were shown to express catalase activity that reduced hydrogen peroxide levels in the local environment [149].

Salt stress

In addition to nutrients and CO_2, water becomes a major challenge for sustainable, industrial scale algal cultivation [3]. Therefore, the replacement of freshwater by low-quality water (e.g., marine water, wastewater) can help to improve both environmental and financial burdens on industrial production. There are numerous examples of halotolerant microalgae that can physiologically adjust to

Downloaded for Sally Bauer (sbauer2@billingsclinic.org) at BILLINGS CLINIC from ClinicalKey.com by Elsevier on July 18, 2024. For personal use only. No other uses without permission. Copyright ©2024. Elsevier Inc. All rights reserved.

environments with osmotic stresses introduced by saline waters via shifted cell metabolism, osmoprotectants, and/or altered ion exchange [150]. For example, previous work has shown that green algae such as *Desmodesmus*, *Chlorella*, *Dunaliella*, *Scenedesmus*, and *Picochlorum* spp. can tolerate elevated salinity and still produce storage compounds (lipids/starches) [150–153], and more recent work has demonstrated the growth of green algae in actual seawater/sea salts, including adaptive evolution for storage compound production in more saline environments [150,154,155]. Moreover, Church and colleagues [156] showed that the salt concentration had more of an effect than the salt type on *Chlorella vulgaris* in terms of both growth and storage compounds.

In the context of salinity stress, microalgae can produce osmoprotectants, and dimethylsulfoniopropionate is considered to be the most abundant and important osmotically active metabolite in phytoplankton, although microalgae can use both nitrogen- and sulfur-containing osmolytes depending on nitrogen levels in the local environment [157,158]. Recently, microalgae species have been shown to utilize either ectoine or proline as alternative osmoprotectants [158,159]. Although saline water provides an alternative water source as well as limiting the growth of invasive species during open cultivation, the impact of resource allocation (carbon and/or nitrogen) to osmoprotectants must be considered when aiming to maximize algal biomass and lipids. However, an additional role for the phycosome could be in the production of osmoprotectants, and recent work has shown microalgal use of bacterially produced ectoine [158]. That said, few studies have tracked the structure and function of microbial communities in saline/salt-stressed outdoor algal cultivations, and future work will be necessary to delineate the potential phycosomal roles in salinity tolerance, particularly in high-alkalinity conditions (i.e., elevated sodium). Interestingly, when scleractinian corals (and the algal symbiont) were exposed to salinity stress, a functional role of osmolyte production was associated with microbiome restructuring [160].

Temperature stress

Temperature stress for photoautotrophic growth can include both climatic- (long-term, seasonal) and weather-related (short-term) changes that must be considered for industrial scale, algal growth facilities. Diurnal temperature swings (in a given day and across seasons) are inherent to most geographic regions selected for maximizing algal growth (i.e., warm, sunny regions), and these can generate extremes in temperature highs and/or lows. Cho and coworkers showed that changes in water temperature over a seasonal cycle affected microalgal biomass productivity by ~10-fold [161], and small temperature changes of 5–10°C can impact biomass allocation (e.g., [162]). Microalgae can modulate phospholipid membrane content in response to temperature changes, and lower temperatures have been shown to increase the production of industrially relevant metabolites such as eicosapentaenoic acid (EPA) and polyunsaturated fatty acids (PUFAs) [163].

Research investigating the comparison between axenic and xenic microalgal cultures and temperature stress is limited, but results from a previous study indicated that a native or non-native microalgal microbiome played a positive role in the microalgal response to heat stress through enhanced chlorophyll fluorescence [164]. Temperature was a dominant factor that affected microbial abundance in xenic microalgal cultures, and the predominant microbial species were related to various seasonal temperature changes [161]. Large, sudden increases in temperatures can promote algal cell death [165], and daily temperature fluxes in outdoor microalgal systems were associated with increased phycosome species richness [166]. However, it is difficult to delineate direct and indirect temperature effects on the respective algal and microbial populations, and it remains unclear how the changes are interrelated; therefore, more work will be necessary

Downloaded for Sally Bauer (sbauer2@billingsclinic.org) at BILLINGS CLINIC from ClinicalKey.com by Elsevier on July 18, 2024. For personal use only. No other uses without permission. Copyright ©2024. Elsevier Inc. All rights reserved.

to better understand the potential role of phycosphere/phycosome in algal growth system resilience to both diurnal temperature changes as well as to different seasonal-regional temperatures.

Learning from nature: ecological engineering for the laboratory and industry

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

However, studies on inter-organismal interactions in engineered microalgal cultivation systems are still relatively rare, particularly at a larger scale, and underscores our nascent understanding of metabolic cooperation within these microbial communities for stable biofuel production [128]. One contributing factor is that typical laboratory practices for enriching and isolating microalgal strains are not optimal for maintaining the associated phycosome/phycosphere. Because isolation protocols generally follow multiple rounds of dilution and enrichment with and without antibiotics, many associated bacteria are likely eliminated. Prolonged axenic cultivation of microalgal species in industrial scale systems is not practical, and the absence of bacteria can negatively influence microalgal physiology and growth even in supportive media. Engineering approaches should therefore be pursued that promote beneficial interactions and minimize detrimental interactions. We use the term 'engineering' here to refer to promoting and maintaining mixed consortia of 'natural' algae and other microorganisms (Eukarya, Bacteria, and Archaea) with desired outputs rather than editing at the genome level.

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

The key issues in addressing gaps in knowledge between natural and engineered consortia in these settings concern (i) whether enriched cultures can capture the necessary diversity of natural microalgal phycospheres/phycosomes, (ii) whether polycultures in managed, open ponds can have similar benefits as those reported in natural habitats, and (iii) whether is it possible to reconstitute and/or design microbial consortia with predictable and controllable outcomes that can be consistently maintained. Although the productivity of natural ecosystems is often measured in terms of biomass production [169], the productivity of a microalgal industrial cultivation is likely assessed by biomass quantity, compositional content (e.g., lipids), stability/robustness, net environmental impact (e.g., water, nutrient, CO_2 requirements), and the overall costs [17]. Therefore, the phycosome and potential impacts should be considered during life-cycle and techno-economic analyses [3].

Use of algal polycultures to improve productivity and stability

In many environments, microorganisms form interactive consortia in which they are more likely to interact with each other than with outside species [170,171]. Termed 'small-world' networks, these consortia are common in natural and man-made systems in which microscale interactions impact overall productivity [172]. In addition, based on modeling of clustered food webs

Downloaded for Sally Bauer (sbauer2@billingsclinic.org) at BILLINGS CLINIC from ClinicalKey.com by Elsevier on July 18, 2024. For personal use only. No other uses without permission. Copyright ©2024. Elsevier Inc. All rights reserved.

[173,174], phycosphere communities can display increased stability compared to randomly assembled food webs and may display increased diversity because extinction rates are more gradual. Sinha and Sinha [174] showed that relationships between species can be independent of both the initial size and connectivity of the network, and that the number of interactive species is a fundamental property of network structure irrespective of biotic or abiotic conditions. The results also suggest that species that interact with too many other species can destabilize network persistence [175].

Two mechanisms are believed to drive diversity–productivity relationships – the selection effect and the complementarity effect [24,28,176]. In the selection effect, the more species in a consortium, the higher the chance of having a species with a specific function. However, simply increasing the number of species does not always lead to increases in yield and stability if species are in the same functional group [18]. To maximize the potential for improving yield, polycultures could be designed based on specific traits/metabolic potential and the functional complementarity between species. Indeed, metabolic dependencies, interactions, and exchanges are being increasingly suggested as major drivers of community structure and function in different systems. Metabolic modeling of >800 subcommunities with known species composition indicated that communities with high phylogenetic diversity tend to consist of species with a low degree of metabolic overlap [177]. These models emphasized metabolic dependencies as a key biotic force that determines microbial communities in nature. Communities with high interaction potential among members are more likely to benefit from complementary biosynthetic capacities and require fewer resources [178]. Therefore, metabolite exchange could be a mechanism that stabilizes phycosphere/phycosome interactions, and communities with metabolic synergy could therefore thrive in nutritionally poor habitats [179]. This principle can guide the design of polycultures through ecological engineering to maximize metabolic capacity and achieve target biomass composition in industrial microalgal cultivation, where the main aim is to maximize outputs (biomass, lipid content) using minimal inputs (e.g., nitrogen, phosphorus, low-quality water). To achieve this goal, it is necessary to identify the mechanisms behind diversity–productivity relationships so that design and control can be attempted.

Choosing multiple microalgae strains as 'core' biomass producers

In the context of managed cultivation, consortia of microalgae with different traits might be more tolerant to changing environmental conditions (light, temperature) and more resistant to invaders [25]. When mono- and polyalgal cultures were evaluated, polycultures exhibited more stable production through time, higher biocrude yields over time, and were more resistant to invasion than monocultures [21,26]. The studies indicated that designing consortia requires characterization and selection of strains based on ecological principles to promote functional diversity [21,26]. A previous study showed that consortia of multiple microalgae resulted in higher biomass production than those of monoalgal cultures when cultivated in wastewater [39]. Interestingly, although four 'standard' UTEX strains (https://utex.org/) were included in the screen for the best consortia, the optimal consortium contained only native strains isolated from the wastewater sources used for cultivation. These native strains may have developed an optimal interaction network with one another and with other indigenous microorganisms, as well as with the local environment. Therefore, identifying and promoting the natural relationships that are characteristic of a given environment (e.g., water or nutrient source) may be an effective strategy for designing stable and productive microalgal consortia.

The positive diversity–productivity relationships could be explained by the efficient use of nutrients and light. Microalgae have different light preferences (wavelength, intensity), and microalgal consortia that contained species with non-overlapping optimal wavelengths had higher lipid content

Downloaded for Sally Bauer (sbauer2@billingsclinic.org) at BILLINGS CLINIC from ClinicalKey.com by Elsevier on July 18, 2024. For personal use only. No other uses without permission. Copyright ©2024. Elsevier Inc. All rights reserved.

and PAR absorbance than polycultures with overlapping light use [24]. In a study on algal nitrogen uptake, algal species differed significantly in their capacity to take up ammonium, urea, and nitrate, and as a result cocultures that differed in nitrogen preferences showed greater complementarity and higher productivity than monocultures [176]. In addition, mixed microalgal communities were also shown to remove inorganic nutrients more rapidly than monoalgal cultures and exhibited increased growth rates [180].

Bacterial consortia as probiotic 'amendments'

Inoculating microalgal cultures with bacterial communities that confer health benefits could be used as a form of phycosome/phycosphere engineering [31]. In photoreactor systems, microalgae and the accompanying bacterial flora were strongly positively correlated [120]. In a separate study, the growth of *Navicula veneta* was positively affected by *Halomonas* NC1, and diatom cell levels were 65% less without the bacterium [181]. When *Chlorella vulgaris* was grown in the presence of different bacteria, all important parameters for biofuel production were higher than those of the corresponding axenic cultures [182]. More recently, Toyama [183] cultured each of three microalgal species *Chlamydomonas reinhardtii*, *Chlorella vulgaris*, and *Euglena gracilis* in the presence and absence of indigenous bacteria in wastewater effluent, and for all three species xenic cultures resulted in higher biomass yields (1.5–2.8-fold) compared to the respective axenic cultures.

The improved biomass production in polyculture may be explained in part by the higher rate of nutrient assimilation and uptake by both microalgal and bacterial members. For example, *P. tricornutum* cells in the presence of bacteria fixed 64% more carbon than axenic cells, whereas bacterial cells that attached to microalgal cells consumed more microalgae-fixed carbon than unattached bacteria [31]. In another study assessing the symbiotic relationship between cyanobacteria and diatoms (e.g., *Climacodium* spp.) in bulk seawater, cyanobacterial cells that attached to diatom cells showed higher nitrogen-fixation rates (171–420-fold) compared to rates estimated for free-living cells, and the majority of the fixed nitrogen was transferred to the diatom [12]. Ortiz-Marquez [184] eliminated the need for providing inorganic nitrogen directly to a microalgal culture by adding *Azotobacter vinelandii*, a nitrogen-fixing bacterium that had been genetically engineered to secrete ammonium into the growth medium. In a recent study, *Janthinobacter* protected *Microchloropsis* from rotifer grazing pressure for short periods of time in outdoor cultures [185]. Although these studies demonstrate both direct and indirect, positive impacts of added bacteria, the explicit use in promoting microalgal growth for biofuel production is still limited owing to the knowledge gaps in interactions of bacteria with microalgae hosts, the potential tradeoff between yield and overall culture health, and the challenges of maintaining stable healthy consortia for different microalgae and/or polycultures. Similarly to the context of human gut microbiomes and person-to-person variability, much more work will be necessary to discern and define 'healthy' phycosomes for different microalgal species under different growth conditions. Some benefits are easier to track than others; for example, an aggregation phenotype could help with biomass dewatering and harvesting [186,187].

Toward designing microalgal consortia with controllable outputs

The enrichment and characterization of microalgal consortia from extreme natural habitats could provide 'simplified' communities with higher productivity and consistent stability. Robust, resilient, adaptable, and productive communities have been established via simple enrichment of native consortia [10,31], or by the assembly of novel synthetic consortia [26,188,189]. Habitats experiencing extreme environmental conditions hold potential for strains with unique traits, for example alkaline systems, that could contribute to functional parameters (i.e., CO_2 delivery).

Downloaded for Sally Bauer (sbauer2@billingsclinic.org) at BILLINGS CLINIC from ClinicalKey.com by Elsevier on July 18, 2024. For personal use only. No other uses without permission. Copyright ©2024. Elsevier Inc. All rights reserved.

Alkaline aquatic systems have been shown to be among the most productive natural ecosystems in the world [57,94], and it is hypothesized that distinct but very well-developed metabolic interactions are at least partially responsible for these high productivities. Microalgae of diverse taxa including *Scenedesmus*, *Navicula*, *Chlorella*, and *Neospongiococcum* spp. can thrive in high-pH environments, and they are valuable resources for cultivation with atmospheric CO_2 [47,59] (A. Vadlamani, PhD Thesis, University of Toledo, 2016; K. Moll, PhD Thesis, Montana State University, 2021). For example, *Chlorella sorokiniana* SLA-04 achieved high biomass productivities (>20g/m^2/day) in open raceway ponds under high pH/high alkalinity without a culture crash over a 2 year period [60,190]. Given the adaptive specialization of such organisms, manipulating cultivation conditions may be a useful approach for selecting stable, beneficial consortia under desired conditions.

Consortium design will also benefit from a thorough understanding of the community composition and metabolic interactions between members, which is being facilitated by next-generation sequencing as well as next-generation physiology and imaging technology [191]. Metagenomic sequencing, for instance, will allow the metabolic potential of the community to be estimated, and can predict possible metabolic relationships between community members. Advanced staining and imaging techniques may make it possible to elucidate how microalgal–bacterial interactions affect carbon, nitrogen, phosphorus, and energy flow for maximum productivity. At the gene expression level, meta-transcriptomic analyses combined with BONCAT (biorthogonal non-canonical amino acid tagging), isotope-specific Raman confocal microspectroscopy, and metabolite analysis can reveal how phycosome interactions influence microalgal physiology, and vice versa, during both short- and long-term cultivations. These omic, chemical, and imaging approaches will shed light on the temporal and spatial dynamics to inform consortia design.

Furthermore, consortia design can be assisted by mathematical modeling, including population-based modeling for predicting interspecies dynamics [192] and metabolic network modeling to predict energy and material flows in a community [193,194]. Ultimately, the performance of a consortium needs to be assessed at the industrial scale at the point of production. Although studies so far suggest that engineering microalgal consortia could improve the productivity and stability of large-scale cultivation, extensive life-cycle and techno-economic assessments (LCAs and TEAs) will be necessary to determine whether these improvements result in more sustainable operation in both economic and environmental terms during anticipated perturbations (e.g., weather fluctuations). From the biofuel processing perspective, bacteria have potential for use in cultivation (provide a growth benefit) [15,25], reduction of invasion, harvesting (induce aggregation) [186,187], and extraction (weakening of the microalgal cell wall) [98,108].

In silico design of phycosphere communities

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

Downloaded for Sally Bauer (sbauer2@billingsclinic.org) at BILLINGS CLINIC from ClinicalKey.com by Elsevier on July 18, 2024. For personal use only. No other uses without permission. Copyright ©2024. Elsevier Inc. All rights reserved.

For phycosome applications, consortium design can be achieved using metabolic predictions of the host algal cell, a native phycosome, a desired microbial community, or a combination. With sufficient metabolic information about the host, exudate composition can be modeled and used to predict how microorganisms may be recruited to the host [74]. Additional approaches are being developed to predict and build systems based upon mathematical models of natural ecosystems [197]. Computational approaches have also been paired with high-throughput culturing techniques such as microfluidics to predict host processes that are integral to recruiting microbiomes [198] or how complementarity of host and microbial growth rate and substrate preferences can be used to train phycosome design algorithms [199]. Phycosomes have been designed by 'letting the host decide' through swapping complex microbiomes from taxonomically distinct host species and by letting the host recruit microbial species to a new microbiome [200]. Used together, *in silico* and *ex situ* tools such as these can strengthen or expand the potential of traditional bottom-up or top-down microbiome design.

Concluding remarks and future perspectives

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

It is evident from the recently published literature that the importance of microalgal microbiomes has been recognized, but more research will be necessary to elucidate mechanistic understanding of phycosphere/phycosome interactions that directly promote productivity and stability at an industrial scale (see Outstanding questions). The challenge can become more complex when polyalgal cultures are considered under different growth conditions, such as high-pH/high-alkalinity systems that have high but consistent pH with or without higher osmolarity. Nevertheless, understanding these interactions is essential for controlling and optimizing the function of industrial ecosystems for maximal societal benefit in terms of direct air capture of CO_2 and the use of low-quality water and nutrients that can produce different value-added products. In fact, natural systems typically operate with low-quality resources via recycling and a combination of functional redundancy and complementarity to offset dynamic stresses. An improved understanding of phototrophic biosystems in different environments and geographic locations could inform the operation of biosystems at the industrial scale for CO_2 capture by taking advantage of ecology and physiology. Remaining challenges include the completion of in-depth physiological studies with accompanying ecology to understand the potential of combined organismal traits, the relationship between community members, and maintaining the consortia over industrially relevant time- and space-scales.

Outstanding questions

What is the relationship between culture diversity and culture productivity? When engineering algal (poly)cultures, are higher levels of diversity necessary to improve the culture stability and productivity?

Are metabolic and ecological interactions more predictable and controllable in more extreme conditions (e.g., high-pH, high-alkalinity cultures)? Can communities be engineered based upon desired functions to occupy functional niche space?

Can natural observations of elevated productivity in alkaline systems translate to the laboratory? To industrial scale systems?

Are bottom-up and/or top-down approaches more feasible and streamlined to achieve desired outcomes of stable and productive algal polycultures?

How can the microalgal industry use phycosomes from naturally productive systems to design consortia cultures with minimal inputs (carbon, nitrogen, phosphorus) and desirable outputs (biomass, lipid, starch)?

Can metabolic modeling be used to estimate and build niche space for stable, photoautotrophic-driven outputs?

How does engineering microalgal–bacterial consortia impact the economics of cultivation models that are often built upon assumptions that cultures are axenic?

Acknowledgments

The algal work by the research groups of R.G. and M.W.F. is supported by the US National Science Foundation (#2125083) and the US Department of Energy (DE-EE0008247, DE-EE0009273, FICUS50943). We thank other members of the Algal Biotechnology Group at Montana State University as well as collaborators at the University of Toledo and the University of North Carolina for helpful discussions.

Declaration of interests

The authors declare no conflicts of interest.

Downloaded for Sally Bauer (sbauer2@billingsclinic.org) at BILLINGS CLINIC from ClinicalKey.com by Elsevier on July 18, 2024. For personal use only. No other uses without permission. Copyright ©2024. Elsevier Inc. All rights reserved.

References

1. Hussain, J. and Rittman, B.E. (2023) Algae as a source of renewable energy: opportunities, challenges, and recent developments. *Sustain. Energy Fuels* 7, 2515–2544
2. Aslan, S. and Kapdan, I.K. (2006) Batch kinetics of nitrogen and phosphorus removal from synthetic wastewater by algae. *Ecol. Eng.* 28, 64–70
3. Fields, M.W. *et al.* (2014) Sources and resources: importance of nutrients, resource allocation, and ecology in microalgal cultivation for lipid accumulation. *Appl. Microbiol. Biotechnol.* 98, 4805–4816
4. Georgianna, D.R. and Mayfield, S.P. (2012) Exploiting diversity and synthetic biology for the production of algal biofuels. *Nature* 488, 329–335
5. Hoffmann, J.P. (1998) Wastewater treatment with suspended and nonsuspended algae. *J. Phycol.* 34, 757–763
6. Widin, S.L. *et al.* (2022) Biodiversity and disease risk in an algal biofuel system: an experimental test in outdoor ponds using a before-after-control-impact (BACI) design. *PLoS One* 17, e0267674
7. Fuentes, J. *et al.* (2016) Impact of microalgae–bacteria interactions on the production of algal biomass and associated compounds. *Mar. Drugs* 14, 100
8. Kazamia, E. *et al.* (2014) An engineered community approach for industrial cultivation of microalgae. *Ind. Biotechnol.* 10, 184–190
9. Smith, V.H. and Crews, T. (2014) Applying ecological principles of crop cultivation in large-scale algal biomass production. *Algal Res.* 4, 23–34
10. Kimbrel, J.A. *et al.* (2019) Host selection and stochastic effects influence bacterial community assembly on the microalgal phycosphere. *Algal Res.* 40, 101489
11. Croft, M.T. *et al.* (2005) Algae acquire vitamin B_{12} through a symbiotic relationship with bacteria. *Nature* 438, 90–93
12. Foster, R.A. *et al.* (2011) Nitrogen fixation and transfer in open ocean diatom–cyanobacterial symbioses. *ISME J.* 5, 1484–1493
13. Geng, H. and Belas, R. (2010) Molecular mechanisms underlying roseobacter–phytoplankton symbioses. *Curr. Opin. Biotech.* 21, 332–338
14. Grant, M.A. *et al.* (2014) Direct exchange of vitamin B_{12} is demonstrated by modelling the growth dynamics of algal–bacterial cocultures. *ISME J.* 8, 1418–1427
15. Kazamia, E. *et al.* (2012) Mutualistic interactions between vitamin B_{12}-dependent algae and heterotrophic bacteria exhibit regulation: algal–bacterial interactions for delivery of vitamin B12. *Environ. Microbiol.* 14, 1466–1476
16. Zehr, J.P. (2015) How single cells work together. *Science* 349, 1163–1164
17. Beyter, D. *et al.* (2016) Diversity, productivity, and stability of an industrial microbial ecosystem. *Appl. Environ. Microbiol.* 82, 2494–2505
18. Newby, D.T. *et al.* (2016) Assessing the potential of polyculture to accelerate algal biofuel production. *Algal Res.* 19, 264–277
19. Mallon, C.A. *et al.* (2015) Microbial invasions: the process, patterns, and mechanisms. *Trends Microbiol.* 23, 719–729
20. Wright, E.S. and Vestigan, K.H. (2016) Inhibitory interactions promote frequent biostability among competing bacteria. *Nat. Commun.* 7, 11274
21. Godwin, C.M. *et al.* (2018) Biodiversity improves the ecological design of sustainable biofuel systems. *GCB Bioenergy* 10, 752–765
22. Bell, T.A.S. *et al.* (2019) Contributions of the microbial community to algal biomass and biofuel productivity in a wastewater treatment lagoon system. *Algal Res.* 39, 101461
23. Narwani, A. *et al.* (2016) Power of plankton: effects of algal biodiversity on biocrude production and stability. *Environ. Sci. Technol.* 50, 13142–13150
24. Stockenreiter, M. *et al.* (2013) Functional group richness: implications of biodiversity for light use and lipid yield in microalgae. *J. Phycol.* 49, 838–847
25. Corcoran, A.A. and Boeing, W.J. (2012) Biodiversity increases the productivity and stability of phytoplankton communities. *PLoS ONE* 7, e49397
26. Carruthers, D.N. *et al.* (2019) Biodiversity improves life cycle sustainability metrics in algal biofuel production. *Environ. Sci. Technol.* 53, 9279–9288
27. Six, C. *et al.* (2007) Light variability illuminates niche-partitioning among marine Picocyanobacteria. *PLoS One* 2, e1341
28. Lindemann, S.R. *et al.* (2016) Engineering microbial consortia for controllable outputs. *ISME J.* 10, 2077–2084
29. Thomas, P.K. *et al.* (2019) A natural algal polyculture outperforms an assembled polyculture in wastewater-based open pond biofuel production. *Algal Res.* 40, 101488
30. Lian, J. *et al.* (2018) The effect of the algal microbiome on industrial production of microalgae. *Microb. Biotechnol.* 11, 806–818
31. Samo, T.J. *et al.* (2018) Attachment between heterotrophic bacteria and microalgae influences symbiotic microscale interactions. *Environ. Microbiol.* 20, 4385–4400
32. Ramanan, R. *et al.* (2016) Algae–bacteria interactions: evolution, ecology and emerging applications. *Biotechnol. Adv.* 34, 14–29
33. Guiry, M.D. (2012) How many species of algae are there? *J. Phycol.* 48, 1057–1063
34. Hanschen, E.R. and Starkenburg, S.R. (2020) The state of algal genome quality and diversity. *Algal Res.* 50, 101968
35. Goemann, C.L.C. *et al.* (2023) Genome sequence, phylogenetic analysis, and structure-based annotation reveal metabolic potential of *Chlorella* sp. SLA-04. *Algal Res.* 69, 102943
36. Pokorny, L. *et al.* (2022) How to verify non-presence-the challenge of axenic algae cultivation. *Cells* 11, 2594
37. Seymour, J.R. *et al.* (2017) Zooming in on the phycosphere: the ecological interface for phytoplankton–bacteria relationships. *Nat. Microbiol.* 2, 17065
38. Teeling, H. *et al.* (2012) Substrate-controlled succession of marine bacterioplankton populations induced by a phytoplankton bloom. *Science* 336, 608–611
39. Hena, S. *et al.* (2015) Cultivation of algae consortium in a dairy farm wastewater for biodiesel production. *Water Res. Ind.* 10, 1–14
40. Teeling, H. *et al.* (2016) Recurring patterns in bacterioplankton dynamics during coastal spring algae blooms. *Elife* 5, e11888
41. Mueller, R.C. *et al.* (2021) An emerging view of the diversity, ecology and function of Archaea in alkaline hydrothermal environments. *FEMS Microbiol. Ecol.* 97, fiaa246
42. Jones, B.E. and Grant, W.D. (2000) Microbial diversity and ecology of alkaline environments. In *Journey to Diverse Microbial Worlds: Adaptation to Exotic Environments* (Seckbach, J., ed.), pp. 177–190, Springer
43. Canale, R.P. and Vogel, A.H. (1974) Effects of temperature on phytoplankton growth. *J. Env. Eng. Div. ASCE* 100, 229–241
44. Varshney, P. *et al.* (2015) Extremophilic micro-algae and their potential contribution in biotechnology. *Bioresour. Technol.* 184, 363–372
45. Talling, J.F. *et al.* (1973) The upper limit of photosynthetic productivity by phytoplankton: evidence from Ethiopian soda lakes. *Freshw. Biol.* 3, 53–76
46. Lopez-Archilla, A.I. *et al.* (2004) Phytoplankton diversity and cyanobacterial dominance in a hypereutrophic shallow lake with biologically produced alkaline pH. *Extremophiles* 8, 109–115
47. Hodgskiss, L.H. *et al.* (2016) Cultivation of a native alga for biomass and biofuel accumulation in coal bed methane production water. *Algal Res.* 19, 63–68
48. Nega, R. *et al.* (2018) Spatial and temporal variations in the diversity of microalgae in Lake Hawassa, Ethiopia. *Int. J. Food Sci. Nutr.* 4, 16–23
49. Chojnacka, K. and Marquez-Rocha, F.J. (2003) Kinetic and stoichiometric relationships of the energy and carbon metabolism in the culture of microalgae. *Biotechnol* 3, 21–34
50. Liang, Y. *et al.* (2009) Biomass and lipid productivities of *Chlorella vulgaris* under autotrophic, heterotrophic and mixotrophic growth conditions. *Biotechnol. Lett.* 31, 1043–1049
51. Deschênes, J.-S. *et al.* (2015) Mixotrophic production of microalgae in pilot-scale photobioreactors: practicability and process considerations. *Algal Res.* 10, 80–86
52. Ruiz, J. *et al.* (2022) Heterotrophic vs autotrophic production of microalgae: bringing some light into the everlasting cost controversy. *Algal Res.* 64, 102698
53. Letcher, P.M. *et al.* (2013) Characterization of *Amoeboaphelidium protococcarum*, an algal parasite new to the cryptomycota isolated from an outdoor algal pond used for the production of biofuel. *PLoS One* 8, e56232

Downloaded for Sally Bauer (sbauer2@billingsclinic.org) at BILLINGS CLINIC from ClinicalKey.com by Elsevier on July 18, 2024. For personal use only. No other uses without permission. Copyright ©2024. Elsevier Inc. All rights reserved.

54. Yamada, T.O. *et al.* (2007) *Chlorella* viruses. *Adv. Virus Res.* 66, 293–336
55. Zhu, Z. *et al.* (2020) Overcoming the biological contamination in microalgae and cyanobacteria mass cultivations for photosynthetic biofuel production. *Molecules* 25, 5220
56. Wang, H. *et al.* (2013) The contamination and control of biological pollutants in mass cultivation of microalgae. *Bioresour. Technol.* 128, 745–750
57. Melack, J.M. (1981) Photosynthetic activity of phytoplankton in tropical African soda lakes. In *Salt Lakes* (Williams, W.D., ed.), pp. 71–85, Springer
58. Gardner, R.D. *et al.* (2012) Use of sodium bicarbonate to stimulate triacylglycerol accumulation in the chlorophyte *Scenedesmus* sp. and the diatom *Phaeodactylum tricornutum*. *J. Appl. Phycol.* 24, 1311–1320
59. Gardner, R.D. *et al.* (2013) Comparison of CO_2 and bicarbonate as inorganic carbon sources for triacylglycerol and starch accumulation in *Chlamydomonas reinhardtii*. *Biotechnol. Bioeng.* 110, 87–96
60. Vadlamani, A. *et al.* (2017) Cultivation of microalgae at extreme alkaline pH conditions: a novel approach for biofuel production. *ACS Sustain. Chem. Eng.* 5, 7284–7294
61. Baattrup-Pedersen, A. *et al.* (2022) Alkalinity and diatom assemblages in lowland streams: how to separate alkalinity from inorganic phosphorus in ecological assessments? *Sci. Total Environ.* 823, 153829
62. Martin, K. *et al.* (2021) The biogeographic differentiation of algal microbiomes in the upper ocean from pole to pole. *Nat. Commun.* 12, 5483
63. Bell, W.H. and Mitchell, R. (1972) Chemotactic and growth responses of marine bacteria to algal extracellular products. *Biol. Bull.* 143, 265–277
64. Amin, S.A. *et al.* (2012) Interactions between diatoms and bacteria. *Microbiol. Mol. Biol. Rev.* 76, 667–684
65. Whipps, J.M. *et al.* (1988) Mycoparasitism and plant disease control. In *Fungi in Biological Control Systems* (Burge, M.N., ed.), pp. 161–187, Manchester University Press
66. Berg, G. *et al.* (2020) Microbiome definition re-visited: old concepts and new challenges. *Microbiome* 8, 103
67. Kawafune, K. *et al.* (2012) Molecular identification of rickettsial endosymbionts in the non-phagotrophic volvocalean green algae. *PLoS ONE* 7, e31749
68. Grossart, H.-P. *et al.* (2005) Marine diatom species harbour distinct bacterial communities: marine diatom species harbour distinct bacterial communities. *Environ. Microbiol.* 7, 860–873
69. Bižić-Ionescu, M. *et al.* (2015) Comparison of bacterial communities on limnic versus coastal marine particles reveals profound differences in colonization: marine and limnic particle-associated bacteria. *Environ. Microbiol.* 17, 3500–3514
70. Eigemann, F. *et al.* (2013) Bacterial community composition associated with freshwater algae: species specificity vs. dependency on environmental conditions and source community. *FEMS Microbiol. Ecol.* 83, 650–663
71. Stocker, R. and Seymour, J.R. (2012) Ecology and physics of bacterial chemotaxis in the ocean. *Microbiol. Mol. Biol. Rev.* 76, 792–812
72. Lambert, B.S. *et al.* (2017) A microfluidics-based in situ chemotaxis assay to study the behaviour of aquatic microbial communities. *Nat. Microbiol.* 2, 1344–1349
73. Raina, J.B. *et al.* (2022) Chemotaxis shapes the microscale organization of the ocean's microbiome. *Nature* 605, 132–138
74. Smriga, S. *et al.* (2016) Chemotaxis toward phytoplankton drives organic matter partitioning among marine bacteria. *Proc. Natl. Acad. Sci. U.S.A.* 113, 1576–1581
75. Sjoblad, R.D. and Mitchell, R. (1979) Chemotactic responses of *Vibrio alginolyticus* to algal extracellular products. *Can. J. Microbiol.* 25, 964–967
76. Miller, T.R. *et al.* (2004) Chemotaxis of *Silicibacter* sp. strain TM1040 toward dinoflagellate products. *Appl. Environ. Microbiol.* 70, 4692–4701
77. Seymour, J.R. *et al.* (2010) Chemoattraction to dimethylsulfoniopropionate throughout the marine microbial food web. *Science* 329, 342–345
78. Mayali, X. *et al.* (2011) Temporal attachment dynamics by distinct bacterial taxa during a dinoflagellate bloom. *Aquat. Microb. Ecol.* 63, 111–122
79. Staats, N. *et al.* (2000) Exopolysaccharide production by the epipelic diatom *Cylindrotheca closterium*: effects of nutrient conditions. *J. Exp. Mar. Biol. Ecol.* 249, 13–27
80. Passow, U. (2002) Production of transparent exopolymer particles (TEP) by phyto- and bacterioplankton. *Mar. Ecol. Prog. Ser.* 236, 1–12
81. Nissimov, J.I. *et al.* (2018) Dynamics of transparent exopolymer particle production and aggregation during viral infection of the coccolithophore, *Emiliania huxleyi*. *Environ. Microbiol.* 20, 2880–2897
82. Martin-Platero, A.M. *et al.* (2018) High resolution time series reveals cohesive but short-lived communities in coastal plankton. *Nat. Commun.* 9, 266
83. Basu, S. and Mackey, K. (2018) Phytoplankton as key mediators of the biological carbon pump: their responses to a changing climate. *Sustainability* 10, 869
84. Buesseler, K.O. and Boyd, P.W. (2009) Shedding light on processes that control particle export and flux attenuation in the twilight zone of the open ocean. *Limnol. Oceanogr.* 54, 1210–1232
85. Ashen, J.B. and Goff, L.J. (2000) Molecular and ecological evidence for species specificity and coevolution in a group of marine algal–bacterial symbioses. *Appl. Environ. Microbiol.* 66, 3024–3030
86. Sapp, M. *et al.* (2007) Species-specific bacterial communities in the phycosphere of microalgae? *Microb. Ecol.* 53, 683–699
87. Krohn-Molt, I. *et al.* (2017) Insights into microalga and bacteria interactions of selected phycosphere biofilms Using metagenomic, transcriptomic, and proteomic approaches. *Front. Microbiol.* 8, 1941
88. Behringer, G. *et al.* (2018) Bacterial communities of diatoms display strong conservation across strains and time. *Front. Microbiol.* 9, 659
89. Tada, Y. *et al.* (2017) Distinct bacterial community and diversity shifts after phytoplankton-derived dissolved organic matter addition in a coastal environment. *J. Exp. Mar. Biol. Ecol.* 495, 119–128
90. Landa, M. *et al.* (2016) Shifts in bacterial community composition associated with increased carbon cycling in a mosaic of phytoplankton blooms. *ISME J.* 10, 39–50
91. Bell, W.H. (1983) Bacterial utilization of algal extracellular products. 3. The specificity of algal–bacterial interaction: extracellular products. 3. *Limnol. Oceanogr.* 28, 1131–1143
92. Fu, H. *et al.* (2020) Ecological drivers of bacterial community assembly in synthetic phycospheres. *Proc. Natl. Acad. Sci. U.S.A.* 117, 3656–3662
93. Morales, M. *et al.* (2020) Rotating algal biofilm versus planktonic cultivation: LCA perspective. *J. Clean. Prod.* 257, 120547
94. Sharp, C.E. *et al.* (2017) Robust, high-productivity phototrophic carbon capture at high pH and alkalinity using natural microbial communities. *Biotechnol. Biofuels* 10, 84
95. Azam, F. and Malfatti, F. (2007) Microbial structuring of marine ecosystems. *Nat. Rev. Microbiol.* 5, 782–791
96. Wang, M. and Yuan, W. (2014) Bacterial lysis of microalgal cells. *J. Sustain. Bioenerg. Syst.* 04, 243–248
97. Sule, P. and Belas, R. (2013) A novel inducer of *Roseobacter* motility is also a disruptor of algal symbiosis. *J. Bacteriol.* 195, 637–646
98. Wagner-Dobler, I. and Biebl, H. (2006) Environmental biology of the marine *Roseobacter* lineage. *Annu. Rev. Microbiol.* 60, 255–280
99. Seyedsayamdost, M.R. *et al.* (2011) The Jekyll-and-Hyde chemistry of *Phaeobacter gallaeciensis*. *Nat. Chem.* 3, 331–335
100. Sharifah, E.N. and Eguchi, M. (2011) The phytoplankton *Nannochloropsis oculata* enhances the ability of *Roseobxacter* clade bacteria to inhibit the growth of fish pathogen *Vibrio anguillarum*. *PLoS One* 6, e26756
101. Henriksen, N. *et al.* (2022) Role is in the eye of the beholder – the multiple functions of the antibacterial compound tropodithietic acid produced by marine Rhodobacteraceae. *FEMS Microbiol. Rev.* 46, fuac007

Downloaded for Sally Bauer (sbauer2@billingsclinic.org) at BILLINGS CLINIC from ClinicalKey.com by Elsevier on July 18, 2024. For personal use only. No other uses without permission. Copyright ©2024. Elsevier Inc. All rights reserved.

102. Bratbak, G. and Thingstand, T.F. (1985) Phytoplankton-bacteria interactions: an apparent paradox? Analysis of a model system with both competition and commensalism. *Mar. Ecol. Prog. Ser.* 25, 23–30
103. Mayali, X. and Azam, F. (2004) Algicidal bacteria in the sea and their impact on algal blooms1. *J. Eukaryot. Microbiol.* 51, 139–144
104. Jung, S.W. *et al.* (2008) *Pseudomonas fluorescens* HYK0210-SK09 offers species-specific biological control of winter algal blooms caused by freshwater diatom *Stephanodiscus hantzschii*. *J. Appl. Microbiol.* 105, 186–195
105. Noh, S.Y. *et al.* (2017) Algicidal activity of the bacterium, *Pseudomonas fluorescensii* SK09, to mitigate *Stephanodiscus hantzschii* (Bacillariophyceae) blooms using field mesocosms. *J. Freshw. Ecol.* 32, 477–488
106. Lenneman, E.M. *et al.* (2014) Potential application of algicidal bacteria for improved lipid recovery with specific algae. *FEMS Microbiol. Lett.* 354, 102–110
107. Demuez, M. *et al.* (2015) Algicidal microorganisms and secreted algicides: new tools to induce microalgal cell disruption. *Biotechnol. Adv.* 33, 1615–1625
108. Meyer, N. *et al.* (2017) Strategies and ecological roles of algicidal bacteria. *FEMS Microbiol. Rev.* 41, 880–899
109. Azam, F. *et al.* (1991) The role of the microbial loop in Antarctic pelagic ecosystems. *Polar Res.* 10, 239–244
110. Fei, C. *et al.* (2020) Quorum sensing regulates 'swim-or-stick' lifestyle in the phycosphere. *Environ. Microbiol.* 22, 4761–4778
111. Rajamani, S. *et al.* (2011) N-ACYL homoserine lactonase inactivation of quorum sensing agaonists produced by *Chlamydomonas reinhardtii* and characterization of a transgenic algae. *J. Phycol.* 47, 1219–1227
112. Lebeau, T. and Robert, J.M. (2003) Diatom cultivation and biotechnologically relevant products. Part II. Current and putative products. *Appl. Microbiol. Biotechnol.* 60, 624–632
113. Davies, D.G. and Marques, C.N.H. (2009) A fatty acid messenger is responsible for inducing dispersion in microbial biofilms. *J. Bacteriol.* 191, 1393–1403
114. Sauer, K. *et al.* (2004) Characterization of nutrient-induced dispersion in *Pseudomonas aeruginosa* PAO1 biofilm. *J. Bacteriol.* 186, 7312–7326
115. Ozer, E.A. *et al.* (2005) Human and murine paraoxonase 1 are host modulators of *Pseudomonas aeruginosa* quorum-sensing. *FEMS Microbiol. Lett.* 253, 29–37
116. Ribalet, F. *et al.* (2008) Differential effect of three polyunsaturated aldehydes on marine bacterial isolates. *Aquat. Toxicol.* 86, 249–255
117. Hitch, T.C.A. *et al.* (2022) Microbiome-based interventions to modulate gut ecology and the immune system. *Mucosal Immunol.* 15, 1095–1113
118. Mayali, X. (2018) Editorial: metabolic interactions between bacteria and phytoplankton. *Front. Microbiol.* 9, 727
119. Nikolaev, Y.A. *et al.* (2011) Effect of bacterial satellites on *Chlamydomonas reinhardtii* growth in an algo-bacterial community. *Microbiology* 77, 78–83
120. Silva-Aciares, F.R. and Riquelme, C.E. (2008) Comparisons of the growth of six diatom species between two configurations of photobioreactors. *Aquac. Eng.* 38, 26–35
121. Amin, S.A. *et al.* (2007) Boron binding by a siderophore isolated from marine bacteria associated with the toxic dinoflagellate *Gymnodinium catenatum*. *JACS* 129, 478–479
122. Amin, S.A. *et al.* (2009) Photolysis of iron–siderophore chelates promotes bacterial–algal mutualism. *Proc. Natl. Acad. Sci. U.S.A.* 106, 17071–17076
123. Hogle, S.L. *et al.* (2017) Direct heme uptake by phytoplankton-associated *Roseobacter* bacteria. *mSystems* 2, e00124
124. Plouviez, M. *et al.* (2018) Nitrous oxide emissions from microalgae: potential pathways and significance. *J. Appl. Phycol.* 31, 1–8
125. Hilton, J.A. *et al.* (2013) Genomic deletions disrupt nitrogen metabolism pathways of a cyanobacterial diatom symbiont. *Nat. Commun.* 4, 1767
126. Kim, B.-H. *et al.* (2014) Role of *Rhizobium*, a plant growth promoting bacterium, in enhancing algal biomass through mutualistic interaction. *Biomass Bioenerg.* 69, 95–105
127. Suleiman, M. *et al.* (2016) Interkingdom cross-feeding of ammonium from marine methylamine-degrading bacteria to the diatom *Phaeodactylum tricornutum*. *Appl. Environ. Microbiol.* 82, 7113–7122
128. Amin, S.A. *et al.* (2015) Interaction and signalling between a cosmopolitan phytoplankton and associated bacteria. *Nature* 522, 98–101
129. Pisman, T.I. *et al.* (2005) Experimental and mathematical modeling of the consumer's influence on productivity of algae in a model aquatic ecosystem. *Adv. Space Res.* 35, 1521–1527
130. Haines, K.C.L. and Guillard, R. (1974) Growth of vitamin B_{12}-requiring marine diatoms in mixed laboratory cultures with vitamin B12-producing bacteria. *J. Phycol.* 10, 245–252
131. Kurata, A. (1986) Blooms of *Uroglena americana* in relation to concentrations of B group vitamins. In *Chrysophytes Aspects and Problems* (Kristiansenm, J. and Andersen, R.A., eds), pp. 185–196, Cambridge University Press
132. Kuo, R.C. and Lin, S. (2013) Ectobiotic and endobiotic bacteria associated with *Eutreptiella* sp. isolated from Long Island Sound. *Protist* 164, 60–74
133. Lupette, J. *et al.* (2016) *Marinobacter* dominates the bacterial community of the *Ostreococcus tauri* phycosphere in culture. *Front. Microbiol.* 7, 1414
134. Warren, M.J. *et al.* (2002) The biosynthesis of adenosylcobalamin (vitamin B_{12}). *Nat. Prod. Rep.* 19, 390–412
135. Helliwell, K.E. *et al.* (2011) Insights into the evolution of vitamin B12 auxotrophy from sequenced algal genomes. *Mol. Biol. Evol.* 28, 2921–2933
136. Giovannoni, S.J. (2012) Vitamins in the sea. *Proc. Natl. Acad. Sci. U.S.A.* 109, 13888–13889
137. Durham, B.P. *et al.* (2015) Cryptic carbon and sulfur cycling between surface ocean plankton. *Proc. Natl. Acad. Sci.* 112, 453–457
138. Wienhausen, G. *et al.* (2017) The exometabolome of two model strains of the Roseobacter group: a marketplace of microbial metabolites. *Front. Microbiol.* 8, 1985
139. de-Bashan, L.E. *et al.* (2011) Cell–cell interaction in the eukaryote-prokaryote model of micro algae *Chlorella vulgaris* and the bacterium *Azospirillium brasilense* immobilized in polymer beads. *J. Phycol.* 47, 1350–1359
140. Lau, S. *et al.* (2009) Auxin signaling in algal lineages: fact or myth? *Trends Plant Sci.* 14, 182–188
141. Segev, E. *et al.* (2016) Dynamic metabolic exchange governs a marine algal–bacterial interaction. *Elife* 5, e17473
142. Peng, H. *et al.* (2021) Comparison of algae growth and symbiotic mechanisms in the presence of plant growth promoting bacteria and non-plant growth promoting bacteria. *Algal Res.* 53, 102156
143. Yu, Z. *et al.* (2017) The effects of combined agricultural phytohormones on the growth, carbon partitioning and cell morphology of two screened algae. *Bioresour. Technol.* 239, 87–96
144. Makridis, P. *et al.* (2006) Microbial conditions and antimicrobial activity in cultures of two microalgae species, *Tetraselmis chuii* and *Chlorella minutissima*, and effect on bacterial load of enriched *Artemia metanauplii*. *Aquaculture* 255, 76–81
145. Geng, H. *et al.* (2008) Genetic dissection of tropodithietic acid biosynthesis by marine Roseobacters. *Appl. Environ. Microbiol.* 74, 1535–1545
146. Mitchell, S.E. *et al.* (2005) Host–parasite and genotype-by-environment interactions: temperature modifies potential for selection by a sterilizing pathogen. *Evolution* 59, 70–80
147. Fels, D. and Kaltz, O. (2006) Temperature-dependent transmission and latency of *Holospora undulata*, a micronucleus-specific parasite of the ciliate *Paramecium caudatum*. *Proc. R. Soc. B* 273, 1031–1038
148. Long, R.A. and Azam, F. (2001) Antagonistic interactions among marine pelagic bacteria. *Appl. Environ. Microbiol.* 67, 4975–4983
149. Hunken, M. *et al.* (2008) Epiphytic bacteria on the Antarctic ice diatom *Amphiprora kufferathii Manguin* cleave hydrogen peroxide produced during algal photosynthesis. *Plant Biol. (Stuttg)* 10, 519–526
150. Arora, N. *et al.* (2019) Elucidating the unique physiological responses of halotolerant *Scenedesmus* sp. cultivated in sea water for biofuel production. *Algal Res.* 37, 260–268

Downloaded for Sally Bauer (sbauer2@billingsclinic.org) at BILLINGS CLINIC from ClinicalKey.com by Elsevier on July 18, 2024. For personal use only. No other uses without permission. Copyright ©2024. Elsevier Inc. All rights reserved.

151. Xia, L. *et al.* (2014) NaCl as an effective inducer for lipid accumulation in freshwater microalgae *Desmodesmus abundans*. *Bioresour. Technol.* 161, 402–409
152. Wang, T. *et al.* (2016) Salt stress induced lipid accumulation in heterotrophic culture cells of *Chlorella protothecoides*: mechanisms based on the multi-level analysis of oxidative response, key enzyme activity and biochemical alteration. *J. Biotechnol.* 228, 18–27
153. Foflanker, F. *et al.* (2016) The unexpected extremophile: tolerance to fluctuating salinity in the green alga *Picochlorum*. *Algal Res.* 16, 465–472
154. Kim, Z.H. *et al.* (2021) Enhanced fatty acid productivity by *Parachlorella* sp., a freshwater microalga, via adaptive laboratory evolution under salt stress. *Biotechnol. Bioproc. E* 26, 223–231
155. Zhao, Y. *et al.* (2022) Stimulating biolipid production from the novel alga *Ankistrodesmus* sp. by coupling salt stress and chemical induction. *Renew. Energy* 183, 480–490
156. Church, J. *et al.* (2017) Effect of salt type and concentration on the growth and lipid content of *Chlorella vulgaris* in synthetic saline wastewater for biofuel production. *Bioresour. Technol.* 243, 147–153
157. Moran, M.A. and Durham, B.P. (2019) Sulfur metabolites in the pelagic ocean. *Nat. Rev. Microbiol.* 17, 665–678
158. Fenizia, S. *et al.* (2020) Ectoine from bacterial and algal origin is a compatible solute in microalgae. *Mar. Drugs* 18, 42
159. Barera, S. and Forlani, G. (2023) The role of proline in the adaptation of eukaryotic microalgae to environmental stress: an underestimated tool for the optimization of algal growth. *J. Appl. Phycol.* 35, 1635–1648
160. Rothig, T. *et al.* (2016) Long-term salinity tolerance is accompanied by major restructuring of the coral bacterial microbiome. *Mol. Ecol.* 25, 1308–1323
161. Cho, D.-H. *et al.* (2015) Organic carbon, influent microbial diversity and temperature strongly influence algal diversity and biomass in raceway ponds treating raw municipal wastewater. *Bioresour. Technol.* 191, 481–487
162. Corredor, L. *et al.* (2021) Effect of temperature, nitrate concentration, pH and bicarbonate addition on biomass and lipid accumulation in the sporulating green alga PW95. *Algal Res.* 53, 102148
163. Jiang, H. and Gao, K. (2004) Effects of lowering temperature during culture on the production of polyunsaturated fatty acids in the marine diatom *Phaeodactylum tricornutum* (Bacillariophyceae). *J. Phycol.* 40, 651–654
164. Samo, T.J. *et al.* (2023) The algal microbiome protects *Desmodesmus intermedius* from high light and temperature stress. *Algal Res.* 75, 103245
165. Bechet, Q. *et al.* (2017) Modeling the impact of high temperatures on microalgal viability and photosynthetic activity. *Biotechnol. Biofuels* 10, 136
166. Schabhüttl, S. *et al.* (2013) Temperature and species richness effects in phytoplankton communities. *Oecologia* 171, 527–536
167. Utami, Y.D. *et al.* (2022) Investigating plant–microbe interactions within the root. *Arch. Microbiol.* 204, 639
168. Chaudhry, V. *et al.* (2021) Shaping the leaf microbiota: plant–microbe–microbe interactions. *J. Exp. Bot.* 72, 36–56
169. Yachi, S. and Loreau, M. (1999) Biodiversity and ecosystem productivity in a fluctuating environment: the insurance hypothesis. *Proc. Natl. Acad. Sci. U.S.A.* 96, 1463–1468
170. D'Souza, G. *et al.* (2018) Ecology and evolution of metabolic cross-feeding interactions in bacteria. *Nat. Prod. Rep.* 35, 455–488
171. Montoya, J.M. and Sol, R.V. (2002) Small world patterns in food webs. *J. Theor. Biol.* 214, 405–412
172. Watts, D.J. and Strogatz, S.H. (1998) Collective dynamics of 'small-world' networks. *Nature* 393, 440–442
173. Sinha, S. (2005) Complexity vs. stability in small-world networks. *Phys. A* 346, 147–153
174. Sinha, S. and Sinha, S. (2005) Evidence of universality for the May–Wigner stability theorem for random networks with local dynamics. *Phys. Rev. E* 71, 020902
175. Wilmers, C.C. (2007) Understanding ecosystem robustness. *Trends Ecol. Evol.* 22, 504–506
176. Mandal, S. *et al.* (2018) Functional divergence in nitrogen uptake rates explains diversity–productivity relationship in microalgal communities. *Ecosphere* 9, e02228
177. Zelezniak, A. *et al.* (2015) Metabolic dependencies drive species co-occurrence in diverse microbial communities. *Proc. Natl. Acad. Sci. U.S.A.* 112, 6449–6454
178. Klitgord, N. and Segre, D. (2010) Environments that induce synthetic microbial ecosystems. *PLoS Comput. Biol.* 6, e1001002
179. Turner, P.E. *et al.* (1996) Tests of ecological mechanisms promoting the stable coexistence of two bacterial genotypes. *Ecology* 77, 2119–2129
180. Stockenreiter, M. *et al.* (2016) Nutrient uptake and lipid yield in diverse microalgal communities grown in wastewater. *Algal Res.* 15, 77–82
181. Avendano-Herrera, R.E. and Riquelme, C.E. (2007) Production of a diatom–bacteria biofilm in a photobioreactor for aquaculture applications. *Aquac. Eng.* 36, 97–104
182. Cho, D.-H. *et al.* (2015) Enhancing microalgal biomass productivity by engineering a microalgal–bacterial community. *Bioresour. Technol.* 175, 578–585
183. Toyama, T. *et al.* (2018) Growth promotion of three microalgae, *Chlamydomonas reinhardtii*, *Chlorella vulgaris* and *Euglena gracilis*, by in situ indigenous bacteria in wastewater effluent. *Biotechnol. Biofuels* 11, 176
184. Ortiz-Marquez, J.C.F. *et al.* (2012) Association with an ammonium-excreting bacterium allows diazotrophic culture of oil-rich eukaryotic microalgae. *Appl. Environ. Microbiol.* 78, 2345–2352
185. Ward, C.S. *et al.* (2021) *Janthinobacter* additions reduce rotifer grazing of microalga *Microchloropsis salina* in biotically complex communities. *Algal Res.* 58, 102400
186. Gardes, A. *et al.* (2011) Diatom-associated bacteria are required for aggregation of *Thalassiosira weissflogii*. *ISME J.* 5, 436–445
187. Wang, H. *et al.* (2012) Novel bacterial isolate from Permian groundwater, capable of aggregating potential biofuel-producing microalga *Nannochloropsis oceanica* IMET1. *Appl. Environ. Microbiol.* 78, 1445–1453
188. Kazamia, E. *et al.* (2012) Synthetic ecology – a way forward for sustainable algal biofuel production? *J. Biotechnol.* 162, 163–169
189. Godwin, C.M. *et al.* (2017) Ecological stoichiometry meets ecological engineering: using polycultures to enhance the multifunctionality of algal biocrude systems. *Environ. Sci. Technol.* 51, 11450–11458
190. Vadlamani, A. *et al.* (2019) High productivity cultivation of microalgae without concentrated CO_2 input. *ACS Sustain. Chem. Eng.* 7, 1933–1943
191. Schaible, G.A. *et al.* (2022) Correlative SIP–FISH–Raman–SEM–NanoSIMS links identity, morphology, biochemistry, and physiology of environmental microbes. *ISME Commun.* 2, 52
192. Bouskill, N.J. *et al.* (2012) Trait-based representation of biological nitrification: model development, testing, and predicted community composition. *Front. Microbiol.* 3, 364
193. Stolyar, S. *et al.* (2007) Metabolic modeling of a mutualistic microbial community. *Mol. Syst. Biol.* 3, 92
194. Taffs, R. *et al.* (2009) In silico approaches to study mass and energy flows in microbial consortia: a syntrophic case study. *BMC Syst. Biol.* 3, 114
195. Briones, A. and Raskin, L. (2003) Diversity and dynamics of microbial communities in engineered environments and their implications for process stability. *Curr. Opin. Biotechnol.* 14, 270–276
196. Lawson, C.E. *et al.* (2019) Common principles and best practices for engineering microbiomes. *Nat. Rev. Microbiol.* 17, 725–741
197. Succurro, A. *et al.* (2017) A diverse community to study communities: integration of experiments and mathematical models to study microbial consortia. *J. Bacteriol.* 199, e00865-16
198. Raina, J.B. *et al.* (2023) Chemotaxis increases metabolic exchanges between marine picophytoplankton and heterotrophic bacteria. *Nat. Microbiol.* 8, 510–521
199. Liu, F. *et al.* (2021) Microfluidic and mathematical modeling of aquatic microbial communities. *Anal. Bioanal. Chem.* 413, 2331–2344
200. Duran, P. *et al.* (2022) Shared features and reciprocal complementation of the *Chlamydomonas* and *Arabidopsis* microbiota. *Nat. Commun.* 13, 406

Downloaded for Sally Bauer (sbauer2@billingsclinic.org) at BILLINGS CLINIC from ClinicalKey.com by Elsevier on July 18, 2024. For personal use only. No other uses without permission. Copyright ©2024. Elsevier Inc. All rights reserved.