Community Ecology of Algal Biofuels: Complementarity and Trait-Based Approaches

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Abstract

Although open outdoor pond systems are the most economically viable option for mass cultivation of algae as a biofuel source, such systems face a number of limitations. Open ponds experience environmental fluctuations (i.e., light levels, nutrient ratios, and temperature), invasion pressure by undesired algal species, pathogen infections, and herbivory by invading zooplankton, all of which may negatively influence the system's overall harvestable yield. Using ecological principles to address the limitations of open-pond cultivation is a promising direction in algal biofuel research. This review surveys the growing body of work on these topics and offers a mechanistic framework for optimizing algal biofuel production while minimizing the negative effects of invasion, infection, and herbivory. High levels of productivity (in terms of biomass and lipids) are crucial for viable biofuel production and can be achieved by increasing algal diversity and assembling communities based on species' eco-physiological traits. Herbivory can be significantly reduced by choosing algal species resistant to grazing or by introducing biotic controls on herbivores. Diverse assemblages of algal species can be constructed to fill in the available ecological niche space, leading not only to high productivity but also reduced invasibility by undesired strains and potentially reduced susceptibility to algal diseases. Optimization of the mass cultivation of algae requires an interdisciplinary approach that includes using ecological principles for designing productive, resistant, and resilient algal communities.

Key words: Traits, microalgae, diversity, open ponds, invasion, top-down, bottom-up, complementarity, productivity, lipids

Introduction

Alternative renewable fuel sources have become an active area of research, as global fossil fuel reserves are estimated to be depleted within the next 60 years and unprecedented anthropogenic combustion of hydrocarbons has led to increased atmospheric carbon dioxide (CO_2) levels and climatic changes.[1–3] One key renewable source is living biomass, or biofuels. But, for biofuel production to be economically viable and environmentally sustainable, the feedstock source must be highly and consistently productive, be scalable to industrial levels, be distinct from food production networks, require little energy and nutrient inputs, and have a low carbon footprint.[4,5] Microalgae are considered among the most promising biomass-based renewable fuels. Microalgae play a number of important roles in the ecosystem, from contributing over half of the global primary production, sequestering large amounts of CO_2, and supplying nutrition for primary consumers.[6–9] They first gained attention as a potential source of biofuel in the mid-1980s through the Department of Energy's Aquatic Species Program (ASP).[10] Fifteen years after the end of the ASP, an interest in algal-based biofuel technology has been reinvigorated. Microalgae are an excellent biofuel crop due to their potentially high levels of productivity, limited land requirements for production, and small carbon footprint from growth to combustion.[11]

Although highly promising, the full potential of algal biofuels has not been realized at the commercial level. A number of limitations have emerged that must be dealt with, e.g., identification of suitable strains and optimization of harvesting and extraction methods. Many studies have developed screening programs to identify algal species that have high biomass lipid content.[9,12,13] Some of these bioprospecting studies found that algal cells can be stimulated to synthesize lipids when exposed to stressful conditions. The most common stressor that can increase lipid synthesis, resulting in higher lipid content, is nitrogen limitation.[14–16] Some species, such as *Botryococcus braunii*, have been identified as having high internal lipid stores ($>80\%$ dry weight), making them ideal candidates for biofuel production.[9] However, there is a trade-off between lipid production and growth, with highest lipid content occurring in slow-growing cells.[17] This trade-off underscores the need for an alternative approach in which highly productive or fast-growing algae could still have higher energy content. Consequently, studies have focused on genetically manipulating species in an attempt to hyper-activate the metabolic pathway responsible for lipid synthesis but still allow for high growth rates.[18,19] Alternatively, there are also ecological options to optimize algal production without creating genetically modified organisms (GMO) that may pose a risk for natural communities.

Through a review of the ecological literature, we will present ideas on how algal biofuel production can be optimized through the promotion of functionally diverse algal assemblages with complementary eco-physiological traits that can increase productivity and reduce invasibility by undesired algae and susceptibility to pathogen infections, while maintaining stable yields through highly variable outdoor environmental conditions.

Our review builds on and extends previous syntheses on the topic by presenting some of our own data analysis and an in-depth discussion of trait-based approaches to assembling algal communities, especially for variable environmental conditions. We also present thoughts on reducing pathogen impacts, discuss light utilization—a key mechanism for niche complementarity in algal biofuel communities that has not been discussed previously–and provide novel insights into how top-down pressures may impact algal crop populations. Finally, we extend these ecological principles to aquaculture applications.

Cultivation Systems for Microalgal Biomass Production

Two main cultivation approaches for microalgal biomass production have been identified; a more industrial approach that uses photobioreactors (PBRs) requires a large amount of infrastructure and technical upkeep, whereas open cultivation is a more agricultural approach that requires less infrastructure and investment. Both have distinct benefits and limitations.[20] PBRs are closed systems of clear plastic or glass tubes that allow solar radiation to penetrate the circulating algal culture. These systems are costly and often plagued by heat production and oxygen buildup. On the other hand, open ponds, or raceway systems, require limited amounts of construction and infrastructure, making them the most economical option available for scaling to industrial (agricultural) levels. However, these open systems are exposed to the natural environment and, therefore, experience environmental fluctuations (e.g., temperature, light) and invasions by unwanted algal species, herbivores, and pathogens (e.g., chytrid fungi). Consequently, for these open-pond systems to be a viable mass-cultivation option monocultures or polycultures of algal species that can persist throughout these fluctuations and invasion pressures while yielding high biomass and lipid content need to be identified. We suggest herein that, by taking into account local environmental conditions and algal trait data, multi-species algal communities that optimize the desired goal functions (e.g., high biomass and/or lipid yield) under given conditions can be assembled, much like piecing together a puzzle (*Fig. 1*). Such communities will not only realize increased energy output but may also limit successful invasion events by undesired (local) algal species.

Diversity-Productivity Relationships in Microalgal Communities

Open-pond systems will be continuously invaded by other organisms with high dispersal rates, such as undesired algae and zooplankton grazers, resulting in a diverse community.[21] However, a diverse community is not necessarily a disadvantage for open-pond cultivation systems and may be more economically feasible than monocultures. Higher species diversity often leads to higher productivity (in terms of biomass and lipid content), as was first observed in grasslands.[22] At least two non-exclusive mechanisms have been proposed for the observed positive diversity-productivity relationship: first, a ''sampling effect'' (*Table 1*), whereby a highly productive species in a set number of possible species has a higher likelihood of being present in a more diverse community, thus increasing the overall biomass.[23] Another proposed mechanism is the ''complementarity effect,'' in which an assortment of species better fills the available niche space than any individual species (*Table 1*), ultimately achieving highly efficient use of available nutrients and leading to an overall increase in productivity (*Fig. 2A*).[23,24] However, some studies in natural and artificial communities have also found a negative diversity-productivity relationship, in which monocultures achieved higher productivity than mixtures of multiple algal species, suggesting the importance of species characteristics (traits), rather than just the number of species.[25,26]

These two mechanisms can act in tandem (in most cases one is emphasized), and result in an overall net biodiversity effect or benefit (*Table 1*). This net biodiversity effect is defined as the observed yield in the mixture minus the theoretical, or expected, yield of the mixture estimated from the mixture composition and the constituent species monoculture yields.[27] Recently, a positive diversity-productivity relationship has been described for microalgal communities that is driven by complementarity, facilitation, and resource-use efficiency.[28–31]

A crucial component of investigating the diversity-productivity relationship is defining diversity, because it can have different meanings. The majority of studies define diversity simply as species richness, which is the total number of species present in the system being studied.[32] However, other aspects of diversity must also be considered, such as functional diversity or functional composition.[32,33] In this case, a ''functional group'' in ecology represents several species grouped based on their common biochemical and/or ecological functions (*Table 1*). In microalgal communities, these functional differences often correspond to major taxonomic groupings, e.g., diatoms, green algae, cyanobacteria, etc., because these taxonomic groups typically differ in their physiologies and biogeochemical roles.[34] Promoting species richness per se as a measure of diversity may result in a species pool consisting of a single functional group. Such an assemblage would have highly overlapping physiological characteristics (traits) (e.g., similar light-harvesting pigments, nutrient requirements), thus reducing the possibility for resource-use complementarity (*Fig. 2A*). For example, in our previous work, a community of three green algae was assembled and no significant increase in total lipid production was detected compared to individual monocultures.[35] Because all the species within the community belong to the same functional group, the polyculture was a functionally redundant mixture, with highly overlapping and similar resource requirements and traits. Functional richness is thus more important than species richness.[36] Therefore, promoting diversity by increasing the number of functional groups or trait differentiation is often more efficient for resource use and, consequently, productivity and harvestable yield.

Diversity, Light, and Lipid Production

Most work on the diversity-productivity relationship has focused primarily on biomass production. Recently, Smith et al. hypothesized that diverse microalgal communities in open pond

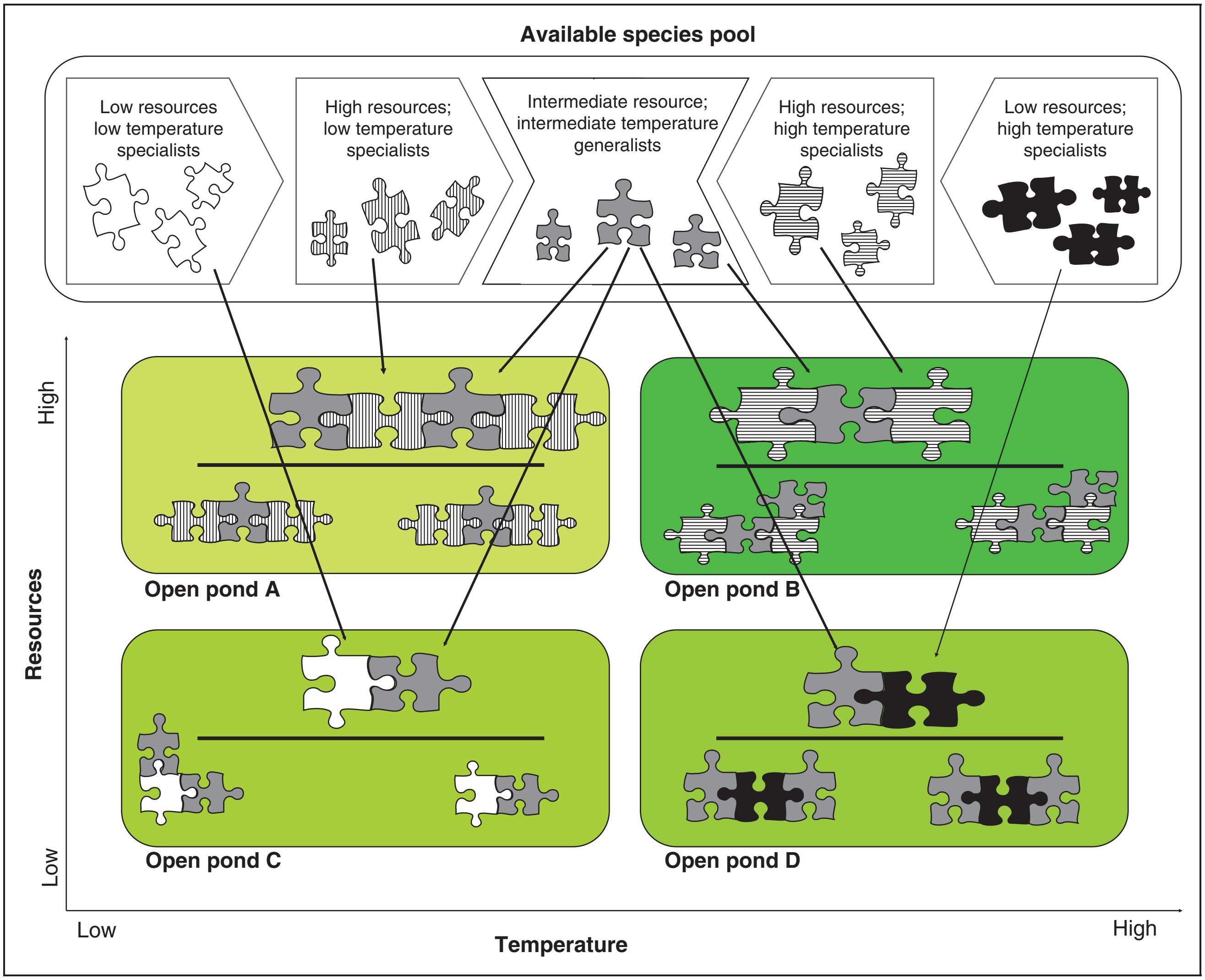

Fig. 1. Open ponds A-D represent hypothetical outdoor ponds experiencing different ranges of temperature and resource (light, nutrients) conditions. The available species pool represents a large number of species with known traits, allowing them to be grouped into categories of specific temperature and resource ranges. Some species are more specialized in their temperature-range tolerances and nutrient demands (i.e., low temperature and low resource adapted). There are also intermediate species, or "generalists," that thrive around the environmental averages, which is an essential attribute as these environments experience fluctuating conditions. When species compositions are pieced-together to optimize trait complementarity for the specific range of local environmental conditions (from both specialists and generalists), these systems can over-yield in terms of productivity even as conditions fluctuate. For example, Pond A's local conditions are high to moderate resource levels (light and nutrients) and low to moderate temperatures. Trait-informed assemblages would be constructed from local taxa that draw from both low thermally adapted, high nutrient-demanding species and generalist species.

bioreactors might store more solar energy as lipids, compared to single species cultures in closed photobioreactors.[37] Shurin et al. also argue that certain species combinations might lead to more robust and productive biofuel systems compared to monocultures.[38] Using diversity as a tool to improve mass cultivation of algae might have important advantages.

Optimizing algal lipid production by applying ecological principles, such as the diversity-productivity theory, has been limited so far to only a few empirical studies.[31,36,39] Lipid production in microalgal communities can be enhanced through two nonexclusive mechanisms. First, increased lipid content could be due to increased biomass production as a result of higher diversity (either species richness or functional richness). Secondly, as Stockenreiter et al. showed, diversity may also influence the biomass-specific algal lipid content, resulting in individual cells having higher than expected internal lipid content, further increasing the overall lipid production.[31] The exact reason for this enhancement is unknown.

An important mechanism for a positive microalgal diversity-productivity relationship may be due to algal species' ability to capture different wavelengths of the photosynthetically available radiation (PAR) spectrum (400–700 nm). About 90%

Table 1. Definitions of Relevant Ecological Terms and Concepts Mentioned in the Text

ECOLOGICAL TERM	DEFINITION
Complementarity effect	A mechanism of species coexistence in which competition for resources is minimized by choosing similar species with different resource requirements and resource-acquisition traits. This differentiation results in a diverse array of species coexisting in an environment in which individually there is little competition and collectively the community efficiently utilizes most, if not all, of the available resources, leading to higher productivity.
Functional group	For algae, a functional group describes a group of algae that all share specific prominent characteristics (biochemical, physiological, ecological).
	For example, diatoms are a functional group of algae that all contain a silicon-based cell wall, or frustule.
Net biodiversity effect (NBE)	The difference between observed yield in a mixture and its expected yield. The expected value is the weighted average (by the actual distribution of algae in the mixture) of the monoculture yields for each species in the mixture.[a]
	NBE = (Observed yield) – (Expected yield)
Niche	The range of all biotic and abiotic environmental factors in which a given species can persist.
Sampling effect	A highly productive species, within a set pool of species, has a higher probability of being included in a more diverse mixture, thereby increasing the overall productivity of diverse communities.
Trophic cascade	A predator impacts a particular ecosystem by suppressing the abundance of that prey item, subsequently releasing the predation pressure on the lower trophic level, leading to positive population growth.
	Zooplankon (+) → (Algae (-)
	Add A Trophic Level: Fish (+) → Zooplankon (-) → Algae (+)

[a]Loreau M, Hector A. Partitioning selection and complementarity in biodiversity experiments. *Nature* 2001;412:72–76.

of carbon is bound up in macromolecules such as proteins, lipids and carbohydrates.[40] Photosynthesis is the key chemical process of carbon fixation to produce macromolecules, and therefore, lipids. Lipid synthesis has been shown to be affected by light. Moreover, light affects lipid metabolism: lipid quality can change with light fluctuations.[35,41] Lipid synthesis and storage strategies in different algal species can change depending on light conditions. Triacylglycerols (TAGs) are usually produced during light periods, and polar lipid synthesis for membranes occurs during dark periods.[42] These findings, however, are restricted to single algal species grown in monoculture; an, understanding of the effects of interactions between light and diversity on lipid production of microalgae remains limited.

Light is traditionally considered to be a homogeneous resource, but it really is a heterogeneous resource made up of different wavelengths. Microalgae have an array of light-harvesting structures (pigments) that greatly outnumber the pigments of terrestrial plants.[43] For each light-harvesting pigment there is a corresponding range of wavelengths of light that can be captured, and the wavelengths different from the pigment's corresponding range cannot be harvested, representing an unused resource (*Fig. 2B*). All microalgae use chlorophyll *a* as their main light-harvesting pigment; however, different algal groups are often characterized by their additional, accessory pigments (e.g., Cyanophyta contain phycoerythrine and/or phycocyanin, Bacillariophyta (diatoms) contain β carotene, etc.). These accessory pigments capture only small portions of the PAR spectrum, but a functionally diverse assemblage of algae would contain a broad range of light-harvesting pigments, resulting in a more efficient and higher overall use of available light compared to a single species (*Fig. 2B*).[44,45] This example highlights an important mechanism to explain the positive microalgal diversity-productivity relationship. Work on this topic is quite limited, but Stockenreiter et al. showed a possible link between light-harvesting efficiency and lipid production in algal communities.[36] The majority of communities with species belonging to different functional groups (e.g., Chlorophyta, Bacillarophyta, Cyanophyta, and Chrysophyta) had a higher lipid content compared to communities consisting only of a single functional group (e.g., Chlorophyta).[36] In functionally diverse communities, a greater variety of pigments present resulted in greater overall light-harvesting ability and higher light-use efficiency. These findings show that certain traits, such as pigment composition, when combined to optimize resource-harvesting capabilities (*Fig. 2B*), may lead to higher lipid production, as there is a correlation between absorbance and lipid content.[36] Additionally, trait variation in light acquisition might be even higher than in nutrient acquisition.[46]

In open-pond cultivation systems, algae experience highly variable light regimes, from extremely low light levels in dense cultures due to self-shading to high, often photoinhibiting levels at the pond surface. Under well-mixed conditions (paddle wheels), algae are only exposed to high irradiance for a very short period of time, which may minimize photoinhibition and still allow high photosynthetic rates. Some algal groups appear to thrive (e.g., diatoms), while others decline (e.g., green algae) under fluctuating light, so choosing appropriate groups for different cultivation conditions may enhance yields.[47]

Trait-Based Approach and Potential Benefits

Assembling a diverse algal community for biofuel production can have a multitude of positive effects. However, these effects will only be realized when species with the right traits are selected for the assemblage. The right traits can include a complementary use of resources such as light and nutrients, or different thermal or pH preferences, for example, so that, in combination, the species outperform the best-performing monocultures in a desired function (e.g., biomass or overall energy content). Therefore, we propose that a trait-based approach to

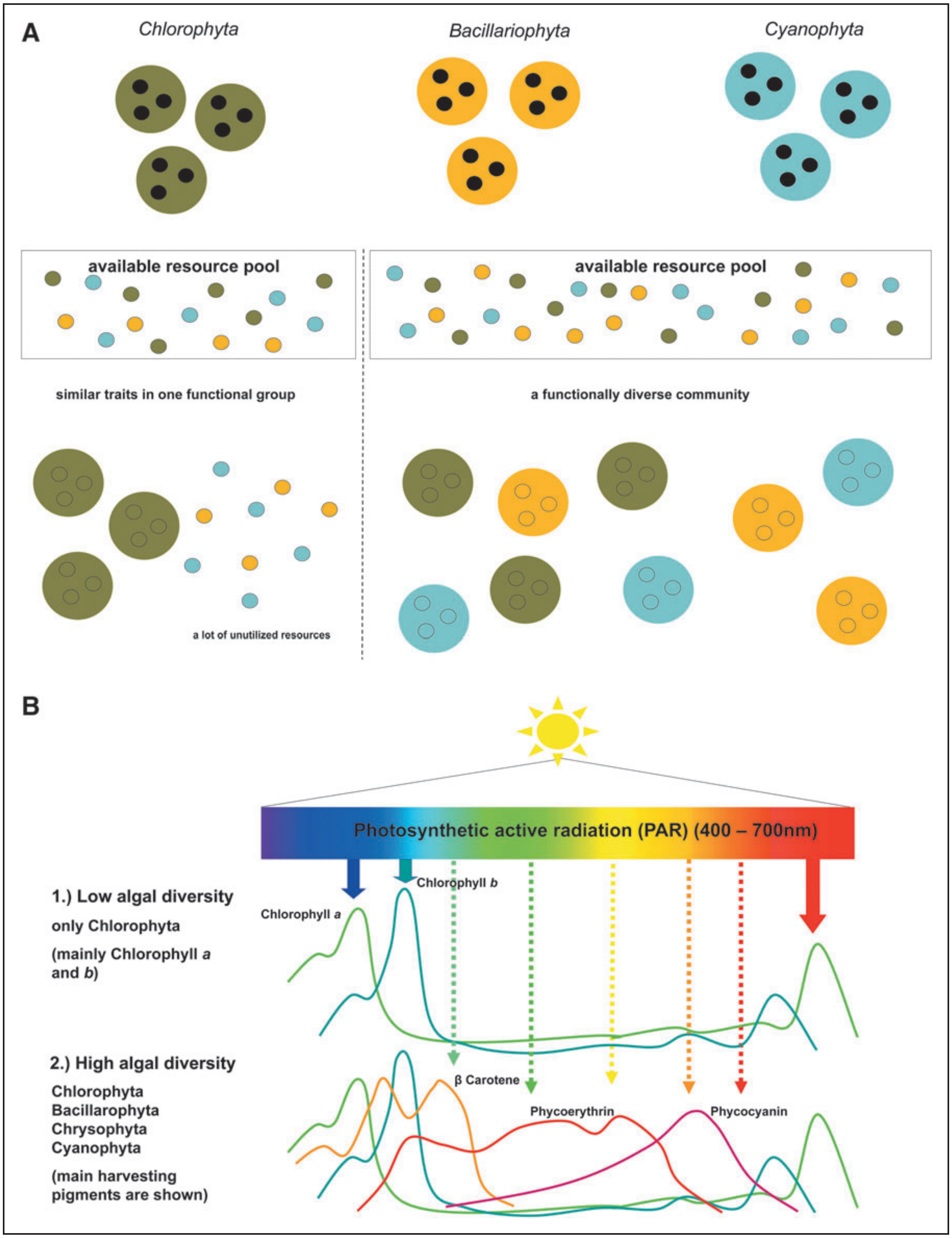

Fig. 2. Investigating the role of trait complementarity. **(A)** Functional group richness increases resource use efficiency. Species from one functional group use mainly similar resources from a given pool leaving a large portion of the resources unused. However, a functionally diverse assemblage utilizes different resources from the same pool, resulting in a higher use of available resources and leading to higher overall production (resource use complementarity). **(B)** The top portion *(1)* illustrates an algal community consisting of a single functional group (here Chlorophyta), which uses primarily Chlorophyll *a* and *b* to capture distinct wavelengths of the PAR spectrum, leaving a largely unused portion of the light resource. The bottom illustration *(2)* shows a diverse algal community consisting of multiple functional groups (Chlorophyta, Bacillariophyta, Crysophyta, Cyanophyta) that are utilizing a diverse collection of light capturing pigmentation, resulting in a larger portion of the PAR spectrum being captured. This light capture optimization through increased functional diversity leads to an efficient use of the entire light resource and can lead to increased algal productivity (i.e., biomass, lipids, energy content).

assembling communities is more efficient than just manipulating species or functional group diversity and should be the next step in algal biofuel research (*Fig. 1*).

Knowing the traits of species is crucial to using trait-based approaches.[48] Many key functional traits of microalgae have been measured, and there are several trait compilations that allow species to be characterized according to their maximum growth rates, cell sizes, and resource requirements.[49–52] The available information can help assemble communities tailored to local environmental conditions with species that have the desired trait values and combinations of traits. For example, species that are good competitors for nitrogen (N) can be placed together with species that are good competitors for phosphorus (P). There is often a trade-off between N and P competitive abilities, and such a trade-off can promote species coexistence and efficient use of these resources.[53] Assemblages of species with complementary traits are expected to perform well under fluctuating conditions. Under light fluctuations, species with high and low light requirements can coexist and utilize varying light efficiently.[54,55] N:P ratios for optimal growth are species-specific and can vary between 20:1 and 50:1 (molar ratio) or more.[56–58] Microalgal biofuel production systems that include combinations of species that vary in their stoichiometric carbon (C):N:P ratios could be used to maximize lipid production per unit of limiting nutrient.

A modular system based on complementary traits for the utilization of resources by microalgae may provide a promising method for optimizing microalgal cultivation for commercial uses under given environmental conditions. Using a trait-based approach, an assemblage of algal species could be put together that would be functionally diverse to increase light-harvesting capabilities, while the individual species within the community would also have different ranges of temperature tolerances, such that the overall system would maintain high growth under fluctuating temperatures. Finally, the species would also be selected to increase the differences in N and P requirements to maintain species coexistence, limit competition, and achieve highly efficient nutrient use. This process would be much like piecing together a multi-dimensional puzzle, such that each species would complement other species to fill the available resource/environmental factor space defined by the local environmental conditions (*Fig. 1*). As this suggests, a different algal assemblage could be constructed for each set of local environmental conditions. The selection of the best community should be aided by trait-based mathematical models that can achieve multi-objective optimization. Such models should include key aspects of algal physiology and ecological interactions and the information on relevant traits. For example, an algal-community model describing the growth dependence of each species on light, nutrients, and temperature can be forced by the specified environmental conditions, and different sets of species with defined traits could be tested. A combination of species that is predicted to maximize a desired output (e.g., biomass, lipid yield) could be selected for experiments and cultivation.

Ecological and physiological trade-offs of microalgal species may also prove to be extremely beneficial in assembling highly productive algal biofuel communities. Algal growth rates inversely relate to cellular lipid content and also total energy content (*Fig. 3A*).[17,59] There is also a trade-off between growth rate and cell size, where species with large cells have low growth rates (*Fig. 3B*).[60] These large-celled species tend to be grazer-resistant, which can help minimize yield loss due to zooplankton grazing, as discussed below (*Fig. 3C*). Thus, a logical extension of this knowledge of trade-offs leads us to posit that larger-celled and, thus, more grazer-resistant species, may have higher levels of overall energy content (*Fig. 3D*). It would be illuminating to test this hypothesis experimentally.

It is important to identify the relevant traits and trade-offs in microalgae that also help explain the mechanisms of species coexistence and diversity.[61] Different environmental conditions, such as varying light intensities and spectral characteristics, nutrient availability, and temperature have distinct impacts on microalgal cellular composition and community structure. Therefore, trying to identify species that grow best under given environmental conditions and then using those species to assemble microalgal communities suitable for different growth environments is a promising strategy. Communities assembled by analyzing a priori how microalgal traits match growth conditions might provide a predictable system for a guaranteed supply of biomass and lipids (*Fig. 1*). Trait-based approaches are being used in terrestrial plant ecology and phytoplankton ecology to explain community structure along major environmental gradients and to predict ecological community responses to global environmental change.[51,52,62] Trait-based approaches use trait information to help predict community assembly and ecological functioning (productivity, stability).[63,64] This framework has been applied to assembling native terrestrial plant communities that resist the establishment of exotic species.[65] This same approach of relying on algal traits to inform community assembly should also be developed and used in microalgal biomass and lipid production to achieve high ecological functioning and reduce invasibility.

Managing Diverse Microalgal Communities in Open Ponds

ENGINEERING ASSEMBLAGES OF MICROALGAL SPECIES TO OPTIMIZE STABILITY AND RESILIENCE

Selecting the optimal combination of traits that will lead to a high lipid yield in diverse communities is an important step towards the mass cultivation of algal biomass for biofuel. However, another critical requirement for the assembled communities is that they maintain stability and resilience.[66] Species with desired traits should be able to coexist stably, and communities should not fluctuate widely in the face of disturbance (stability), and they should be able to rebound after it (resilience). Often, competition for resources can lead to competitive exclusion of inferior competitors and decreased diversity, the so-called competitive exclusion principle.[67] Using species with complementary traits, e.g., good nitrogen and light competitors, may promote coexistence.

Several studies have shown that diversity increases not only productivity in terms of biomass and/or lipids, but the stability of algal communities as well.[29-,31,36,39] A classic study by Tilman

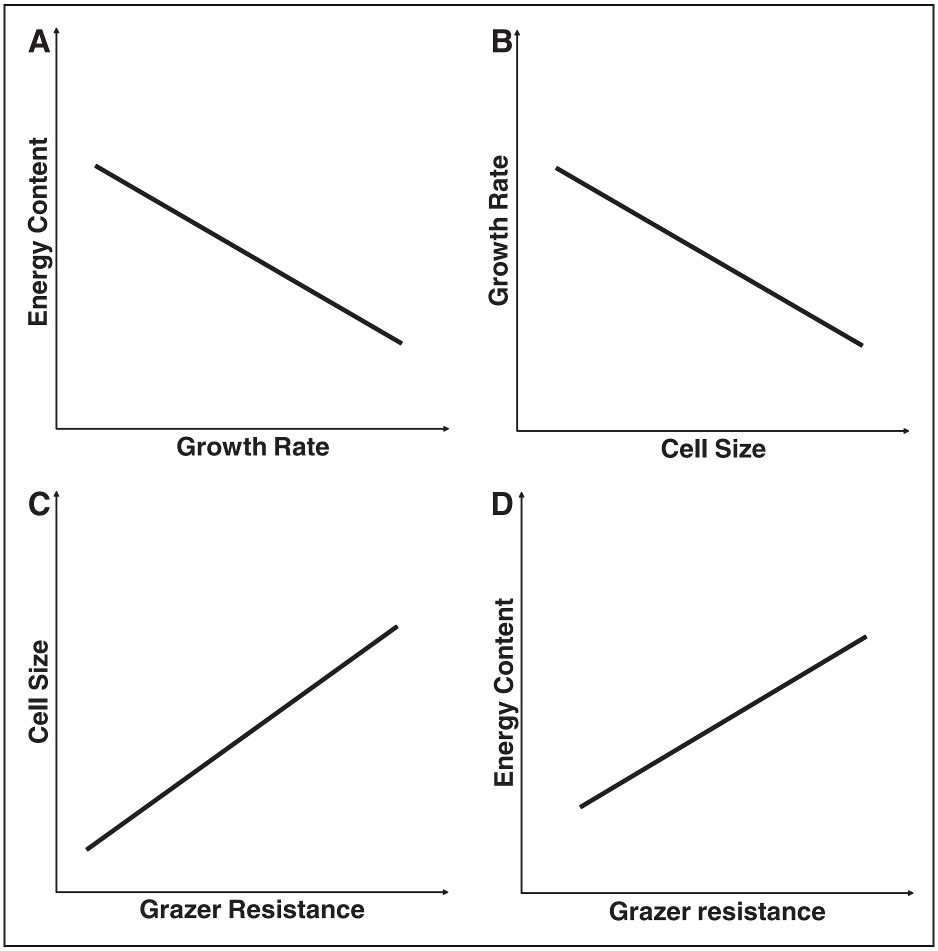

Fig. 3. (A) The energy content of algae is species-specific and highly variable. However, total energy content tends to be inversely proportional to the growth rate of algae.[17,59] **(B)** Growth rates of smaller algae are observed to be higher than larger-sized algae.[60] **(C)** Individual cell size is proportional to resistance to grazing pressures. Larger cells have higher grazer resistance. Colonizing of small algae (increasing total size) and developing anti-predatory accouterments (spines, thick sheaths) effectively increase overall size and thus grazer resistance. Investment in thick, carbon-rich sheaths may increase overall energy content of the cells without increasing total internal cellular lipid content. **(D)** Drawing from the logical progression of **A-C,** one could hypothetically assume that algae with high grazer resistance might have higher energy content.

and Downing found that, in grasslands, higher levels of species diversity resulted in more stable biomass levels when communities experienced environmental perturbation (drought) and greater resilience, so that community biomass returned to the pre-disturbance level faster.[68] The hypothesized mechanism for stability and resilience is that diverse communities had more drought-resistant species, thus underscoring the importance of certain trait combinations. A follow-up study by Tilman et al. supported and extended their earlier results, showing that diversity helps hedge against temporal variation resulting in consistent biomass yields.[69] Corcoran and Boeing showed that both species composition and species richness are important in driving patterns of stability.[70] Additionally, a recent study by Cardinale et al. determined that biodiversity enhanced both productivity and stability, but these effects were independent of one another.[71]

Cultivating algal communities in outdoor ponds poses a serious challenge to maintaining desired species composition, since these ponds experience fluctuating environmental conditions—especially light and temperature—on multiple temporal scales (daily and seasonal). Fluctuations could have dramatic effects on the species assemblages. If they exceed the environmental tolerances of the desired species, the conditions could lead to culture crashes, invasion, and establishment of undesired algal species—potentially resulting in lower productivity. Recent studies have concluded that in a diverse community experiencing a single or multiple environmental stressors, species composition and species-specific responses were crucial in predicting the overall community stability.[72,73] Assembling communities based on traits should help buffer against environmental fluctuations. For example, having species with different temperature optima should enable high growth to be maintained under temperature fluctuations (*Fig. 1*).

Fluctuating environmental conditions experienced by algae in outdoor ponds may also affect the overall lipid production and fatty acid composition. Nalley et al. found that light fluctuations caused changes in fatty acid composition in mixed- and single-species microalgae cultures, and these changes were species-specific.[35] Maintaining a consistent fatty acid feedstock is a crucial component of maintaining a commercially viable biodiesel product, so understanding the implications of these environmental fluctuations is essential.[74]

Invasions

Outdoor algal ponds experience not only fluctuating environmental conditions but also face constant invasion from windborne or waterfowl-hitchhiking algae and their grazers (zooplankton). Several studies on terrestrial systems showed that another benefit of diversity is an increased resistance to invasion pressures.[75–77] The historical beginning of this diversity-invasion hypothesis comes from Elton, who states, ''[T]he balance of relatively simple communities of plants and animals is more easily upset than that of richer ones; that is…more vulnerable to invasions.'' [78] Elton's observations were supported by MacArthur's modeling work, which concluded that higher levels of occupied niche space results in lower successful establishment rates by invaders.[79] This phenomenon has become known as ''resident biotic resistance.''

Multiple empirical studies have supported this earlier work by finding an inverse relationship between resident species richness and invader biomass, likely due to filled niches in diverse communities.[75,76] However, some researchers find that promoting diversity may not enable communities to become completely resistant to invasion but would only constrain the abundance of already established invasive species.[80]

In outdoor open-pond systems, algal assemblages can be constructed to optimize the overall productivity for biofuel generation, but these assemblages may be susceptible to invasions by undesired algae and could quickly change to unproductive systems. Developing an understanding of how these open pond systems respond to invasion pressure—or more specifically, to invader establishment—is essential for consistent maintenance of desired levels of productivity. Drawing from the resident biotic-resistance theory, creating diverse assemblages of algal species with high levels of niche complementarity should constrain the growth of invading species, thus reducing the impact of the inevitable undesired algal invasion on productivity.

Another topic of concern is the potential for reverse invasion, in which algal species from open-pond systems invade the surrounding natural water bodies. An advantage of tailoring a diverse algal community to the local environmental conditions is that it can be assembled of species that most likely are already present in the surrounding natural systems. GMOs, however, can have a high potential for an accidental reverse invasion event. Accidental releases of strongly competitive GMO strains could drastically disrupt natural communities.[81]

Biotic Controls

TOP–DOWN APPROACH

Ecological interactions in open pond systems follow the same principles of natural ecosystems. However, it is likely that artificial systems will have simpler food webs than most natural lakes and ponds, resulting in communities that are more sensitive to perturbations, as we have no knowledge of the strength of their interaction links. May demonstrated that more complex food webs are prone to be less stable; however, it is the strength of interaction links that describe the stability of a food web.[82,83] Also, May's results may not be definitive, as the stability criteria were applied to individual species, rather than the aggregate community, and the theoretical communities he used were randomly assembled, which is not the case for natural communities. Ecological interactions between microalgal communities and herbivorous zooplankton in artificially assembled food webs have to be studied in more detail to integrate them into commercial production systems and optimize biomass yields.

Top-down control of producer biomass by higher trophic levels is one such process. Herbivorous zooplankton can invade open cultivation ponds, creating a simple two-level food web (primary producers and consumers). Herbivory by zooplankton can have tremendous impacts on the phytoplankton community. The direction and strength of zooplankton grazing impacts depend significantly on cell or colony size of phytoplankton species, as size influences phytoplankton edibility. Small, highly edible phytoplankton usually decline in the presence of herbivorous zooplankton, and the algal community shifts towards dominance by large, poorly edible algae.[37,84] *Daphnia*, in particular, can promote the growth of large, inedible, and fast-sinking algae that could be harvested more easily than small species. If large, fast-sinking species have higher lipid content (*Figs. 3B-C*), lipid content and yield can be influenced by introducing grazers that benefit large species within the desired microalgal groups. Grazing periodicity (e.g., diel vertical migration) and the initial algal composition can also mediate the responses to grazing.[85] Drawing from the ecological principles of the top-down control and trophic cascades, undesirable reductions in algal biomass can be minimized by introducing zooplanktivorous fish that decrease zooplankton and, thus, release phytoplankton from grazing pressure.[37]

PATHOGENS

Pathogen infections, specifically by chytrid fungi, are another threat to algal ponds. Chytrid fungi have been shown to alter the competitive abilities of algal species that could impact community composition and species succession.[86] Chytrid infections can lead to pond-wide epidemics, completely decimating phytoplankton species within days to weeks of observed infection and crashing the biomass in both natural and algal biofuel open ponds.[38,86] One approach to controlling chytrid infection is the application of fungicide, which can lead to a sharp reduction of the chytrid population.[38] Another approach could be promoting a diverse assemblage of algal species, thus reducing the densities of potential hosts that would result in lower infection susceptibility, especially for the pathogens with high host specificity.[38,86]

Other Microalgal Applications: Aquaculture

The production of biofuel is only one of many possible applications of microalgal cultivation. Several other applications, such as health food, animal feed, fertilizers, and bioplastics, are also important.[87–90] Producing feedstock is one widely used algal application; currently, 30% of the algal biomass produced goes into the feed market, with algae mainly used for zooplankton and/or fish feed in aquaculture.[91] The food quality of microalgae is crucial for transferring energy to higher trophic levels and is determined by the cellular amounts of carbohydrates, lipids, and proteins as well as the carbon-to-nutrient ratio. Low carbon-to-nutrient ratios (in most cases due to high levels of phosphorus and/or nitrogen) result in high-quality food for herbivorous zooplankton.[92,93] Another major factor in food quality is the fatty acid composition—expressed in terms of essential omega 3-polyunsaturated fatty acids (ω3-PUFAs) of primary producers—because animals are incapable of synthesizing certain ω3-PUFAs de novo.

Most modern aquaculture systems focus on particular microalgal strains that have the desired properties. However, in natural ecosystems, zooplankton and algae-eating fish are exposed to a variety of microalgal species living in mixed communities. Phytoplankton ω3-PUFA content positively correlates with nutrient concentration (phosphorus) in the system.[8] In addition, as Stockenreiter et al. showed, microalgal diversity influences their fatty acid composition, especially by increasing the essential ω3-

PUFAs such as α-linolenic acid.[39] Therefore, higher nutrient input and diversity of algal aquaculture communities may increase energy transfer efficiency from primary producers to consumers and, thus, increase aquaculture productivity.

Conclusions

Open-pond algal systems are currently viewed as the most economically viable cultivation system for the mass production of algae-derived biofuel. These systems will face a number of environmental pressures, from fluctuating conditions (light, temperature, nutrient ratios) to herbivory, infections, and invasions by undesired algal species. One approach would be the artificial selection or genetic modification of algal strains that can persist under extremely harsh growth conditions, such as high salinity, so that no other organisms (competitors, pathogens, or herbivores) can become established in such ponds. We argue that a more effective approach would be to use fundamental ecological principles to design algal assemblages with desired properties for outdoor open ponds (*Fig. 1*). By promoting algal diversity within these systems and selecting species with complementary traits, biomass and lipid yields may exceed those of highly productive monocultures. These artificial algal assemblages can better weather environmental fluctuations and remain stable through invasion, herbivory, and pathogen infection events, all while yielding consistent biofuel feedstock. However, further work is needed to determine the optimal assemblages for local environmental conditions, as well as to focus on scale-up to industrial-scale levels. We believe that a number of benefits can be realized through diversity and trait-based research, ultimately taking this developing technology a step closer to industrial-scale reality.

Acknowledgments

This work was supported by National Science Foundation (NSF) grant CBET-1134215 (to E. Litchman and C.A. Klausmeier), and an NSF GK-12 graduate fellowship (to J.O. Nalley). This is Kellogg Biological Station contribution No. 1716.

Author Disclosure Statement

No competing financial interests exist.

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