

REVIEW AND SYNTHESIS

Industrial-strength ecology: trade-offs and opportunities in algal biofuel production

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Abstract

Microalgae represent one of the most promising groups of candidate organisms for replacing fossil fuels with contemporary primary production as a renewable source of energy. Algae can produce many times more biomass per unit area than terrestrial crop plants, easing the competing demands for land with food crops and native ecosystems. However, several aspects of algal biology present unique challenges to the industrial-scale aquaculture of photosynthetic microorganisms. These include high susceptibility to invading aquatic consumers and weeds, as well as prodigious requirements for nutrients that may compete with the fertiliser demands of other crops. Most research on algal biofuel technologies approaches these problems from a cellular or genetic perspective, attempting either to engineer or select algal strains with particular traits. However, inherent functional trade-offs may limit the capacity of genetic selection or synthetic biology to simultaneously optimise multiple functional traits for biofuel productivity and resilience. We argue that a community engineering approach that manages microalgal diversity, species composition and environmental conditions may lead to more robust and productive biofuel ecosystems. We review evidence for trade-offs, challenges and opportunities in algal biofuel cultivation with a goal of guiding research towards intensifying bioenergy production using established principles of community and ecosystem ecology.

Keywords

Bioenergy, chemical ecology, diversity, GMOs, micro-algae, phytoplankton, stoichiometry, trade-offs.

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INTRODUCTION

Appropriation of the metabolic products of microorganisms as sources of food, energy and specialised organic compounds represents a growing form of agriculture. One of the most tantalising prospects is generating new sources of transportation energy using the fatty acids and other organic compounds produced by phytoplankton. Being microscopic, algae have far higher rates of biomass production than terrestrial plants and therefore have much smaller footprints for land required for producing energy in quantities required for the global economy (Chisti 2007; Mata *et al.* 2010; Georgianna & Mayfield 2012). However, extrapolating experimental results from the laboratory or field to make predictions about the performance of algae in industrial-scale aquaculture are fraught with uncertainties. Much of the research aimed at commercialising algal biofuels to date has focused primarily on manipulating genetics and cellular metabolism to maximise the yield of energy-rich compounds. Here, we review aspects of the ecology of phytoplankton that are relevant to their cultivation as bioenergy crops. Insights from algal ecology reveal a number of challenges limiting their

potential production of biofuels, and also suggest opportunities for ecological approaches to managing their productivity, biochemical profiles and pests.

WHAT CONTROLS ALGAL PRODUCTIVITY?

The biotic and abiotic factors that govern the productivity of natural phytoplankton communities are well established: nutrients, light, temperature and losses to grazers. Fertilisation with inorganic nutrients is a sure route to establishing dense algal populations; however, the identity of the primary growth-limiting nutrient(s) remains a source of vigorous debate in aquatic ecology (Conley *et al.* 2009; Harpole *et al.* 2011). While excess phosphorus loading is a major cause of eutrophication of surface waters (Carpenter *et al.* 1998), the role of nitrogen is contentious (Smith 2003; Schindler *et al.* 2008; Conley *et al.* 2009). The importance of inorganic nitrogen supply to algal production depends on the favourability of conditions required for N₂ fixation (Conley *et al.* 2009) and denitrification (McCrackin & Elser 2010). Co-limitation by multiple resources can arise through physiological or ecological mechanisms, and has been

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demonstrated frequently in fertilisation experiments with both aquatic and terrestrial autotrophs (Harpole *et al.* 2011). Light becomes limiting due to self-shading under eutrophic conditions where nutrients are plentiful and algal biomass is high, resulting in reduced nutrient uptake efficiency by algal cells. Thus, the resources that potentially limit algal biomass production can vary greatly depending on local environmental conditions.

In addition to influencing the production of algal biomass, the relative supplies of different mineral nutrients and light can have strong effects on the species composition, elemental stoichiometry and biochemistry of phytoplankton, and therefore their value as biofuel feedstocks. Large supplies of P relative to N may favour competitive dominance by heterocystous N₂-fixing cyanobacteria (Smith 1983; Schindler *et al.* 2008), which have low cellular lipid contents (Griffiths & Harrison 2009; Rodolfi *et al.* 2009). However, a range of other environmental factors including salinity (Conley *et al.* 2009), temperature (Johnk *et al.* 2008; Paerl & Huisman 2008) and light (Pinto & Litchman 2010) determine whether N₂-fixers become prevalent in phytoplankton communities at any given N : P supply ratio. In addition, many but not all species of eukaryotic algae accumulate lipid in their cells under conditions of N-starvation when carbon is primarily used to produce either starches or lipids instead of proteins (Spoehr & Milner 1949; Rodolfi *et al.* 2009). This intraspecific variation in cellular lipids represents a trade-off between optimising the yields of total algal biomass vs. lipids in large-scale production facilities designed to harvest lipids for downstream transesterification into liquid biodiesel.

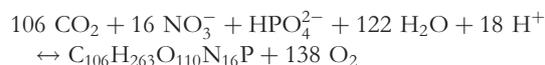
Productivity patterns in natural ecosystems indicate that fertilisation strategies need to be carefully considered to avoid excess nutrient loading that can contribute to downstream eutrophication, shift the competitive balance between targeted algal crop species and invasive algal weeds, and maintain both rapid growth and a favourable lipid content of algal biomass. Water from domestic wastewater treatment plants or livestock operations offers a potential source of cheap nutrients, and also provides the ancillary benefit of recycling of nutrients that might otherwise be discharged into surface waters (Craggs *et al.* 2011; Sturm *et al.* 2012). Wastewater or sea water are likely to be the only viable sources for growing algal biofuels in many of the arid, high irradiation regions that could offer the highest production without displacing arable land. However, the variable water chemistry and microbial communities contained in these water supplies may preclude the possibility for maintaining monocultures of selected or engineered algal lineages with desirable properties for biofuels as sterilisation of the inflowing water would be infeasible and cost prohibitive.

Estimating the nutrient demands of large-scale algal biofuel production

The resource requirements of algal biofuel production have a major impact on the economics and sustainability of biomass cultivation systems (DOE 2009; Georgianna & Mayfield 2012). Large quantities of inorganic nutrients will be essential to support billion gallon per year-levels of liquid biofuel productivity. However, the life cycle impacts of these resource inputs on algal biomass cultivation have only recently been considered (Clarens *et al.* 2010; Pate *et al.* 2011).

Our knowledge of nutrient physiology and ecological stoichiometry can help inform estimates of the nutrient supply rates that will

be needed to support commercial-scale algal biofuels. Phytoplankton use photosynthesis to convert light energy into algal biomass that typically has the following elemental stoichiometry (Stumm & Morgan 1981):



The typical carbon (C) to nitrogen (N) to phosphorus (P) stoichiometry of C₁₀₆ : N₁₆ : P₁ by moles for algal biomass is commonly referred to as the Redfield ratio (Redfield 1958). Although the Redfield ratio is not universal, this 'average' cellular stoichiometry allows quantitative predictions to be made about the quantities of carbon, nitrogen and phosphorus required for algal production. Pate *et al.* (2011) used Redfield C : N : P stoichiometry to estimate the nutrient requirements of future commercial-scale algal biodiesel production. They calculated the N and P required to produce one metric tonne of dry algal biomass as follows. Converted to a dry mass basis, the C : N : P ratio in algae becomes

$$\begin{aligned} \text{C : N : P : by mass} &= (106 \text{ mol C} * 12 \text{ g C/mol C}) : \\ & (16 \text{ mol N} * 14 \text{ g N/mol N}) : \\ & (1 \text{ mol P} * 31 \text{ g P/mol P}) \\ & = 41 \text{ g C} : 7.2 \text{ g N} : 1 \text{ g P} \end{aligned}$$

Assuming that cellular carbon is on average 50% of algal dry weight, Pate *et al.* (2011) concluded that each metric tonne of dry algal biomass produced requires 88 kg of elemental N and 12 kg of elemental P if it adheres to Redfield ratio stoichiometry. Their conclusions were pessimistic for the future commercialisation of algal biofuels: the projected nutrient requirements needed to support their lowest scenario target production level (10 billion gallons of algal bio-oil feedstock per year, assuming 50% oil content in the harvested biomass) represented 44% of the total US consumption of N from ammonia, and 20% of the total US consumption of P from phosphate rock, in 2006.

The above calculations are likely to be sensitive to the potential to recycle N and P contained in algal biomass after extracting lipids, and to significant deviations from Redfield stoichiometry that have been observed (Sterner *et al.* 2008). The Redfield Ratio characterises diverse natural algal assemblages, however, and the species that make up those communities can vary considerably in elemental composition (Geider & La Roche 2002). Stoichiometry at the community level is also variable within and among habitats. Sterner *et al.* (2008) analysed more than 2000 measurements of the chemical content of suspended particulate matter from freshwater and marine ecosystems worldwide, and found that a non-Redfield stoichiometry of C₁₆₆ : N₂₀ : P₁ by moles best described the elemental composition of algae. This conclusion implies a significantly *higher* average nutrient use efficiency for algal biomass production (NUE, defined here as the number of moles of carbon fixed into algal biomass per mole of cellular N or P) than predicted by the Redfield ratio. Algal communities in small freshwater lakes, which should resemble the assemblages that would develop over time in open, freshwater raceway pond bioreactors exposed to invasions by wild algae, exhibited even higher average elemental ratios of C : P = 224 by moles and C : N = 10 by moles. These values imply that higher nitrogen- and phosphorus-use efficiencies could potentially be achieved in algal biomass production systems than assumed based on the Redfield ratio (Pate *et al.* 2011). However, the slopes of the lines relating C

content to both N and P content of seston were < 1 , indicating that the most productive water bodies (which likely bear closer resemblance to highly productive bioenergy communities) had stoichiometry closer to Redfield ratios than oligotrophic lakes.

Recent stoichiometric data from pilot-scale outdoor bioreactors indicate greater P demands but lower N requirements for biofuel production than predicted by Redfield ratios. Sturm *et al.* (2012) performed a pilot-scale algal production experiment using four bioreactors with pre-chlorination effluent withdrawn from the final clarifier of the Lawrence, KS, domestic wastewater treatment plant. Each bioreactor had a volume of 10 m^3 that was continuously aerated without CO_2 supplementation and a hydraulic residence time of 10 days after being seeded with a diverse plankton inoculum from a nearby lake. The molar stoichiometry of the dry algal biomass produced by these outdoor bioreactors was $\text{C}_{67} : \text{N}_{3.9} : \text{P}_1$, indicating far lower phosphorus-use efficiency than predicted either from the classical Redfield stoichiometry or from Sterner *et al.*'s (2008) average of $\text{C} : \text{P} = 224$ (Fig. 1a). In contrast, the biomass produced contained a much higher average $\text{C} : \text{N}$ ratio of $17 : 1$ by moles and thus a much greater nitrogen-use efficiency than predicted either by Redfield ($\text{C} : \text{N} = 6.6 : 1$) or Sterner *et al.* ($\text{C} : \text{N} = 10 : 1$, Fig. 1b). Further research is required to determine the appropriate $\text{C} : \text{N}$ and $\text{C} : \text{P}$ ratios for calculating the nitrogen and phosphorus demands of large-scale algal biofuel efforts, and must take into account large deviations from Redfield elemental stoichiometry that have been identified.

Top-down control of production

Many of the same traits that make algae highly productive also increase their susceptibility to a wide range of herbivores. Consumers including micro- and meso-zooplankton exert strong control over algal production and density (Brooks & Dodson 1965), influencing seasonal dynamics of production (Sommer 1989). Heterotrophs appropriate on average ca. 50% of net primary production in phytoplankton communities, a $3\times$ greater fraction than their counterparts in terrestrial ecosystems (Cebrian 1999). The stronger

top-down control of aquatic production may arise from the closer stoichiometric agreement between phytoplankton, which have low $\text{C} : \text{N}$ and $\text{C} : \text{P}$ ratios in their cells, and their heterotrophic consumers (Elser *et al.* 2000; Cebrian *et al.* 2009). The greater productivity of biofuels by phytoplankton relative to terrestrial crop plants therefore comes at a cost of greater susceptibility to pest outbreaks and crashes due to grazing.

In addition to being more effective herbivores, the consumers of algae also differ from many terrestrial pests in being highly dispersive and cosmopolitan in their distribution (Shurin 2000; Finlay 2002). Unicellular and metazoan heterotrophs rapidly colonise even very small water bodies (Jenkins & Buikema 1998; Cáceres & Soluk 2002; Cohen & Shurin 2003). Park *et al.* (2011) noted that attempts to grow algal monocultures in open ponds for periods longer than three months all failed due to contamination by wild algae and/or zooplankton. Preventing contamination by consumers in large, open outdoor biofuel ponds will therefore likely rely more on limiting the success and impact of colonists than preventing their dispersal. Biocontrol schemes invoking trophic cascades from fish predators have been proposed (Smith *et al.* 2010; Kazamia *et al.* 2012) and may prove effective for large crustacean grazers. However, more specialised microbial pathogens like fungi (Ibelings *et al.* 2004), viruses (Suttle 2005) or prokaryotes (Kang *et al.* 2005) can exert substantial control over algal productivity, especially in low diversity systems. These consumers may not be subject to strong top-down control via vertebrate predators, although some such as chytrids may be vulnerable to grazing by crustacean zooplankton (Kagami *et al.* 2004; Hamilton *et al.* 2012). The impacts of microconsumers of algae may be better minimised by management strategies that manipulate biomass harvesting strategy, water chemistry or utilise mixed-species algal assemblages.

Test raceway ponds at Sapphire Energy's industrial test facility in Las Cruces, NM, USA (Fig. 2) illustrate the vulnerability of cultivated algae to invasion by contaminating microorganisms. Fig. 3 shows fluctuations in the density of one cultivated algal strain (*Scenedesmus sp.*) and pathogenic fungi (chytrids) detected by quantitative PCR in three replicate ponds. Densities of chytrids (as measured by 'cycle threshold', the number of PCR amplification cycles necessary to detect a given sequence) increased in concert with algae until application of a chemical fungicide on day 33 of the trial (indicated by the arrow) resulted in a sharp drop in chytrid abundance. The dynamics closely resemble those of wild chytrids which have been shown to terminate blooms of their diatom hosts in lakes (Ibelings *et al.* 2004; Gsell *et al.* 2013). Chytrids are a ubiquitous component of freshwater ecosystems, but their role in aquatic food webs and productivity is largely unknown (Kagami *et al.* 2007). Chytrids display considerable variation in host ranges, with some infecting many hosts and others restricted to individual species, strains or even host life cycle stage (Ibelings *et al.* 2004; Kagami *et al.* 2007). Biofuel monocultures may be more susceptible to epidemics than diverse communities in the same way as other agricultural crops, and disease spread has been shown to be slowed in the presence of genetically diverse cultivars (Tooker & Frank 2012). Fig. 3 illustrates some of the variability in the dynamics of algae and their pathogens. While the patterns of population abundance cannot conclusively implicate chytrids as controlling factors of algal density, they show the vulnerability of biofuel ponds to contamination by wild pathogens and indicate that crop protection needs to be a major research priority for algal bioenergy.

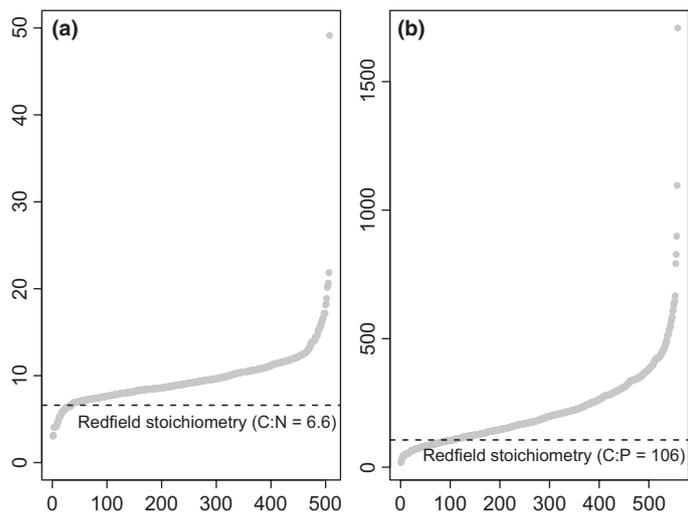


Figure 1 Ranked values of (a) algal carbon:phosphorus stoichiometry ($\text{C} : \text{P}$ ratios, by moles) and (b) algal carbon:nitrogen ($\text{C} : \text{N}$) in natural freshwater systems from the database of Sterner *et al.* (2008).

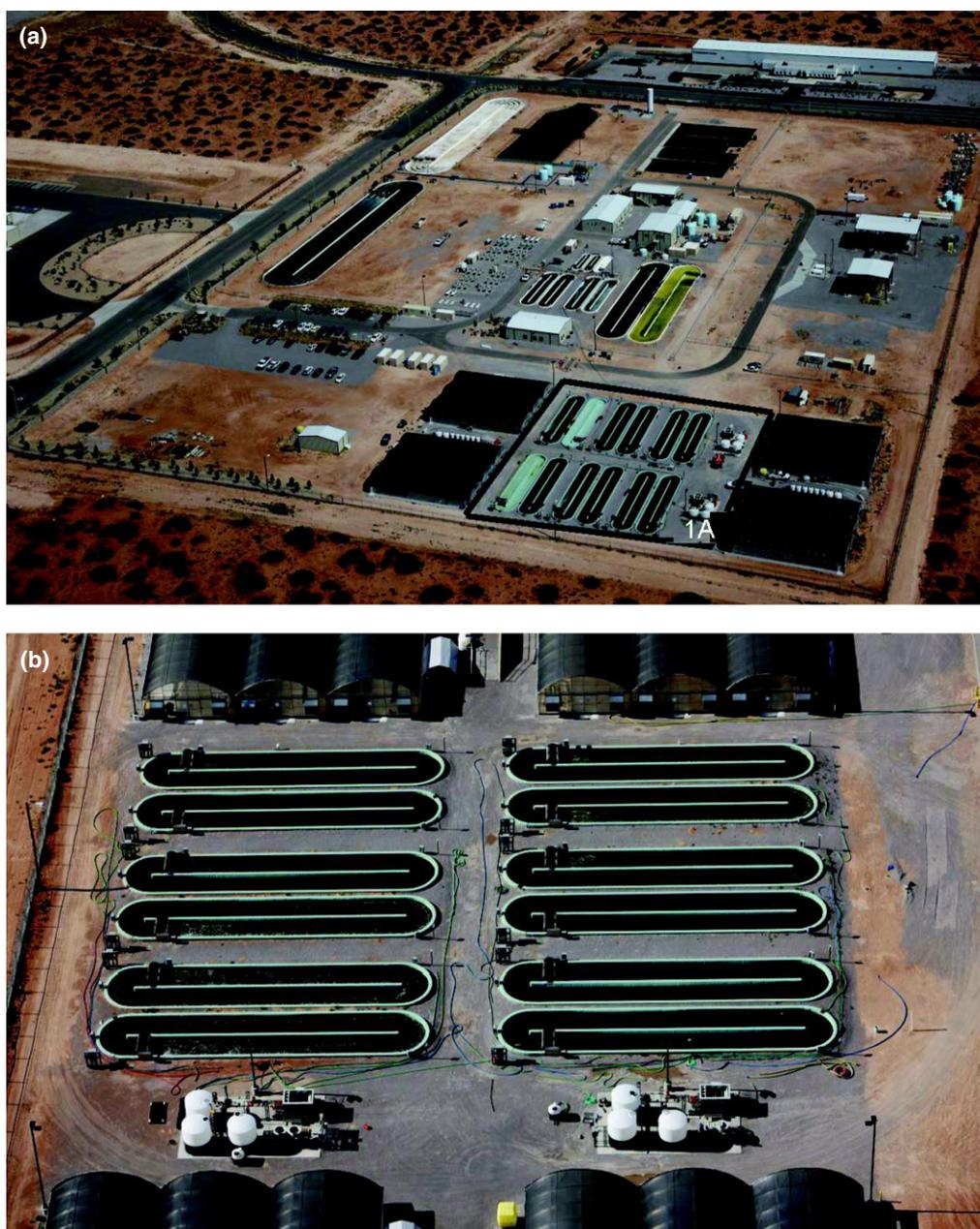


Figure 2 (a) shows an aerial view of the Sapphire Energy field test site in Las Cruces, New Mexico. (b) shows some of the 30.5 m raceways from which the data in Fig. 3 were generated.

What determines the biochemical composition of algae?

Algae exhibit tremendous variation in elemental and biochemical composition both within and between species, and in response to environmental conditions. This plasticity in composition is an important aspect of their use as biofuel feed stock. Under favourable growth conditions, algae synthesise polar lipids for structural and signalling functions which reside mainly in the plasma membrane and organelles (Solovchenko 2012). In stressful environmental conditions, particularly under N-starvation, however, many microalgae activate neutral lipid biosynthetic pathways towards the formation and accumulation of neutral lipids, especially triacylglycerols (TAG, Hu *et al.* 2008). Cells exposed to nitrogen limitation often decrease in protein content and increase carbohydrate and/or lipid storage (Mandal

& Mallick 2009; Feng *et al.* 2011; Jiang *et al.* 2012). Phosphorus limitation has also been found to enhance lipid accumulation in *Nannochloropsis* sp. (Rodolfi *et al.* 2009) and *Monodus subterraneus* (Khozin-Goldberg & Cohen 2006), as has stress from high salinity (Takagi *et al.* 2006), low pH and high light intensity (Damiani *et al.* 2010). Thus, while nitrogen starvation seems to be a general mechanism for inducing neutral lipid production across species, other environmental stresses also affect biochemical composition in idiosyncratic and species-specific ways.

Growth conditions also affect the chemical properties of TAGs in ways that influence their quality as a liquid fuel feedstock. For instance, the fuel properties of biodiesel (which is produced by the transesterification of TAGs) are determined by chain length and degree of unsaturation of fatty acid esters (Knothe 2005). Biodiesel

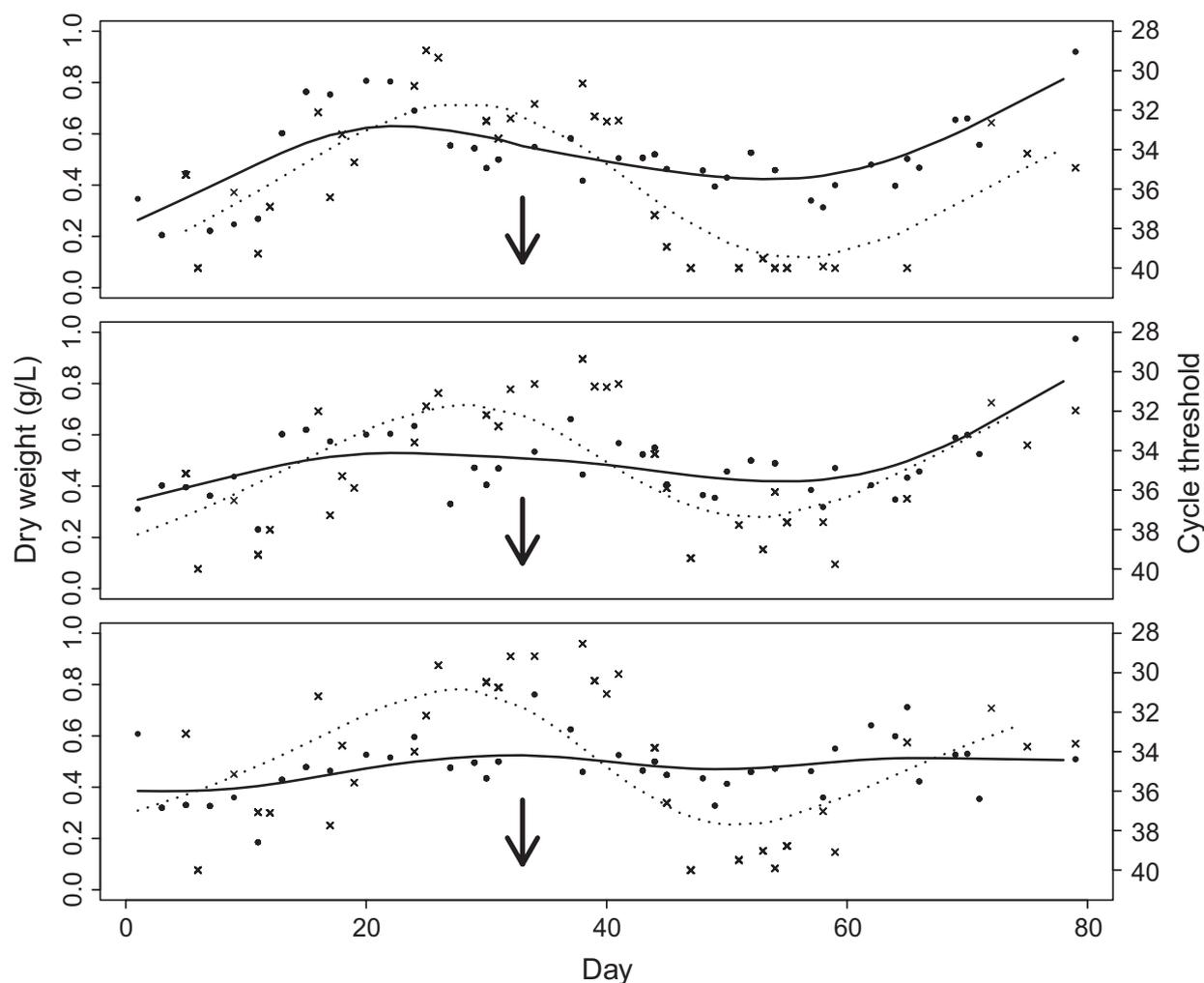


Figure 3 Dynamics of a cultivated micro-alga (dry weight in g/L, solid points and lines, left y-axis) and pathogenic chytrid fungi (CT, cycle threshold for PCR detection, x-symbols and dashed lines) in three replicate raceway ponds (~ 130 m² surface area) at Sapphire Energy. Chytrids were controlled with a chemical fungicide introduced on the date indicated by arrow. Dry weight of algae indicated by solid lines and circles, indicated on the left y-axis. The 'x' symbols and dashed lines indicate chytrid CT values (shown on the right y-axis). The General Additive Models shown by the lines are all significant ($P < 0.05$).

derived from TAGs with high amounts of un-saturated fatty acids shows better lubricity and cold-temperature flow properties. On the other hand, increasing oxidative stability and ignition quality (as measured by the cetane number) requires decreasing the relative content of unsaturated fatty acids. Fuel quality is therefore optimised at intermediate levels of saturation. A good compromise can be reached by generating fuel high in the mono-unsaturated fatty acids, such as oleate or palmitoleate, and low in both saturated and poly-unsaturated fatty acids (Durrett *et al.* 2008). Several aspects of the algal cultivation environment affect the degree of lipid saturation. For example, low temperatures generally induce greater unsaturation in fatty acids in microalgae (Khotimchenko & Yakovleva 2005), while nitrogen deficiency results in the biosynthesis of storage TAGs enriched in saturated and mono-unsaturated fatty acids (Mandal & Mallick 2011). The quality of biodiesel produced therefore can be manipulated through the growth conditions imposed during algal cultivation; however, less is known about how other properties such as algal population growth rate and susceptibility to grazing may influence biofuel quality.

Ecological trade-offs

As in most organisms, traits of phytoplankton that determine fitness are not independent, but rather are often correlated. These correlations often represent trade-offs arising from mechanistic constraints based on principles of cell biology (Litchman *et al.* 2007). For example, cell size and surface area to volume ratio (SA/V) control major fundamental functions in phytoplankton. Small-celled microalgae have higher SA/V and more efficient nutrient utilisation and, thus, may be better nutrient competitors than larger cells (Chisholm 1992). However, storage capabilities, including lipid storage, are higher in large-celled microalgae (Litchman *et al.* 2009). Growth rate is unimodally related to cell size, peaking around $10^2 \mu\text{m}^3$ in volume (Marañón *et al.* 2013). High growth rates also appear to be correlated with low lipid production (Smith *et al.* 2010), and, therefore, fast-growing microalgae may not be the best candidates for biofuel production. Trade-offs may also occur in competitive ability for different nutrients, e.g. nitrogen and phosphorus, or even in multidimensional trait space between competi-

tive abilities for different nutrients and grazer resistance (Edwards *et al.* 2011). Light is also an essential resource and its utilisation is governed by physiological trade-offs. Algal species utilise different parts of the wavelength spectrum of light depending on the presence of particular accessory photosynthetic pigments (Stomp *et al.* 2004). If the same cell cannot produce all accessory pigments, then trade-offs may occur in the ability to harvest different wavelengths. These kinds of trade-offs may limit the potential to engineer or select algal species for biofuel production that maximise a desired function, such as lipid content, growth rate or photosynthetic efficiency. Such trade-offs have major implications for the selection of strains, and for the design of growing conditions and harvesting technologies. Understanding trade-offs is critical to maximising the performance of cultivated strains in terms of multiple functions, including growth, lipid concentration, nutrient demands and resistance to enemies.

Can genetic modification overcome trade-offs?

The development of genetically modified algae for commercial biofuel production is still in its infancy but proceeding rapidly (Georgianna & Mayfield 2012), and, to our knowledge, none has been approved for outdoor cultivation. Just as modern agricultural crops emerged from their wild progenitors, first through long-term selection and then by advanced breeding methods, the development of algae for commercially viable biofuel production depends on research to develop effective crop enhancement strategies. However, scientists are rapidly improving their ability to programme the behaviour of microbes for specific applications including biofuel production (Brenner *et al.* 2008; Kilian *et al.* 2011). A growing capacity to sequence genomes is also facilitating the identification, cloning and manipulation of genes, and also bringing the power of translational tools to bear in algal biotechnology. Much of this research is concentrated upon manipulating their genetics and cellular metabolism to increase cellular lipid concentrations, increase growth rates or confer biotic resistance against consumers. For instance, Simkovsky *et al.* (2012) identified the genetic basis for resistance to protozoan grazing in a filamentous cyanobacteria and proposed its use as a basis for crop protection strategies. Rosenberg *et al.* (2008) concluded that metabolic engineering may be necessary in order for microalgae to achieve commercialisation as biofuel crops. Gressel (2008) outlined seven targets for genetic modification to improve the performance of algal biofuel crops, including the acquisition of herbicide resistance, simultaneously maximising growth and lipid content, improving the efficiency of photosynthesis and CO₂ utilisation, and improving strain biosafety by engineering an inability to survive in natural ecosystems.

We suggest that faith in the ability of genetic modification to overcome all biological limitations on algal biofuel productivity is inconsistent with the concept of trade-offs. For instance, it may not be feasible to engineer microalgae to simultaneously resist herbivore grazing and also be ecologically non-competitive to survive if accidentally released into the wild, or to simultaneously achieve high lipid yields and low cellular nutrient demands. In addition, microalgae are likely to evolve resistance to herbicides at a much faster rate than terrestrial crops because they have orders of magnitude shorter generation times (ca. 1–2 days, vs. 1 year) and larger population sizes. Bull & Collins (2012) outline several evolutionary challenges that will likely be associated with the use of algae as biofuel feed-

stock. They note that algae are grown as a continuously reproducing population and harvested periodically to maintain high yields, in a manner more similar to managed wild fish or game populations than terrestrial crop plants. They conclude that this propagation and harvesting method subjects cultivated algae populations to strong selection pressures which, in many cases, may produce traits that are at odds with maintaining algae suited for biofuel production. In particular, there is the strong possibility of selection for weedy mutants that grow fast or escape harvesting, as well as genetic drift and loss of selected or engineered traits. We agree with Bull & Collins (2012) that a clear understanding of evolutionary principles are needed to design algal growth and harvesting methods that reduce the negative impact of selection.

We also agree with Snow & Smith (2012) that although genetically modified microalgae are unlikely to persist if accidentally released into natural ecosystems, thorough ecological and evolutionary assessments nonetheless will be needed to test this assumption. Invasive microbes and their potentially transformative effects on invaded ecosystems are often cryptic and almost certainly impossible to reverse (Litchman 2010). The benefits of genetic modification of algae for biofuel purposes therefore must be weighed against environmental risks which, although likely to be small, are virtually unknown at present.

Can diversity overcome ecological trade-offs and enhance productivity?

The organismal trade-offs outlined above imply that algal biofuel ecosystems may not be able to achieve optimal productivity and stability by growing monocultures. In addition, the ecological and evolutionary instability of monocultures suggest that it will be very challenging or impossible to maintain single-species pond communities in the long term. Synthetic biology and genetic modification may be able to overcome some of the trade-offs in performance of different organismal functions by algae; however, they also may potentially incur poorly understood environmental risks and create difficult ethical and legal challenges (Snow & Smith 2012). In addition, performance trade-offs may arise from fundamental constraints on cell biology and physiology that may not be amenable to modification through genetic manipulation. These observations lead to the implication that engineering synthetic algal communities and their environment may prove to be a more effective route to maintaining productive and resilient algal biofuel industrial ecosystems than genetic modification (Goldman & Brown 2009; Kazamia *et al.* 2012). Community engineering and genetic modification are not mutually exclusive approaches, and organismal traits that are not achievable within the same algal strain may be possible within a constructed multi-species phytoplankton community including wild, domesticated or engineered taxa. In the following section, we discuss the potential to overcome trade-offs and limitations by exploiting algal community assembly and biodiversity to maintain biofuel pond productivity and resilience against contamination and grazing.

Diversity & productivity

The conclusion that species diversity promotes ecosystem productivity and stability has become canonised as one of the basic tenets of ecology (Cardinale *et al.* 2011). This principle has been applied to biofuel production by grassland plants, which produce similar

amounts of energy as crops such as corn or soybean crops without inputs of fertilisers, pesticides or water (Tilman *et al.* 2006). In addition, Stockenreiter *et al.* (2012) showed that both natural and constructed algal assemblages of high diversity produced more biomass and more total neutral lipids than those containing fewer species (see also Corcoran & Boeing 2012). These studies provide encouraging indications that the use of polycultures may be an effective approach to enhancing algal bioenergy production.

Diversity can enhance productivity either through sampling effects in which diverse communities are more likely to include the most productive species under any set of conditions, or through niche differentiation and complementarity in resource use (Loreau & Hector 2001). Transgressive overyielding occurs when polycultures produce more biomass than monocultures of any of their constituent taxa, and results from facilitation or complementarity among species that allows a diverse community to acquire resources and convert them to biomass more efficiently (Fox 2005). Non-transgressive overyielding is the outcome of sampling effects and represents situations where polycultures are more productive on average than monocultures but do not out-produce the best single species. The distinction between overyielding and the sampling effect is important in the context of biofuels because benefits are only derived from co-culturing multiple taxa when polycultures out-perform their respective monocultures. If the sampling effect is most important, as has been shown in a meta-analysis of biodiversity–ecosystem function experiments (Cardinale *et al.* 2006), then monocultures can produce equivalent yield to polycultures if the most productive monoculture can be identified *a priori*.

Although polycultures may fail to out-perform highly productive monocultures in tests of algal diversity effects on biofuel productivity, there may still be some reason to expect benefits to be realised from co-culturing diverse taxa. First, the best performing single taxon may be difficult to pinpoint in advance, given inherent environmental uncertainty in weather and water chemistry. Diversity may reduce temporal variability in productivity through the portfolio effect (Doak *et al.* 1998) by increasing the likelihood that a productive species will be present under prevailing conditions. In this scenario, diversity acts as a form of insurance against unavoidable environmental stochasticity. Second, species-rich communities may also be less susceptible than monocultures to the effects of top-down control via grazers or pathogens. Because algal taxa vary greatly in their quality as food resources for different consumers, a diverse assemblage is more likely to contain inedible taxa that can resist many of the grazers that are likely to invade biofuel ponds (Duffy 2002). In addition, the presence of inedible prey can indirectly lower the attack rate on vulnerable prey by reducing the consumer's foraging rate and increasing handling time. Kratina *et al.* (2007) showed that the presence of inedible species in protozoan communities dramatically decreased the attack rate of predators on their preferred prey. Hillebrand & Cardinale (2004) showed evidence for these effects in a synthesis of experiments where consumers of benthic algae exerted stronger top-down control on low diversity periphyton assemblages. This principle might be applicable to algal biofuel communities if diverse algae decrease the likelihood of population crashes due to invasion by wild grazers.

We performed a laboratory experiment to test the idea that algal diversity enhances biomass production and nutrient uptake, and invasion by grazing zooplankton. We cultured communities consisting of 1, 2 or 5 species of phytoplankton (belonging to the groups

Chlorophyta, Cyanophyta, Bacillariophyta and Heterokontophyta) with 10 unique combinations at each richness level drawn randomly from a pool of 10 species, as well as the full community of all 10 species (Fig. 4). These 31 unique diversity and composition treatments were crossed with the addition of *Daphnia pulex* grazers on day 25 of the experiment, and each treatment was replicated three times in 200-mL batch cultures. A full description of the methods of the experiment is given in the Online supporting information.

We found that mean total community biovolume increased with the number of species in a pattern most consistent with sampling effects. The two, five and 10 species polycultures mostly had final biovolume on par with, but not greater than, that of the most productive monocultures (Fig. 4). Fig. 5 shows the \log_e of the ratio of final biovolume in the polycultures to both the mean (Fig. 5a) and maximum (Fig. 5b) of the biovolume yield of the component species in monoculture. On average, the polycultures yielded 28.7% more biovolume than the mean of the component species in monoculture, but 19.9% less than the most productive species alone. However, one exception was a five species combination consisting of two cyanobacteria and three chlorophytes (mixture ABEHG in Figs 4 and 5, consisting of *Aphanothece sp.*, *Synechococcus elongatus*, BL0910 (an unidentified green alga), *Neochloris oleabundans* and *Chlorococoum sp.*). This combination yielded 15% more biovolume than any of the other polycultures or monocultures, and more than 2× as much as any of the five species when grown alone. This result indicates that overyielding occurs but is a relatively rare outcome, as has been shown in experiments with other groups of organisms (Cardinale *et al.* 2011). Identifying overyielding combinations of species is key to the application of community engineering to algal bioenergy.

In addition, the species mixtures on average acquired more nutrients from their medium and resisted invasion by grazers better than monocultures. Dissolved phosphate concentration, a measure of free resources not absorbed from the environment by algae, declined with the number of species seeded. Finally, the daily survival of introduced *Daphnia* grazers declined markedly at high algal diversity even though the total biomass of algal food resources increased. Although *Daphnia* survived in many cultures, low survival in nearly all of the high-diversity polycultures prevented us from testing the effects of algal species richness on the grazing impact of the consumer. The results of this experiment support the contentions that algal diversity promotes both high biomass yield and dissolved P acquisition, while simultaneously providing some resistance against invasion by grazing consumers.

Although polycultures on average showed increased biovolume yield and dissolved phosphorus acquisition, and decreased grazer survival, the specific combinations of species that showed high values for one of these functions often did not necessarily show high values for the others. The treatment mean values for *Daphnia* survival were uncorrelated with either biovolume yield ($n = 31$, Pearson $r = -0.30$, $P = 0.10$), or dissolved P concentration ($r = 0.18$, $P = 0.34$), while biovolume and dissolved P were marginally negatively correlated ($r = -0.35$, $P = 0.05$). The weakness of the correlation between the three functions we measured indicates that polycultures that optimise one important function for bioenergy (e.g. biomass yield) may be suboptimal in terms of other crucial functions (e.g. grazing resistance). This finding suggests that the 'multivariate dominance effect' (Duffy *et al.* 2003), or the capacity of diverse assemblages to simultaneously increase rates of multiple ecosystem functions, may be weak in algae. The patterns agree with

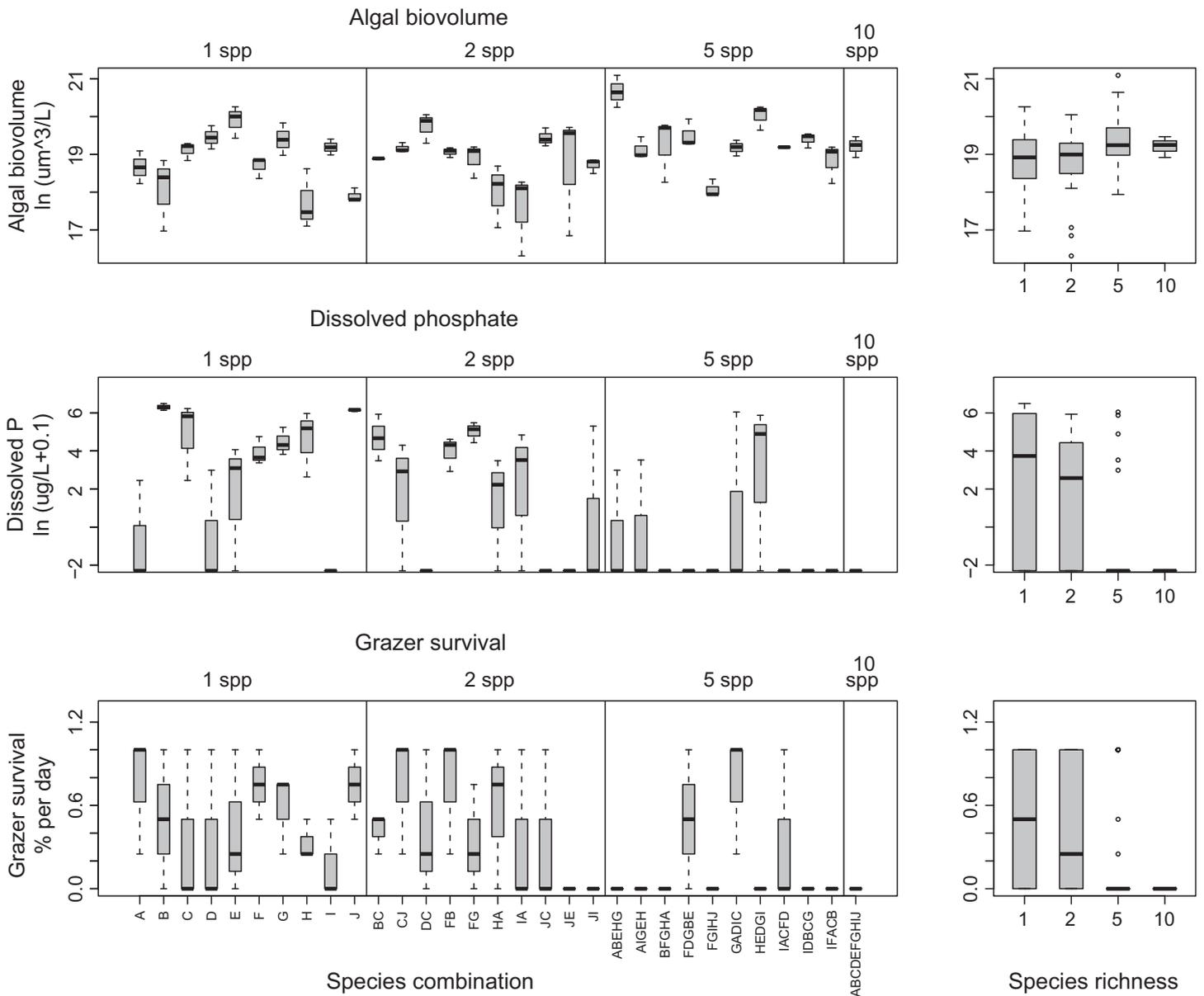


Figure 4 The results of a laboratory experiment growing multiple combinations of 1, 2, 5 and 10 species of microalgae. Each box on the x-axis of the left panels represents a unique microalgal species combination which was assigned by random draws from the full 10 species pool. The right panels show the distributions for each richness level. Rows show total algal biovolume (top), dissolved phosphate concentration in the medium (middle) and daily survival rate of *Daphnia* grazers on day 29 of the experiment (bottom). ANOVAs with species richness, *Daphnia*, and species combination nested within richness as factors found that both richness and species combination had significant effects on biovolume and PO_4 concentration ($P < 0.0001$), but that addition of *Daphnia* had no effect. Richness, but not species combination, also had a significant effect on *Daphnia* survival ($P = 0.02$). The codes for the species making up the communities are shown on Fig. 5.

Gamfeldt *et al.* (2013) and Zavaleta *et al.* (2010) who show that some ecosystem services are negatively correlated with one another, preventing simultaneous optimisation of rates along multiple axes.

Although strong evidence supports the idea that species diversity promotes high ecosystem productivity (Cardinale *et al.* 2006), the utility of polycultures for enhancing productivity or stability of highly managed agricultural or bioenergy ecosystems remains largely untested. Using diversity as an operational approach to intensify yield in industrial-scale bioenergy requires much greater predictive understanding of the species combinations and environmental conditions that give rise to productive, efficient and robust assemblages.

Different communities containing the same number of species show tremendous variation in biomass productivity depending on the identities of the species present (Fig. 4). The challenge in applying principles relating biodiversity and ecosystem function to industrial bioenergy is therefore not simply to understand the relationship between diversity and production. Rather, the important task is to identify combinations of species that are productive and robust against vagaries of the weather and the inevitable contamination by wild taxa.

Another reason that haphazardly assembled polycultures may not benefit biofuel yield is the potential for underyielding, the situation

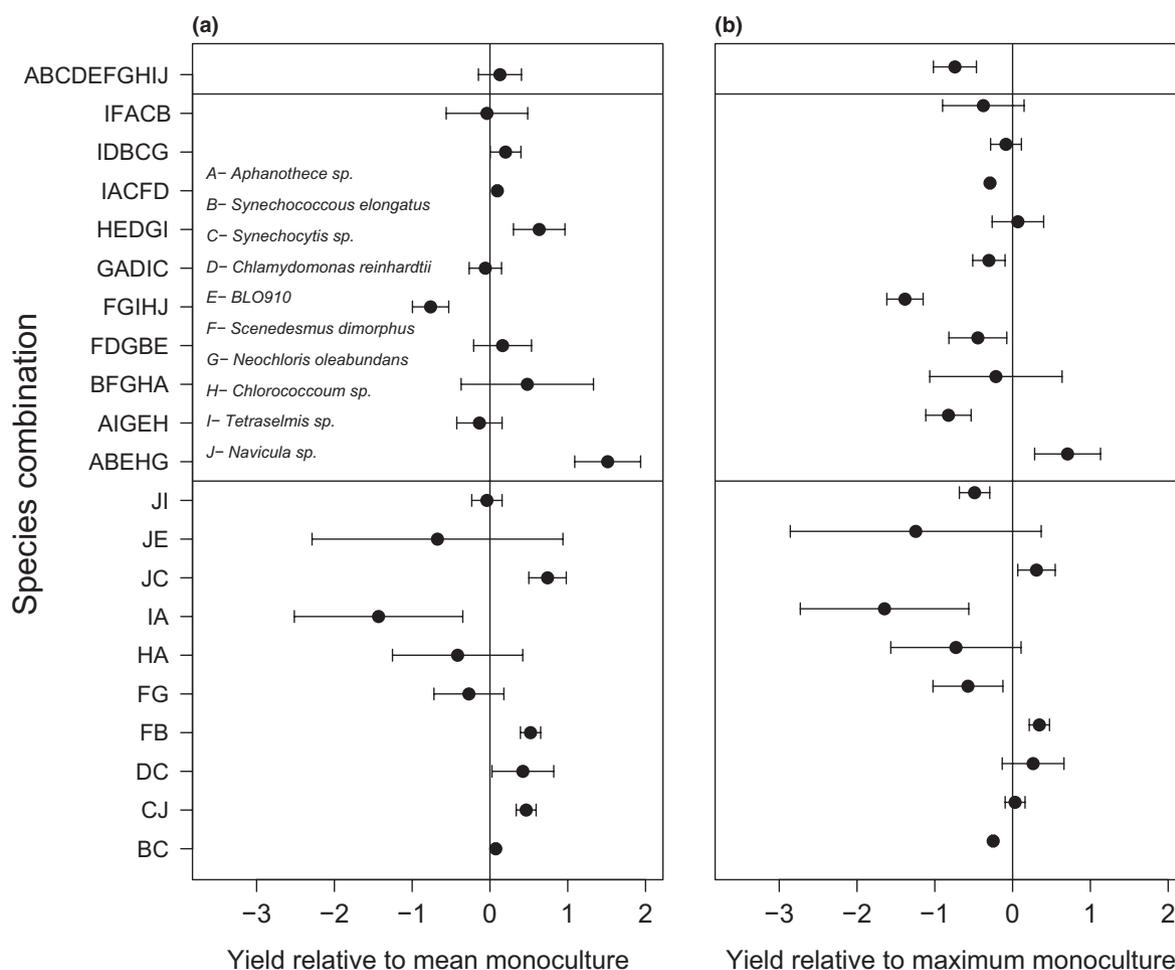


Figure 5 Final biovolume yields of species mixtures relative to the average biovolume of the component species in monoculture (a) and the maximum of the component monocultures (b). Each point is the \log_e of the ratio of the polyculture yield to either the mean or the maximum biovolume of the species making up the mixture when in monoculture.

where combinations of species are *less* productive than the average monoculture. Although underyielding is a relatively uncommon outcome in published studies that manipulated diversity (Cardinale *et al.* 2006), a number of examples show that many mixtures perform poorly relative to their component monocultures. Schmidtke *et al.* (2010) found that among eight species of algae cultured in the laboratory, the fastest growing species competitively displaced slower growers, but produced less biomass at steady state, implying a trade-off between maximum exponential growth at low density and carrying capacity. Jiang (2007) found evidence of similar negative selection effects among bacteria where the taxa that dominated in polyculture were often less productive, resulting in underyielding. Thus, some species in diverse mixtures may have negative effects on community production. The data shown in Fig. 5 also show a number of examples where polycultures yielded less final biovolume than either the maximum or the average of the component species on their own (negative values of yield relative to the mean or maximum monoculture). Together, these results suggest that polyculture cannot be applied as a blind insurance policy because mixtures of species can yield either much more or much less biomass than any of their component monocultures.

Chemical ecology of algal aquaculture

Aquatic plants and animals produce a variety of biomolecules that play critical roles in almost every aspect of the functioning of aquatic communities and ecosystems. Compounds not involved in basic biochemical functions are referred to as secondary metabolites, and play critical roles in interactions within and between aquatic species. Phytoplankton secondary metabolites can suppress grazers and competitors (Hay 2009), and synchronise cell death during decline of phytoplankton blooms (Vidoudez & Pohnert 2008). An understanding of phytoplankton chemical ecology may have applications to the design and implementation of artificial algal communities for biofuel production and may provide valuable tools for crop protection.

Phytoplankton have evolved diverse morphological and chemical adaptations to grazing pressure (Long *et al.* 2007). Cyanobacteria, long recognised for their role in freshwater harmful algal blooms, produce structurally diverse secondary metabolites including microcystins that have toxic and inhibitory effects on zooplankton grazers (Wiegand & Pflugmacher 2005). Chemical defences have also been documented in other taxa. For example, copepod grazers showed

reduced feeding activity and reproductive success when exposed to the toxic haptophyte *Prymnesium parvum* (Sopanen *et al.* 2006), strongly suggesting chemical mediation. *Botryococcus braunii*, a species of interest as a biofuel crop, produces high levels of hydrocarbon fatty acids in the form of triterpenes (up to 30–40% dry mass) that have suppressive effects on some zooplankton (Chiang *et al.* 2004). Harnessing these compounds in one form or another may be a viable approach to crop protection in biofuel aquaculture.

Incorporating chemically defended, herbivore-resistant phytoplankton into biofuel pond communities may confer crop protection through an 'associational refuge' for more herbivore-susceptible crop species. Similar strategies have been proven effective in mixed cultivars of plant crops (Tooker & Frank 2012). An associational refuge is an interaction where a susceptible species benefits from protection against grazing conferred by growing in association with a defended species. Associational refuges have been well documented in macroalgae (Duffy & Hay 1994; Levenbach 2008). To our knowledge, there are no well-documented cases of associational refuges in phytoplankton communities. However, compounds released by chemically defended phytoplankton have negative impacts on both zooplankton herbivores and phytoplankton competitors (Chiang *et al.* 2004; Ianora & Miralto 2010). Therefore, allelopathy and defensive chemistry, the main ingredients of associational refuges, occur in phytoplankton communities. Studies documenting the presence or absence of these interactions are ripe areas for future research in chemical ecology of phytoplankton communities. Defensive traits are the targets of many efforts to select or engineer defended strains of algae for bioenergy (Gressel 2008; Georgianna & Mayfield 2012; Simkovsky *et al.* 2012). However, it may not be necessary to incorporate defensive traits into the genome of crop taxa if their benefits can be realised by cultivating strains in the presence of defended taxa. The costs and benefits of such a strategy depends on the degree of crop protection conferred, the competitive interactions between the defended taxa and the crop, and the losses to grazers.

HOW CAN ECOLOGY CONTRIBUTE TO INDUSTRIAL BIOTECHNOLOGY? OPPORTUNITIES FOR ECOLOGICAL INNOVATION

Major technological advances are required in order for microalgae-based biofuels to be cost-competitive with fossil fuels (DOE 2009; Mata *et al.* 2010; Georgianna & Mayfield 2012). Much of the research focus in this effort concerns understanding the genetics and cell biology of lipid production and quality, nutrient use, and crop protection. However, many of the challenges in advancing bioenergy technology are ecological, including minimising nutrient demands and protecting cultivated algae against a broad spectrum of natural enemies. We suggest that these technological advancements must also be accompanied by equally well-focused efforts to incorporate key principles of ecology and evolutionary biology into the design and operation of these bioenergy production systems. Biofuel engineers need to use fundamental ideas from ecological and evolutionary theory to help guide future production system design. Likewise, ecologists should view engineered biosystems as valuable new platforms for ecological research and opportunities to apply ecological principles to technological questions (Graham & Smith 2004). Constructing reliable microbial communities for biofuel productivity provides the ultimate test of our understanding of engineered ecosystems.

A diversity of algal crop taxa, whether engineered using synthetic biology or selected by traditional methods using standing genetic variation from wild populations, will likely prove essential to commercialisation of algal biofuels. Diversity need not be maintained at the local scale of the growth pond. Instead, a strategy of crop rotation through inoculation with new strains may be used to maintain productivity in the face of fluctuations due to weather or contamination. The rapid pace of microbial aquaculture demands immediate response to fluctuating conditions and performance (Fig. 3), as well as a deep knowledge of the multivariate traits of cultivated strains and taxa. While elite strains may provide optimum performance under a given set of local conditions, this high level of production will likely erode over ecological time due to genetic drift and selection, contamination and environmental variability. In addition, it is likely that all algal taxa are inherently vulnerable to grazing or infection by at least some wild pest species. We agree with Kazamia *et al.* (2012) that a robust community of multiple algal species growing in a carefully designed and carefully constructed consortium offers the potential both for increased biomass yields, and for the crop protection that will be necessary to achieve commercial-scale production. The published literature suggests that polyculture may be a viable approach to algal biofuels, especially in outdoor growth ponds subject to variable weather and contamination with wild pests, and using growth media with variable chemistry such as wastewater or marine sources. However, much more research is needed to identify the specific combinations of taxa and growing conditions that will give rise to the greatest levels of productivity and resilience.

A trait-based approach to understanding strain selection and engineering may be powerful for constructing bioenergy agro-ecosystems. Assembling polycultures from species with known, complementary trait values may be much more successful at intensifying biomass yield and stabilising variation in this yield than creating the randomly generated species combinations that are commonly used in biodiversity experiments. Understanding the mechanistic basis for overyielding and resilience against environmental variation and top-down control is essential to engineering a robust bioenergy community. These trait-based approaches to optimising algal biofuel polycultures should be explored both theoretically, with trait-based models of algal communities, and experimentally through exploration of the many potential species combinations. Understanding the combinations of species composition and environmental conditions that optimise biomass productivity and biochemical composition also provides a rigorous test of our practical knowledge of algal ecology.

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AUTHORSHIP

JBS conceived and designed the review; RLA, GK, RM, SM and VS collected data; all authors wrote and edited the manuscript.

REFERENCES

- Brenner, K., You, L.C. & Arnold, F.H. (2008). Engineering microbial consortia: a new frontier in synthetic biology. *Trends Biotechnol.*, 26, 483–489.

- Brooks, J.L. & Dodson, S.I. (1965). Predation, body size, and composition of the plankton. *Science*, 150, 28–35.
- Bull, J.J. & Collins, S. (2012). Algae for biofuel: will the evolution of weeds limit the enterprise? *Evolution*, 66, 2983–2987.
- Cáceres, C.E. & Soluk, D.A. (2002). Blowing in the wind: a field test of overland dispersal and colonization by aquatic invertebrates. *Oecologia*, 131, 402–408.
- Cardinale, B.J., Srivastava, D.S., Duffy, J.E., Wright, J.P., Downing, A.L., Sankaran, M. *et al.* (2006). Effects of biodiversity on the functioning of trophic groups and ecosystems. *Nature*, 443, 989–992.
- Cardinale, B.J., Matulich, K.L., Hooper, D.U., Byrnes, J.E., Duffy, E., Gamfeldt, L. *et al.* (2011). The functional role of producer diversity in ecosystems. *Am. J. Bot.*, 98, 572–592.
- Carpenter, S.R., Caraco, N.F., Correll, D.L., Howarth, R.W., Sharpley, A.N. & Smith, V.H. (1998). Nonpoint pollution of surface waters with phosphorus and nitrogen. *Ecol. Appl.*, 8, 559–568.
- Cebrian, J. (1999). Patterns in the fate of production in plant communities. *Am. Nat.*, 154, 449–468.
- Cebrian, J., Shurin, J.B., Borer, E.T., Cardinale, B.J., Ngai, J.T., Smith, M.D. *et al.* (2009). Producer nutritional quality controls ecosystem trophic structure. *PLoS ONE*, 4, e4929.
- Chiang, I.Z., Huang, W.Y. & Wu, J.T. (2004). Allelochemicals of *Botryococcus braunii* (Chlorophyceae). *J. Phycol.*, 40, 474–480.
- Chisholm, S.W. (1992). Phytoplankton size. In *Primary Productivity and Biogeochemical Cycles in the Sea*. (eds Falkowski, P.G., Woodhead, A.D.). Plenum Press New York, pp. 213–237.
- Chisti, Y. (2007). Biodiesel from microalgae. *Biotechnol. Adv.*, 25, 294–306.
- Clarens, A.F., Resurreccion, E.P., White, M.A. & Colosi, L.M. (2010). Environmental life cycle comparison of algae to other bioenergy feedstocks. *Environ. Sci. Technol.*, 44, 1813–1819.
- Cohen, G.M. & Shurin, J.B. (2003). Scale-dependence and mechanisms of dispersal in freshwater zooplankton. *Oikos*, 103, 603–617.
- Conley, D.J., Paerl, H.W., Howarth, R.W., Boesch, D.F., Seitzinger, S.P., Havens, K.E. *et al.* (2009). Ecology controlling eutrophication: nitrogen and phosphorus. *Science*, 323, 1014–1015.
- Corcoran, A.A. & Boeing, W.J. (2012). Biodiversity increases the productivity and stability of phytoplankton communities. *PLoS ONE*, 7, e49397.
- Craggs, R.J., Heubeck, S., Lundquist, T.J. & Benemann, J.R. (2011). Algal biofuels from wastewater treatment high rate algal ponds. *Water Sci. Technol.*, 63, 660–665.
- Damiani, M.C., Popovich, C.A., Constenla, D. & Leonardi, P.I. (2010). Lipid analysis in *Haematococcus pluvialis* to assess its potential use as a biodiesel feedstock. *Bioresour. Technol.*, 101, 3801–3807.
- Doak, D.F., Bigger, D., Harding, E.K., Marvier, M.A., O'Malley, R.E. & Thomson, D. (1998). The statistical inevitability of stability-diversity relationships in community ecology. *Am. Nat.*, 151, 264–276.
- Duffy, J.E. (2002). Biodiversity and ecosystem function: the consumer connection. *Oikos*, 99, 201–219.
- Duffy, J.E. & Hay, M.E. (1994). Herbivore resistance to seaweed chemical defense: the roles of mobility and predation risk. *Ecology*, 75, 1304–1319.
- Duffy, J.E., Richardson, J.P. & Canuel, E.A. (2003). Grazer diversity effects on ecosystem functioning in seagrass beds. *Ecol. Lett.*, 6, 637–645.
- Durrett, T.P., Benning, C. & Ohlrogge, J. (2008). Plant triacylglycerols as feedstocks for the production of biofuels. *Plant J.*, 54, 593–607.
- Edwards, K.F., Klausmeier, C.A. & Litchman, E. (2011). Evidence for a three-way tradeoff between nitrogen and phosphorus competitive abilities and cell size in phytoplankton. *Ecology*, 92, 2085–2095.
- Elser, J.J., Fagan, W.F., Denno, R.F., Dobberfuhl, D.R., Folarin, A., Huberty, A. *et al.* (2000). Nutritional constraints in terrestrial and freshwater food webs. *Nature*, 408, 578–580.
- Feng, P.Z., Deng, Z.Y., Hu, Z.Y. & Fan, L. (2011). Lipid accumulation and growth of *Chlorella zofingiensis* in flat plate photobioreactors outdoors. *Bioresour. Technol.*, 102, 10577–10584.
- Finlay, B.J. (2002). Global dispersal of free-living microbial eukaryote species. *Science*, 296, 1061–1063.
- Fox, J.W. (2005). Interpreting the 'selection effect' of biodiversity on ecosystem function. *Ecol. Lett.*, 8, 846–856.
- Gamfeldt, L., Snäll, T., Bagchi, R., Jonsson, M., Gustafsson, L., Kjellander, P. *et al.* (2013). Higher levels of multiple ecosystem services are found in forests with more tree species. *Nat. Commun.*, 4, 1430.
- Geider, R.J. & La Roche, J. (2002). Redfield revisited: variability of C: N: P in marine microalgae and its biochemical basis. *Eur. J. Phycol.*, 37, 1–17.
- Georgianna, D.R. & Mayfield, S.P. (2012). Exploiting diversity and synthetic biology for the production of algal biofuels. *Nature*, 488, 329–335.
- Goldman, R.P. & Brown, S.P. (2009). Making sense of microbial consortia using ecology and evolution. *Trends Biotechnol.*, 27, 3–4.
- Graham, D.W. & Smith, V.H. (2004). Designed ecosystem services: application of ecological principles in wastewater treatment engineering. *Front. Ecol. Environ.*, 2, 199–206.
- Gressel, J. (2008). Transgenics are imperative for biofuel crops. *Plant Sci.*, 174, 246–263.
- Griffiths, M.J. & Harrison, S.T.L. (2009). Lipid productivity as a key characteristic for choosing algal species for biodiesel production. *J. Appl. Phycol.*, 21, 493–507.
- Gsell, A.S., Domis, L.N.D., Naus-Wiezer, S.M.H., Helmsing, N.R., Van Donk, E. & Ibelings, B.W. (2013). Spatiotemporal variation in the distribution of chytrid parasites in diatom host populations. *Freshw. Biol.*, 58, 523–537.
- Hamilton, P.T., Richardson, J.M.L. & Anholt, B.R. (2012). Daphnia in tadpole mesocosms: trophic links and interactions with *Batrachochytrium dendrobatidis*. *Freshw. Biol.*, 57, 676–683.
- Harpole, W.S., Ngai, J.T., Cleland, E.E., Seabloom, E.W., Borer, E.T., Bracken, M.E.S. *et al.* (2011). Nutrient co-limitation of primary producer communities. *Ecol. Lett.*, 14, 852–862.
- Hay, M.E. (2009). *Marine Chemical Ecology: Chemical Signals and Cues Structure Marine Populations, Communities, and Ecosystems*. Annual Review of Marine Science, In, pp. 193–212.
- Hillebrand, H. & Cardinale, B.J. (2004). Consumer effects decline with prey diversity. *Ecol. Lett.*, 7, 192–201.
- Hu, Q., Sommerfeld, M., Jarvis, E., Ghirardi, M., Posewitz, M., Seibert, M. *et al.* (2008). Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and advances. *Plant J.*, 54, 621–639.
- Ianora, A. & Miralto, A. (2010). Toxicogenic effects of diatoms on grazers, phytoplankton and other microbes: a review. *Ecotoxicology*, 19, 493–511.
- Ibelings, B.W., De Bruin, A., Kagami, M., Rijkeboer, M., Brehm, M. & van Donk, E. (2004). Host parasite interactions between freshwater phytoplankton and chytrid fungi (Chytridiomycota). *J. Phycol.*, 40, 437–453.
- Jenkins, D.G. & Buikema, A.L.J. (1998). Do similar communities develop in similar sites? a test with zooplankton structure and function. *Ecol. Monogr.*, 68, 421–443.
- Jiang, L. (2007). Negative selection effects suppress relationships between bacterial diversity and ecosystem functioning. *Ecology*, 88, 1075–1085.
- Jiang, Y.L., Yoshida, T. & Quigg, A. (2012). Photosynthetic performance, lipid production and biomass composition in response to nitrogen limitation in marine microalgae. *Plant Physiol. Biochem.*, 54, 70–77.
- Johnk, K.D., Huisman, J., Sharples, J., Sommeijer, B., Visser, P.M. & Stroom, J.M. (2008). Summer heatwaves promote blooms of harmful cyanobacteria. *Glob. Change Biol.*, 14, 495–512.
- Kagami, M., Van Donk, E., de Bruin, A., Rijkeboer, M. & Ibelings, B.W. (2004). Daphnia can protect diatoms from fungal parasitism. *Limnol. Oceanogr.*, 49, 680–685.
- Kagami, M., de Bruin, A., Ibelings, B.W. & Van Donk, E. (2007). Parasitic chytrids: their effects on phytoplankton communities and food-web dynamics. *Hydrobiologia*, 578, 113–129.
- Kang, Y.H., Kim, J.D., Kim, B.H., Kong, D.S. & Han, M.S. (2005). Isolation and characterization of a bio-agent antagonistic to diatom, *Stephanodiscus hantzschii*. *J. Appl. Microbiol.*, 98, 1030–1038.
- Kazamia, E., Aldridge, D.C. & Smith, A.G. (2012). Synthetic ecology: a way forward for sustainable algal biofuel production? *J. Biotechnol.*, 162, 163–169.
- Khotimchenko, S.V. & Yakovleva, I.M. (2005). Lipid composition of the red alga *Tichocarpus crinitus* exposed to different levels of photon irradiance. *Phytochemistry*, 66, 73–79.
- Khazin-Goldberg, I. & Cohen, Z. (2006). The effect of phosphate starvation on the lipid and fatty acid composition of the fresh water eustigmatophyte *Monodus subterraneus*. *Phytochemistry*, 67, 696–701.

- Kilian, O., Benemann, C.S.E., Niyogi, K.K. & Vick, B. (2011). High-efficiency homologous recombination in the oil-producing alga *Nannochloropsis* sp. *Proc. Natl. Acad. Sci. USA*, 108, 21265–21269.
- Knothe, G. (2005). Dependence of biodiesel fuel properties on the structure of fatty acid alkyl esters. *Fuel Process. Technol.*, 86, 1059–1070.
- Kratina, P., Vos, M. & Anholt, B.R. (2007). Species diversity modulates predation. *Ecology*, 88, 1917–1923.
- Levenbach, S. (2008). Community-wide ramifications of an associational refuge on shallow rocky reefs. *Ecology*, 89, 2819–2828.
- Litchman, E. (2010). Invisible invaders: non-pathogenic invasive microbes in aquatic and terrestrial ecosystems. *Ecol. Lett.*, 13, 1560–1572.
- Litchman, E., Klausmeier, C.A., Schofield, O.M. & Falkowski, P.G. (2007). The role of functional traits and trade-offs in structuring phytoplankton communities: scaling from cellular to ecosystem level. *Ecol. Lett.*, 10, 1170–1181.
- Litchman, E., Klausmeier, C.A. & Yoshiyama, K. (2009). Contrasting size evolution in marine and freshwater diatoms. *Proc. Natl. Acad. Sci. USA*, 106, 2665–2670.
- Long, J.D., Smalley, G.W., Barsby, T., Anderson, J.T. & Hay, M.E. (2007). Chemical cues induce consumer-specific defenses in a bloom-forming marine phytoplankton. *Proc. Natl. Acad. Sci. USA*, 104, 10512–10517.
- Loreau, M. & Hector, A. (2001). Partitioning selection and complementarity in biodiversity experiments. *Nature*, 412, 72–76.
- Mandal, S. & Mallick, N. (2009). Microalga *Scenedesmus obliquus* as a potential source for biodiesel production. *Appl. Microbiol. Biotechnol.*, 84, 281–291.
- Mandal, S. & Mallick, N. (2011). Waste utilization and biodiesel production by the green Microalga *Scenedesmus obliquus*. *Appl. Environ. Microbiol.*, 77, 374–377.
- Marañón, E., Cermeño, P., Lopez-Sandoval, D.C., Rodriguez-Ramos, T., Sobrino, C., Huete-Ortega, M. *et al.* (2013). Unimodal size scaling of phytoplankton growth and the size dependence of nutrient uptake and use. *Ecol. Lett.*, 16, 371–379.
- Mata, T.M., Martins, A.A. & Caetano, N.S. (2010). Microalgae for biodiesel production and other applications: a review. *Renew. Sustain. Energy Rev.*, 14, 217–232.
- McCrackin, M.L. & Elser, J.J. (2010). Atmospheric nitrogen deposition influences denitrification and nitrous oxide production in lakes. *Ecology*, 91, 528–539.
- Paerl, H.W. & Huisman, J. (2008). Climate: blooms like it hot. *Science*, 320, 57–58.
- Park, J.B.K., Craggs, R.J. & Shilton, A.N. (2011). Wastewater treatment high rate algal ponds for biofuel production. *Bioresour. Technol.*, 102, 35–42.
- Pate, R., Klise, G. & Wu, B. (2011). Resource demand implications for US algae biofuels production scale-up. *Appl. Energy*, 88, 3377–3388.
- Pinto, P.D. & Litchman, E. (2010). Interactive effects of N:P ratios and light on nitrogen-fixing abundance. *Oikos*, 119, 567–575.
- Redfield, A.C. (1958). The biological control of chemical factors in the environment. *Am. Sci.*, 46, 205–221.
- Rodolfi, L., Zittelli, G.C., Bassi, N., Padovani, G., Biondi, N., Bonini, G. *et al.* (2009). Microalgae for oil: strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor. *Biotechnol. Bioeng.*, 102, 100–112.
- Rosenberg, J.N., Oyler, G.A., Wilkinson, L. & Betenbaugh, M.J. (2008). A green light for engineered algae: redirecting metabolism to fuel a biotechnology revolution. *Curr. Opin. Biotechnol.*, 19, 430–436.
- Schindler, D.W., Hecky, R.E., Findlay, D.L., Stainton, M.P., Parker, B.R., Paterson, M.J. *et al.* (2008). Eutrophication of lakes cannot be controlled by reducing nitrogen input: Results of a 37-year whole-ecosystem experiment. *Proc. Natl. Acad. Sci. USA*, 105, 11254–11258.
- Schmidtko, A., Gaedke, U. & Weithoff, G. (2010). A mechanistic basis for underyielding in phytoplankton communities. *Ecology*, 91, 212–221.
- Shurin, J.B. (2000). Dispersal limitation, invasion resistance, and the structure of pond zooplankton communities. *Ecology*, 81, 3074–3086.
- Simkovsky, R., Daniels, E.F., Tang, K., Huynh, S.C., Golden, S.S. & Brahamsha, B. (2012). Impairment of O-antigen production confers resistance to grazing in a model amoeba-cyanobacterium predator-prey system. *Proc. Natl. Acad. Sci. USA*, 109, 16678–16683.
- Smith, V.H. (1983). Low nitrogen to phosphorus ratios favor dominance by blue-green algae in lake phytoplankton. *Science*, 221, 669–671.
- Smith, V.H. (2003). Eutrophication of freshwater and coastal marine ecosystems: a global problem. *Environ. Sci. Pollut. Res.*, 10, 126–139.
- Smith, V.H., Sturm, B.S.M., deNoyelles, F.J. & Billings, S.A. (2010). The ecology of algal biodiesel production. *Trends Ecol. Evol.*, 25, 301–309.
- Snow, A.A. & Smith, V.H. (2012). Genetically engineered algae for biofuels: a key role for ecologists. *Bioscience*, 62, 765–768.
- Solovchenko, A.E. (2012). Physiological role of neutral lipid accumulation in eukaryotic microalgae under stresses. *Russ. J. Plant Physiol.*, 59, 167–176.
- Sommer, U. (ed.) (1989). *Plankton Ecology: Succession in Plankton Communities*. Springer-Verlag, Berlin, Germany.
- Sopanen, S., Koski, M., Kuuppo, P., Uronen, P., Legrand, C. & Tamminen, T. (2006). Toxic haptophyte *Prymnesium parvum* affects grazing, survival, egestion and egg production of the calanoid copepods *Eurytemora affinis* and *Acartia bifilosa*. *Mar. Ecol. Prog. Ser.*, 327, 223–232.
- Spoehr, H.A. & Milner, H.W. (1949). The chemical composition of chlorella - effect of environmental conditions. *Plant Physiol.*, 24, 120–149.
- Stern, R.W., Andersen, T., Elser, J., Hessen, D.O., Hood, J.M., McCauley, D.E. *et al.* (2008). Scale-dependent carbon: nitrogen: phosphorus seston stoichiometry in marine and freshwaters. *Limnol. Oceanogr.*, 53, 1169–1180.
- Stockenreiter, M., Graber, A.K., Haupt, F. & Stibor, H. (2012). The effect of species diversity on lipid production by micro-algal communities. *J. Appl. Phycol.*, 24, 45–54.
- Stomp, M., Huisman, J., de Jongh, F., Veraart, A.J., Gerla, D., Rijkeboer, M. *et al.* (2004). Adaptive divergence in pigment composition promotes phytoplankton biodiversity. *Nature*, 432, 104–107.
- Stumm, W. & Morgan, J.J. (1981). *Aquatic Chemistry: An Introduction Emphasizing Chemical Equilibria in Natural Waters*. Wiley, New York, NY.
- Sturm, B.S.M., Peltier, E., Smith, V.H. & DeNoyelles, F.J. (2012). Controls of microalgal biomass and lipid production in municipal wastewater-fed bioreactors. *Environ. Prog. Sustain. Energy*, 31, 10–16.
- Suttle, C.A. (2005). Viruses in the sea. *Nature*, 437, 356–361.
- Takagi, M., Karseno & Yoshida, T. (2006). Effect of salt concentration on intracellular accumulation of lipids and triacylglyceride in marine microalgae *Dunaliella* cells. *J. Biosci. Bioeng.*, 101, 223–226.
- Tilman, D., Hill, J. & Lehman, C. (2006). Carbon-negative biofuels from low-input high-diversity grassland biomass. *Science*, 314, 1598–1600.
- Tooker, J.F. & Frank, S.D. (2012). Genotypically diverse cultivar mixtures for insect pest management and increased crop yields. *J. Appl. Ecol.*, 49, 974–985.
- U.S. DOE (Department of Energy). (2010). National Algal Biofuels Technology Roadmap. DOE, Washington, pp. 126.
- Vidoudez, C. & Pohnert, G. (2008). Growth phase-specific release of polyunsaturated aldehydes by the diatom *Skeletonema marinoi*. *J. Plankton Res.*, 30, 1305–1313.
- Wiegand, C. & Pflugmacher, S. (2005). Ecotoxicological effects of selected cyanobacterial secondary metabolites a short review. *Toxicol. Appl. Pharmacol.*, 203, 201–218.
- Zavaleta, E.S., Pasari, J.R., Hulvey, K.B. & Tilman, G.D. (2010). Sustaining multiple ecosystem functions in grassland communities requires higher biodiversity. *Proc. Natl. Acad. Sci. USA*, 107, 1443–1446.

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