Trends in Plant Science

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A Chemical Perspective on Microalgal–Microbial Interactions

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The exchange of chemical compounds is central to the interactions of microalgae with other microorganisms. Although foundational for many food webs, these interactions have been poorly studied compared with higher plant–microbe interactions. Emerging insights have begun to reveal how these interactions and the participating chemical compounds shape microbial communities and broadly impact biogeochemical processes.

Microalgal–Microbial Partnerships

Aquatic photosynthetic organisms primarily comprise eukaryotic microalgae and cyanobacteria and account for approximately half of the carbon fixation on Earth [1]. As primary producers, these photoautotrophs form the basis of aquatic food webs. For example, oceanic phytoplankton serve as the primary food source for zooplankton and thus is at the base of the food pyramid for all marine animals. Algae are also responsible for toxic blooms that negatively impact ecosystems, fishery resources, and human well-being and can lead to economic losses in the millions of dollars [2]. Biotechnologically, algae are being exploited for the production of biofuels and high-value products [3,4]; while this work has often focused on the use of pure cultures, an appreciation of the importance of studying and pursuing mixed cultivation in industrial settings will likely grow. Natural associations between eukaryotic algae and other microbes have been known for decades [5] and in many cases attempts to remove bacteria and fungi from microalgal cultures have failed, suggesting a dependence or close association of these organisms in their natural environment. To date, our understanding about the breadth, ecological significance, and chemical complexity of these partnerships has been limited, but new tools and strategies are being developed to shed more light on these multifaceted interactions (Figure 1A).

Emerging Concepts

Modes of Interaction

Nutritional interdependence provides a basis for understanding many microalgal–microbial associations (Figure 1B). As with some land plants, marine diatoms can derive their source of nitrogen by associating with diazotrophic cyanobacteria [6]. Haptophytes (prymnesiophytes) can also derive fixed nitrogen in association with a unicellular diazotrophic cyanobacterium, UCYN-A, that cannot fix CO_2 [7]. In return for fixed nitrogen, the haptophyte partner provides an as-yet-unidentified source of fixed carbon. Such symbioses between microalgae and nitrogen-fixing cyanobacteria are likely to be major determinants of marine productivity in oligotrophic waters [7].

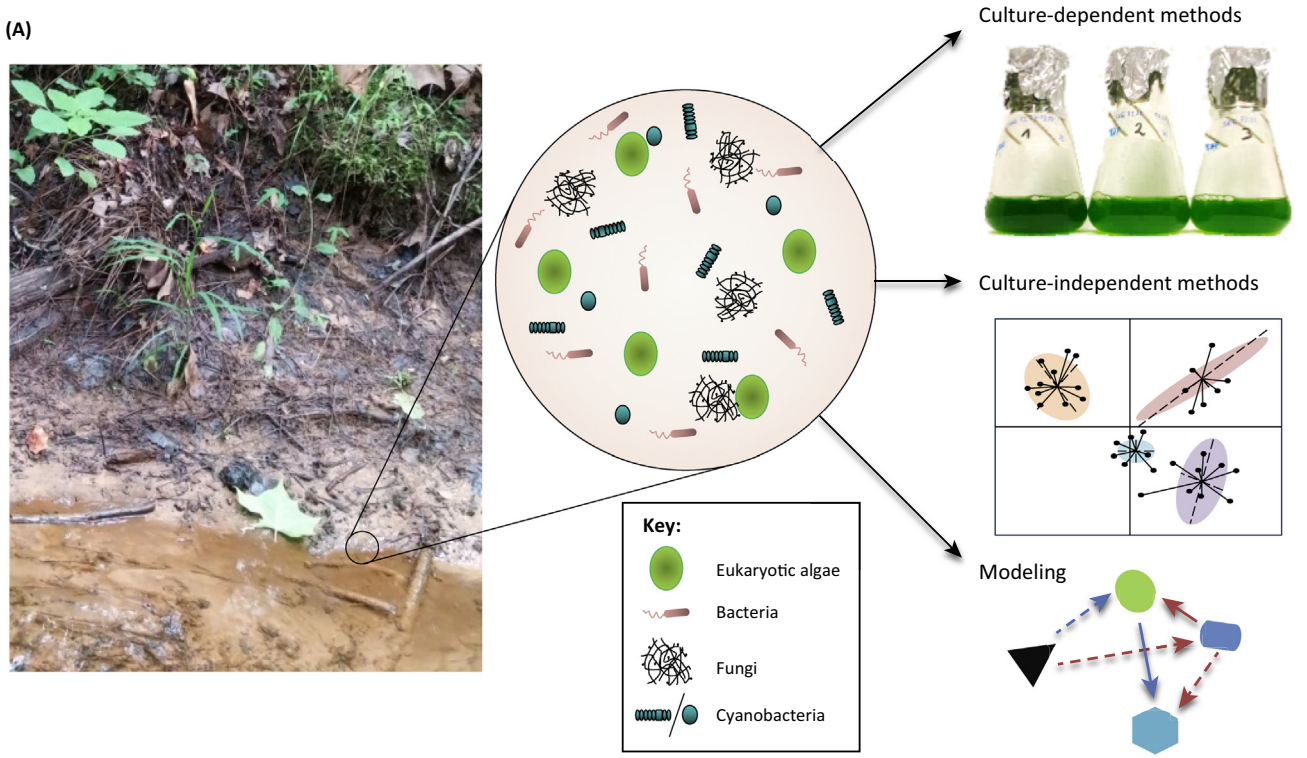
Many microalgae depend on vitamin B_{12} from heterotrophic bacteria in exchange for fixed organic carbon (Figure 1B), which may affect the composition and productivity of microalgae-containing communities [8]. A mixed culture comprising a B_{12}-auxotrophic green alga, *Lobomonas rostrata*, and a B_{12}-providing bacterium, *Mesorhizobium loti*, was found to equilibrate at a cell ratio of approximately 1:30, although this ratio could be altered by the addition of external carbon or vitamin B_{12} [9]. In addition to a direct exchange of beneficial nutrients, cooperative interactions may occur indirectly via protection from detrimental factors or inhibition relief (e.g., defense against stress or the degradation of noxious waste products and toxins; Figure 1C).

In the past several years, a different type of microalgal–microbial interaction has come into focus: the production of and response to signaling chemicals and toxins. For example, some microalgae produce negative allelochemicals to compete with other microalgae or cyanobacteria within biofilms [10]. Other microalgae produce quorum-sensing mimics, probably as a means to interfere with bacterial communication [11]. The bloom-forming haptophyte *Emiliania huxleyi* collaborates with α-proteobacteria of the Roseobacter clade to provide organic carbon and sulfur in the form of dimethylsulfoniopropionate (DMSP) in return for antibiotics effective against other bacteria [12]. This mutualistic phase is terminated by a pathogenic phase involving the bacterial production of algicidal toxins. Such biphasic patterns may also govern the interactions of Roseobacter with dinoflagellates and may explain natural patterns of algal bloom formation and collapse [13].

Other antagonistic interactions include the encapsulation of haptophytes (*Phaeocystis* spp.) by *Acantharia*, a group of grazing zooplankton, in exchange for dimethylated sulfur compounds [14]. Physical association may also help to ensure generational persistence of partnerships (Figure 1C). To discern the types of interaction involved, a careful molecular characterization of microalgal–microbial associations is critical.

Division of Labor: Metabolic Complementation and Cooperative Biosynthesis

In laboratory co-culture experiments, the *Chlamydomonas* genus of green algae was found to trade nitrogen for carbon with a broad range of free-living ascomycetous fungi, including several genetically tractable model species (e.g., *Saccharomyces cerevisiae*, *Aspergillus nidulans*, *Neurospora crassa*) (Figure 1B) [15]. Although induced by artificial conditions, the phylogenetic breadth of this mutualism and the physical associations of alga with filamentous fungi (that resemble those



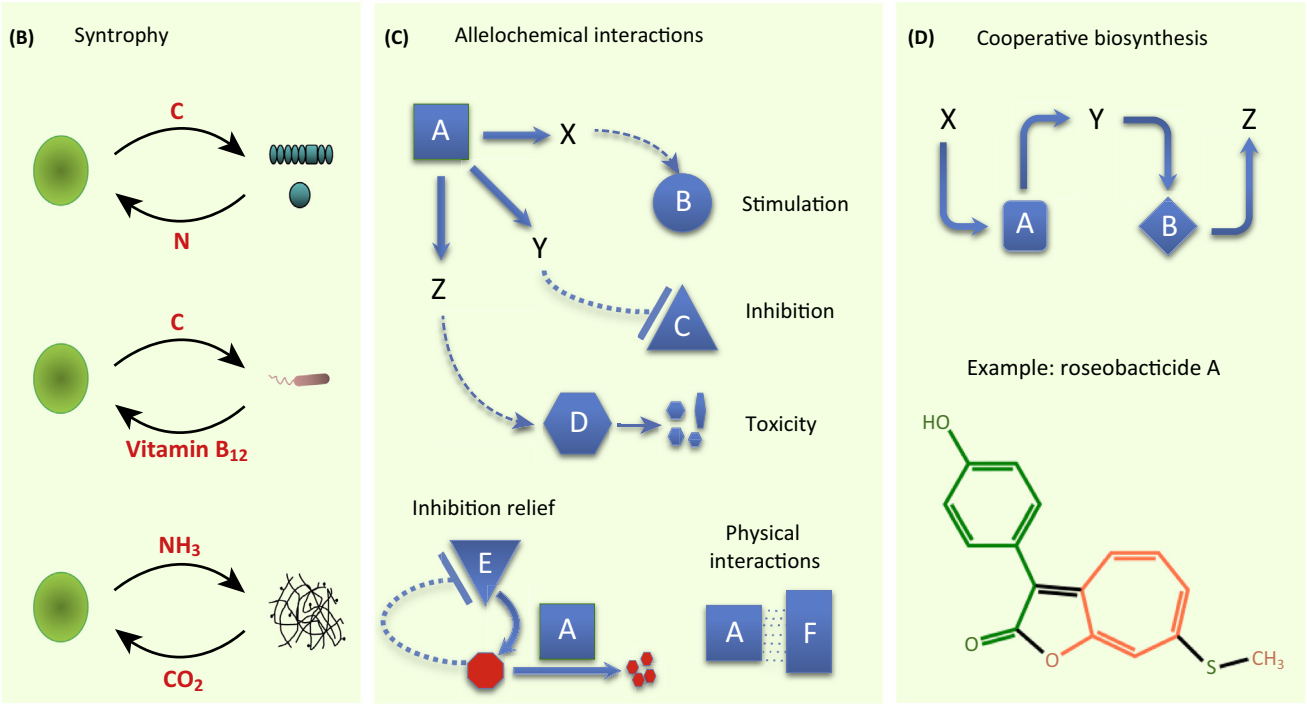


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Figure 1. The Interactions between Microalgae and Other Microorganisms Can Be Studied by Various Approaches and Comprise Various Types. (A) Analysis of microbial communities. Microalgae are present in bustling microbial communities with a multitude of interactions. A traditional approach to understanding these interactions would be to co-culture microalgae with a specific microbe of interest and to compare the growth and physiological parameters in axenic versus mixed cultures. Culture-independent methods like metagenomics and metabolomics can be employed to survey the complexity of microorganisms and their interactions in the wild, especially when the microbes are difficult to culture. Combining experimental observations, bioinformatics, and modeling tools are important for making sense of

seen in extant lichens) supports the notion that many more associations between fungi and microalgae remain to be discovered in the wild; moreover, these associations may be important for our understanding of the evolution of these two taxa.

In the haptophyte-associating diazotrophic cyanobacterium UCYN-A mentioned above [7], photosystem II (PSII) is absent. This leads to a partitioning of nitrogen fixation and photosynthetic functions among different microbial 'modules', thus making it possible for nitrogen fixation to occur in the presence of light (oxygen generated within the same cell by PSII would poison nitrogenase activity). This is functionally analogous to the division of tasks between different cell types in heterocystic nitrogen-fixing cyanobacteria. Both nitrogen fixation and vitamin B_{12} biosynthesis are confined to bacteria and archaea and several microalgae can profit from these specialized metabolic capabilities of bacterial partners while providing fixed carbon in return. It will be important to delineate potential overlap in the different nutrient exchanges described above; for example, could some microalgae obtain both vitamin B_{12} and a source of nitrogen from the same bacterium?

In addition to metabolic complementation, examples of cooperative biosynthesis of secondary metabolites were recently discovered [12,16]. In a case mentioned earlier, Roseobacter incorporates both bacterial and algal building blocks to synthesize the roseobacticides, which are toxic for *E. huxleyi* (Figure 1D) [12]. Thus, microalga-based cooperation can endow communities with new biosynthetic capabilities. It remains to be seen whether cooperative biosynthesis operates within lichens in the production of novel secondary metabolites and is a common theme among other fungal–algal associations to be discovered.

Lose a Gene, Gain a Genome

Gene function loss is often viewed as an unfortunate or restricting consequence, although examples presented above suggest that this need not be true. Without the loss or absence of PSII in the diazotrophic cyanobacterium UCYN-A [7], the ecological division of labor of nitrogen fixation and photosynthesis between UCYN-A and its haptophyte partner may not have been possible. Similarly, in the fungal–algal mutualism between *Chlamydomonas* and filamentous fungi, loss of nitrite utilization ability in fungal mutants enabled the birth of stable, obligate symbiotic composites in which algal cells physically attached to fungal hyphae [15]. Without this loss in gene function, such new entities may not persist, given that the fungus could grow independently of (and faster than) the alga. In the case of vitamin B_{12}-mediated mutualisms, the loss of B_{12}-independent forms of methionine synthase in some algae [8] not only may have created a dependency on bacteria for vitamins but may have enabled access to other, as-yet-unknown bacterial resources. Thus, gene loss may facilitate the 'acquisition of a genome' via mutualism and the creation of new forms and functions that serve as substrates for further evolutionary adaptation. The order and timing of gene loss versus genome complementation as a function of ecological context is a topic that merits more active research.

Antagonism in Mutualism

Although well appreciated within the plant–microbe and macroorganism literature on mutualisms [17], the idea that various degrees of antagonism underlie net cooperative associations in microalgal–microbial mutualisms is becoming clear only as the specific biochemical details of association are elucidated. The *E. huxleyi*–Roseobacter interaction described above is a classic example of the delicate balance between beneficial and pathological partner behavior and of the complex 'chemical dance' that can exist between microalgae and bacteria [12]. For the synthetic fungal–algal mutualisms created between *Chlamydomonas* and saprotrophic fungi like *A. nidulans*, it was posited that the physical association observed between alga and fungus is a consequence of fungal cell-wall-remodeling enzymes that partially digest the algal cell wall [15]. Although physical attachment and thinning of algal cell walls may facilitate nutrient exchange, a fragile balance may exist where too much digestion may lead to the death of the algal partner. The degree of this fungal–algal mutualism could also be tuned by environmental context or genetic background [15]. In both of these examples, a regulated compromise is key for cooperative fitness. Although the existence of antagonism may be masked by an overall beneficial relationship and the mutual net positive fitness of partners, a change in environmental context may reveal this antagonism and fundamentally alter the ledger weights for positive and negative interactions.

microalgal–microbial interactions in complex microbial communities and establishing a framework for making predictions. (B) Examples of syntrophy involving microalgae. Depicted are the reciprocal exchange of fixed carbon and nitrogen between diatoms and filamentous cyanobacteria [6] and between a haptophyte and a unicellular cyanobacterium [7] (top), fixed carbon and vitamin B_{12} between various microalgae and heterotrophic bacteria (middle) [8], and NH_3 and CO_2 between *Chlamydomonas* and various fungi (bottom) [15]. (C) Modes of cooperative and antagonistic microbial interactions. In the course of allelochemical interactions (top), microorganism A may secrete compounds that stimulate, inhibit, or even kill other microorganisms (compounds X, Y, and Z, respectively). Microorganism A may also support microorganism E via the degradation of noxious waste products and toxins (inhibition relief, bottom left) and interactions may also involve physical contact (bottom right). (D) Cooperative biosynthesis. For the biosynthesis of a final compound Z, microorganism B modifies an intermediary compound Y from microorganism A (top). Cooperative biosynthesis differs from induced biosynthesis (not shown), where compound Y only induces the expression of biosynthesis genes in B and is not used as a building block for compound Z. The example (bottom) shows roseobacticide A, synthesized by Roseobacter in association with the haptophyte *Emiliania huxleyi*; portions derived from bacterial (orange) or algal (dark green) building blocks are highlighted.

Box 1. Outstanding Questions

How do microalgae and other microorganisms recognize each other and how do they physically associate? Why do they associate? How are these interactions regulated and what chemical signals and receptors are involved in these processes? What is the identity of the exchanged nutrients?

What are the natural functions of bioactive compounds such as antibiotics or toxins? How and why is their production triggered, regulated, and correlated with microbial community dynamics?

How specific and how dynamic are microalgal–microbial interactions? How and why are associations modulated by the presence of additional microorganisms (multipartner systems) and can these effects be predicted?

To what extent do spatial colocalization, physical association, or temporal expression windows drive the evolution of these interactions and shape the genomes of the microorganisms involved?

How and why are these interactions formed and shaped by environmental conditions? What are the major factors and mechanisms that govern diversity within microbial communities?

What are the ecological consequences of microalgal–microbial interactions and of their dysfunction?

How can we quantify the fitness cost and benefit of microalgal–microbial interactions and use this to make quantitative predictions about partner stoichiometry, community stability, and ecosystem function?

How can studies on microalgal–microbial interactions help us to understand the origins and evolution of secondary and tertiary symbioses?

Concluding Remarks and Future Outlook

Recent investigations on microalgal–microbial associations have helped to shed light on this neglected, although ecologically significant, suite of interactions. These studies also raise important questions that may help guide future research (Box 1). For example, it is often unclear how microalgae recognize other microorganisms, how they physically associate, and how environmental conditions modulate their behavior. It is also unknown how the bipartite microalgal–microbial associations studied to date fit within the broader context of their natural community, in which there may be additional, simultaneously interacting microorganisms. For example, recent data suggest that bacteria that are stably associated with lichens make important contributions to lichen metabolism and stress control [18]. Mechanistic clarity about important system features and processes is likely to come from first studying simple, well-defined systems of interacting microorganisms.

Methodological advances in flow cytometry and single-cell omics will soon make it routinely possible to analyze the genome expression programs of individual cells within microbial communities and shed light on the interactions of microalgae with microbes in their native contexts. Mass spectrometry imaging will be particularly useful for chemically analyzing microbe-covered surfaces with a spatial resolution of less than 1 μm [19]. Continuous-culture studies and evolution experiments using simplified or engineered communities are likely to yield fundamental insights into the processes and mechanisms that also apply to complex, natural communities. Results from the application of these approaches are expected to facilitate the manipulation of microbial communities in nature or in algaculturing facilities as well as support the development of critically needed new lead compounds for bio- and agrotechnology.

Acknowledgments

The authors thank Ute Holtzegel (Friedrich Schiller University, Jena) for helpful comments on the manuscript. E.F.Y.H. thanks Michael McDonald (Harvard University) for inspiring the phrase 'lose a gene, gain a genome' and Michael Clear (University of Mississippi) for his assistance with the UM Field Station site photograph used in Figure 1. M.M., S.S., P.A., and D.S. are grateful for support from the DFG-funded Collaborative Research Centre ChemBioSys (SFB 1127). P.A. is a fellow of the International Leibniz Research School (ILRS), which is under the umbrella of the Jena School for Microbial Communication (JSMC).

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http://dx.doi.org/10.1016/j.tplants.2015.09.004

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