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The ecology of algal biodiesel production

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Sustainable energy production represents one of the most formidable problems of the 21st century, and plant-based biofuels offer significant promise. We summarize the potential advantages of using pond-grown microalgae as feedstocks relative to conventional terrestrial biofuel crop production. We show how pond-based algal biofuel production, which requires significantly less land area than agricultural crop-based biofuel systems, can offer additional ecological benefits by reducing anthropogenic pollutant releases to the environment and by requiring much lower water subsidies. We also demonstrate how key principles drawn from the science of ecology can be used to design efficient pond-based microalgal systems for the production of biodiesel fuels.

Algae-based biofuels hold great promise

Because of dramatic recent variations in oil prices and strong global concerns about climate change, biologically-produced fuels have increasingly been identified as potential alternative energy sources [1]. Biofuels are currently being promoted by many as one of the most promising pathways to reducing the world's dependence on fossil fuels, lowering CO_2 emissions, and in some cases, supporting local agriculture and developing economies [2]. In particular, the synthesis of biofuels from renewable biological resources is seen as a highly desirable means of meeting aviation and other worldwide transportation demands. Intense interest has focused on the biofuel promise of photosynthetic plants, which produce storage lipids in the form of triacyglycerols (TAGs) that can be used to synthesize biodiesel fuels via simple transesterification reactions [3,4]. Diesel fuel already holds a dominant position in the refined petroleum products market, and even in countries where gasoline is the primary liquid fuel, diesel vehicles have unique importance across a wide range of economic sectors [5]. The EU for example intends to replace 5.75% of all transportation fossil fuels with biofuels by 2010 and 10% by 2020, and major developed countries worldwide have similarly ambitious goals [6].

Trends in the global production of biodiesel reflect these goals: annual production rose from near zero levels in 1991 to almost 2 billion gallons in 2006, with a steeply exponential growth of 43% per year between 2001 and 2006 [7]. However, a major biodiesel dilemma already exists: even if it were advisable to turn this important food resource into fuel, the global annual production of TAGs from oilseed crops could not meet current the diesel demands of 44 billion gallons per year by the United States alone [8], and it has been estimated that the combined production of biofuels from traditional oil crops plus waste cooking oils and fats cannot offset the world's demand for transportation fuels [9].

Other biofuel feedstocks will thus be needed to meet the world's future energy demands, and biodiesel from microalgae could represent the only renewable source of oil that can meet global transportation fuel needs [10]. Microalgal biofuels are produced from the lipid content of the algal cells, which potentially can serve as the feedstocks for many high energy density transportation fuels, including biodiesel as well as green diesel, green jet fuel and green gasoline; the remaining algal biomass can also be converted to biofuels through either biochemical or thermochemical conversion routes [11].

Although the relative merits of cultivated terrestrial plant biomass versus microalgae as feedstocks for biofuel production are still a subject of debate [12–15], microalgae have numerous characteristics that favor their use as a biofuel source (Box 1). Conventional terrestrial plants are relatively inefficient in capturing light, converting less than 0.5% of the solar energy received at typical mid-latitudes into plant biomass; in contrast, the photosynthetic efficiency of microalgae potentially can exceed 10% [16]. In addition, microalgae require far less land, can be converted to liquid fuels using simpler technologies than needed to convert cellulose, and have secondary uses that fossil fuels do not provide [17]. Algal biodiesel can easily be used in unmodified diesel engines, and it has significant advantages over conventional diesel fuel because it is renewable, biodegradable and might produce lower emissions of sulfur oxides and particulates when burned [3]. In addition, microalgae are microscopic in size and can be grown continuously in well-mixed liquid cultures, potentially providing the benefits of controlled high-output productivity that are seen in industrial fermentation [18].

If microalgae are to be capable of meeting future global transportation fuel demands, sustainable and cost-effective systems for their large-scale cultivation must be put into place. Extensive research has focused on the selection and bioengineering of microalgal strains that can be grown in transparent photobiobioreactors (PBRs; e.g. [19])). However, we are unaware of any high-production PBR that is currently in sustainable operation for biofuel production, and conclude that the commercially viable use of PBRs is

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not likely at the present time. Whereas we acknowledge that debate about the relative advantages of PBRs versus pond bioreactors is likely to continue [4], we propose, as do others [19,20], that outdoor ponds are feasible for use as economically viable systems for sustained, high-volume, microalgae-based biofuel production. Outdoor pond bioreactors are a versatile and highly desirable alternative to PBRs, and they can simultaneously be used for high-throughput energy production, resource recovery and wastewater reclamation [21].

Because outdoor ponds are open to the environment, they contain diverse assemblages of natural algae and zooplankton; their behavior and operation is both subject to, and informed by, known ecological principles. Ecologists can therefore make critical intellectual contributions to the development of algal biofuels, particularly in conjunction with bioengineers, but to date there has been limited exchange of ideas between ecologists and bioengineers (however, see [22,23]). Here, we describe several ecological benefits of producing microalgae-based biofuels relative to crop-based feedstocks and we present several ecological principles that can help guide the successful mass production of microalgae.

We do not intend to present an exhaustive review of the challenges associated with land-based biofuel feedstock production, or to detail the technologies that will be required by biofuel production from crop or algal feedstocks. Rather, we highlight key issues of concern for land-based biofuel crops and several important ecological principles that should help to maximize the potential of algal-based biofuel production systems. First, we briefly describe the advantages of using microalgae as feedstocks relative to land-based, biofuel crop production. We then discuss how pond-based algal biofuel production, which generally requires significantly less space than land-based biofuel efforts, can offer additional ecological benefits by reducing pollutant releases to the environment and by requiring much lower water subsidies. Finally, we outline three key ecological principles that can inform the design and successful operation of pond-based microalgal systems for biofuel production.

Environmental advantages of algal biofuels
In order to be a viable alternative energy source, a biofuel should provide a net energy gain, have environmental benefits, be economically competitive and be producible in large quantities without reducing food supplies [24]. In the subsections below we illustrate how the use of microalgae as feedstocks for biodiesel production can provide significant environmental benefits by reducing the land, pollutant and water footprints of biofuel production.

Reductions in ecological impact and land footprint
The potential for biofuel feedstock production to create greater environmental damage than benefit is a critical challenge to establishing a successful advanced bioenergy industry [25]. The National Non-Food Crops Centre (NNFCC) of the UK has produced calculators that allow growers to assess whether it is economically feasible to produce their own fuel, invest in anaerobic digestion, or switch from cereals to non-food crops [26]. However, these calculators do not yet take into account the potential environmental costs that would be associated with such production decisions. We share the concerns of Johnston and Holloway [5] that until more efficient methods of biofuel production become commercialized, the growth of biodiesel will eventually have strong impacts both on global food supplies and on the long-term sustainability of agricultural production.

Quantifying the land use changes associated with intensive biofuel feedstock production relies upon many assumptions [27,28], but it is clear that the accelerated cultivation of terrestrial plant biomass for biofuels will have an exceptionally large land footprint. For example, the United States has the fourth largest absolute biodiesel potential of the 119 countries studied by Johnston and Holloway [5]. However, recent work has suggested that the projected year 2016 demand for corn ethanol alone would require 43% of all U.S. land used for corn production in 2004 [29]. A related study concluded that the annual corn production needed to satisfy one half of all U.S. transportation fuel needs would require an area equivalent to more than eight times the U.S. land area that is presently used for crop production [4]. Other land-based crops would require less cropland, based on their oil content: oil palm (24% of current cropland area), coconut (54%), jatropha (77%), canola (122%) and soybean (326%) [4]. Moreover, recent work indicates that the ability of countries to grow terrestrial crops explicitly for the production of biofuels such as ethanol and biodiesel is significantly overestimated [30], contributing to concerns that these biofuels are not feasible options for providing a significant fraction of global fuel demand.

The above studies take on added importance when we consider that cultivation of terrestrial biofuel crops can

Table 1. Comparison of estimated biodiesel production efficiencies from vascular plants and microalgae.[a]

Biodiesel feedstock	Area needed to meet global oil demand (10^6 hectares)	Area required as a percent of total global land	Area required as a percent of total arable global land
Cotton	15 000	101	757
Soybean	10 900	73	552
Mustard seed	8500	57	430
Sunflower	5100	34	258
Rapeseed/canola	4100	27	207
Jatropha	2600	17	130 (0)[b]
Oil palm	820	5.5	41
Microalgae (10 g/m^2/day, 30% TAG)	410	2.7	21 (0)[c]
Microalgae (50 g/m^2/day, 50% TAG)	49	0.3	2.5 (0)[c]

[a]Modified with rounding from [10].
[b]Jatropha is mainly grown on marginal land.
[c]Assuming that microalgal ponds and bioreactors are located on non-arable land.

contribute to the loss of natural ecosystems and their associated biodiversity [2]. For example, oil palm is one of the most extensively cultivated feedstocks worldwide, and expansion of its cultivation poses significant threats to tropical ecosystems [31]. Replacing intact terrestrial ecosystems with biofuel crops also can induce significant increases in greenhouse gas (GHG) fluxes. For example, converting rainforests, peatlands, savannas, or grasslands to produce food-based biofuels in Brazil, Southeast Asia, and the United States would create an undesirable 'biofuel carbon debt' that could release 17–420 times more CO_2 than the annual GHG reductions that such biofuels could provide by displacing fossil fuel combustion [32]. Using a worldwide agricultural model to estimate emissions from land-use change, Searchinger *et al.* [29] reported that corn-based ethanol could double GHG emissions over 30 years, and generate a net increase in GHG for 167 years. Their work also indicates that, if cultivated on U.S. corn lands, biofuel production from switchgrass would increase GHG emissions by 50%. Although the models used by studies such as those above require simplifying assumptions that might not always be completely accurate [27], their projections create significant concerns about the potential impacts of land-based biofuels on global land use patterns.

Many of the above concerns could be greatly reduced if microalgae were to be used as biofuel feedstocks. In comparison with terrestrially-based biofuel crops, microalgal production results in a far smaller land footprint because the oil productivity of many microalgae can exceed all other oil crops. For example, depending upon the lipid content of the harvested algae, the production of microalgal oil needed to satisfy 50% of U.S. transportation fuel needs would require a surface area of water equivalent to only 1–3% of all U.S. land currently used for agricultural crops [2,4]. A recent comparison of the global biodiesel production capacity of seven vascular plants versus microalgae was equally impressive: relative to the estimated biodiesel production from the three best performing terrestrial crops, in liters per hectare per year, estimates for microalgae-based biodiesel production were 2–16x higher [10]. The corresponding reductions in land footprint were very large, suggesting that current global oil demands could potentially be met by microalgae produced on less than 21% of the world's total arable land area (Table 1). An analysis by Pienkos and Darzins [11] indicates that even low-productivity microalgae can potentially produce more

than ten times the plant oil provided by soybeans (633 vs. only 48 gallons per acre per year); the contrast is even greater for medium-productivity (> 2500 gallons per acre per year) and for high-productivity algae (>10 000 gallons per acre per year).

Reductions in pollutant footprint
In addition to greatly reducing the land footprint of bioenergy production, the use of microalgae as a biofuel feedstock can potentially yield significant pollution control benefits. For example, Hill et al. [24] used life-cycle accounting analyses to explore the environmental, economic, and energetic costs and benefits of biodiesel and ethanol biofuels. When compared directly with ethanol, they concluded that soybean-based biodiesel released just 1.0%, 8.3%, and 13% of the agricultural nitrogen, phosphorus, and pesticide pollutants, respectively, per net energy gain.

Using these same three criteria, we expect that microalgae-based biodiesel should be superior both to ethanol and to soybean-based biodiesel. Microalgal production requires no external subsidies of herbicides or insecticides, and thus generates no associated pesticide waste streams; moreover, the economic costs typically associated with agricultural pesticide applications would also be zero. Furthermore, both the economic costs and the water pollution typically associated with agricultural fertilizers would be eliminated if microalgae production was driven by nutrient feeds supplied from confined animal feeding operation waste lagoons, human sewage, or other concentrated liquid waste sources.

High Rate Algal Ponds (HRAPs) and similar systems are already in use worldwide as nutrient removal systems for N- and P-rich wastewaters (e.g. [33–35]). Microalgae grown in bioreactors supplied with nutrient-rich wastewater absorb pollutants that are physically removed when the suspended microalgal cells are harvested for biofuel production [21,36]. Residual nitrogen and phosphorus concentrations in the filtrate obtained from dewatering the algal biomass will be far lower than in the original wastewater feed [37], thus greatly assisting in eutrophication management and control (Box 2). In addition, the harvested microalgae will contain significant quantities of organic compounds and heavy metal contaminants [38,39] absorbed from wastewater streams that would otherwise be directly discharged into surface and ground-

Box 2. Nutrient control of algal biomass

It has been established for more than 150 years that the biomass production of plants can be limited by the nutrient that is provided in the least quantity relative to cellular demands for growth: this theory is commonly known as Liebig's Law of the Minimum [77]. Although a brief but sharp controversy arose in the 1970s about the relative roles of carbon (C), nitrogen (N), and phosphorus (P) as regulators of phytoplankton growth and productivity, research worldwide has demonstrated unequivocally that N and P are most likely to be the dominant growth-limiting nutrients for microalgae in aquatic ecosystems. Our current concept of nutrient limitation implies that the growth of plants in a given ecosystem should be proportional to the rate of supply of the primary growth-limiting nutrients, and that the control of algal biomass can be accomplished by regulating the loading of these key nutrients to the ecosystem in question [78–80].

For freshwater ecosystems, the key nutrient has been identified as P. The local supplies of other essential resources such as carbon dioxide [78] and mineral elements other than P such as silicon or iron have only relatively minor effects on the yield of algal biomass produced at a given supply of P. However, nitrogen can become biomass-limiting at low N:P supply ratios [46,60,79–83]. The practical importance of this knowledge is clear: regardless of their geographical location on the globe, aquatic ecosystems that are increasingly enriched with N and P will in turn exhibit increasingly abundant microalgal growth, although the shape of this response might be strongly curvilinear at the highest nutrient enrichment levels [46,82,83]. As a result, the control of eutrophication worldwide has become focused on the restriction of nutrient inputs into our surface waters, both from point sources such as industrial and wastewater treatment plant effluents, and from non-point sources such as agricultural runoff. The management of eutrophication has substantial economic as well as ecological benefits [84,85].

waters, thereby adding to the environmental benefits of using microalgae rather than terrestrial crops for biofuel production. Physically connecting algal biodiesel production to wastewater feeds thus provides a low-cost supply of critically important resources (in the forms of water and nutrients), creates strong economic and environmental incentives and provides a pathway for the development and eventual commercialization of this technology [36].

In addition, microalgae represent one of the few technologies that can be used effectively for the biological capture and utilization of CO_2 emitted by fossil-fuel-driven power plants: CO_2 captured from power plants can be introduced into outdoor ponds and partially recaptured in the form of algal biomass. Benemann and colleagues [36,37] have concluded that simple pond-based CO_2 biosequestration systems should have fewer and more predictable process variables than would terrestrial plant-based sequestration systems (which also would be strongly affected by local variations in soil quality and precipitation), thereby allowing greater flexibility with regards to the geographical placement of the microalgal bioreactors. Moreover, he noted that the rapid growth rates of microalgae (which have generation times of hours to days) should allow the research and development of such systems to be completed in years rather than decades.

Reductions in water demand footprint
Although biofuels are currently only a marginal additional stress on regional and local scale water supplies, future acceleration of biofuels production could cause very significant water quantity problems [40]. Relative to conven-

tional energy sources, a much greater volume of water is required to produce an equivalent amount of energy from biofuels, and thus will be very important to mitigate the impacts of biofuel production on water resources, given the large surface and groundwater demands that are associated with agricultural stages of a biofuel life cycle [41]. Large-scale global expansions of terrestrial energy crop production could lead to large increases in evapotranspiration appropriation for human uses that could be equivalent in magnitude to present evapotranspiration rates from global cropland [42]. As emphasized by Gerbens-Leenes *et al.* [43], the discussion of whether agricultural crops can or should be used for energy production must therefore be extended to discussions of how we can most effectively use our increasingly limited water supplies.

Linking algal biodiesel production with anthropogenic wastewater production directly addresses these water resource allocation concerns: for the foreseeable future, humans will continue to generate immense flows of nutrient-rich wastewater that can potentially be directly mated to microalgae-based energy production. Unlike terrestrial crops, no irrigation subsidy would be required, and the high quality of the filtrate obtained from dewatering the harvested algal biomass should meet or surpass current standards for wastewater discharges, thereby preserving water quality as well as water quantity.

Concepts and principles from ecology
The successful, large-scale generation of biodiesel from microalgal feedstocks will require viewing the algal production facilities as biologically diverse bioreactors that will obey the known rules of ecology. In the three subsections below we illustrate how the application of core concepts and principles from ecology and ecological physiology

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Figure 1. Dense algal growth in four pilot-scale tank bioreactors fed by treated wastewater from the Lawrence, Kansas (USA) wastewater treatment plant (photo by B. Sturm). Each fiberglass bioreactor has an operating volume of ten cubic meters of water, and is operated as an air-mixed, flow-through vessel. Nutrient-rich wastewater inflows are pumped in through the clear plastic hose (blue clamp), and water outflow occurs through the white plastic pipe shown at the waterline. These bioreactors are intended to be operated year-round, as the temperature of the inflowing wastewater is consistently ca. 10 °C.

can provide important new insights into the design and operation of these systems.

Nutrient limitation of algal production

As in freshwater lakes, the productivity of microalgae grown in outdoor bioreactors (e.g. Figure 1) will be limited by their supply of growth-limiting nutrients [44]. The resulting algal biomass production can be predicted by our limnological knowledge base, which has uncovered strong relationships between algal biomass and the nutrient concentrations in lakes worldwide (Box 2). A remarkably strong relationship exists between algal biomass (expressed as concentrations of the photosynthetic pigment chlorophyll a, mg L^{-1}) and measured concentrations of total phosphorus (TP, mg L^{-1}) in the water column [45,46]. As in eutrophication science, such empirical relationships can be used to estimate the biomass of microalgae that can be generated by outdoor bioreactors containing known concentrations of total phosphorus. When the lipid content of this biomass is also known (e.g. the lipid:chlorophyll a ratio), corresponding estimates for annual biodiesel production by these pond bioreactors can in turn be calculated.

Physiological controls of algal biomass and lipid production

Lipid productivity, the mass of lipid that can be produced per day, is dependent upon plant biomass production as well as the lipid content of this biomass [4]. Algal biodiesel production will therefore be limited not only by the standing crop of microalgae, but also by its lipid content, which can vary from <1% to >50% dry weight [3,47].

Given that a strong and predictable response of microalgal biomass to phosphorus enrichment has consistently been exhibited by freshwater ecosystems worldwide (Box 2), it can be expected that the volumetric lipid content (in mg L^{-1}) of water contained in algal bioreactors should also in general increase with an increase in the total phosphorus content of the system, as has been reported for lakes by Berglund *et al.* [48]. However, both the quantity and the quality of lipids produced will vary with the identity of the algal species that are present in the water, as well as with site-specific growth conditions. This variability probably reflects modifications in the properties of cellular membranes, and alterations in the relative rates of production and utilization of storage lipids [49].

In the presence of moderate temperatures and sufficient light, many dozens of studies during the past several decades have revealed that algal lipid content is particularly sensitive to conditions of nutrient limitation [3]. For example, silicon-starved diatoms can contain almost 90% more lipids than silicon-sufficient cells [47]. However, silicon will be a growth-limiting nutrient only for the limited subset of microalgal species that have an absolute requirement of this element for their cellular growth. A stronger stimulation of lipid production occurs in response to conditions of nitrogen limitation, which potentially can occur in all known microalgae. Nitrogen-starved cells can contain as much as four times the lipid content of N-sufficient cells [3,47,50–53], and maximizing the lipid pro-

duction of pond bioreactors should therefore depend on their operators' ability to reliably and consistently induce N-limitation in the resident algal cells.

Resource-ratio theory and the principles of ecological stoichiometry (e.g. [54,55]) provide additional new insights into the control of algal biomass and lipid production in pond bioreactors. As demonstrated by Rhee [56], the nutrient limitation status of microalgae can be directly controlled by regulating the ratio of nitrogen and phosphorus (N:P) supplied in the incoming nutrient feed: nitrogen limitation occurs at N:P supply ratios that lie below the optimal N:P ratio for microalgal growth, whereas phosphorus limitation occurs at ratios that exceed this ratio. A transition between N- and P-limitation of phytoplankton growth typically occurs in the range of N:P supply ratios between ca. 20:1 to ca. 50:1 by moles [57,58]. Such shifts between N- and P-limitation have extremely important implications for algal biofuel production because diverse species of microalgae grown under nitrogen-limited conditions (i.e. low N:P supply ratios) can exhibit as much as three times the lipid content of cells grown under conditions of phosphorus limitation (high N:P supply ratios) [59].

Both the total phosphorus concentration as well as the total nitrogen concentration in the nutrient feeds to pond bioreactors should therefore impact algal biodiesel production, because the N:P ratio of incoming nutrients will strongly influence algal biomass production [46,56,60] as well as the cellular lipid content. Given the inverse relationship observed between N:P and cellular lipids [59], and the positive, hyperbolic relationship observed between N:P and microalgal biomass [56], we conclude that optimal lipid yields (in terms of mass of lipid produced per unit bioreactor volume per day) should occur at intermediate values of the N:P supply ratio. From the strong apparent interactions between the effects of nitrogen and carbon dioxide availability on microalgal lipids (e.g. [61]), we also conclude that the effects of N:P supply ratios on volumetric lipid production might be even greater if the bioreactors are simultaneously provided with supplemental CO_2 (cf. Figure 2).

In addition to their N:P supply ratio, the hydraulic residence time (HRT, in days) of pond bioreactors will also influence total lipid production because the lipid content of microalgal cells is strongly determined by the per capita population growth rate (r, day^{-1}). For example, at very low growth rates (< ca. 0.1 day^{-1}), cellular lipids in four very different species of microalgae were found to be 2–3 times higher than at higher growth rates [62]. Because the microalgal biomass achieved in outdoor bioreactors should be highest at long HRTs [63], and because the harvestability of this algal biomass might also be highest at long HRTs [64], we conclude that lipid yields from continuous-flow outdoor pond bioreactors could potentially be maximized by using HRTs longer than ca. 10–15 days (corresponding to microalgal growth rates <0.1 day^{-1}).

Species diversity, solar energy fixation and nutrient removal efficiency

Outdoor pond bioreactors for microalgal production will be continuously invaded by airborne and animal-borne pro-
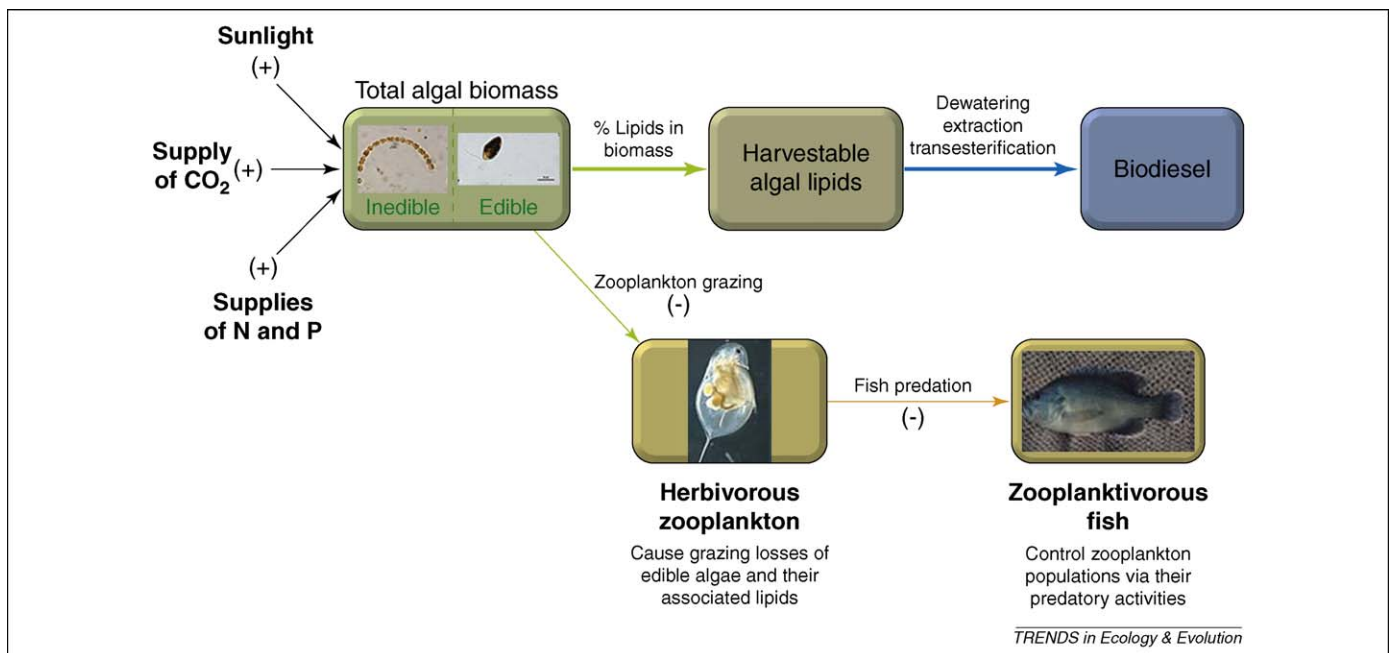
Figure 2. Natural biotic communities in outdoor bioreactors require the external provision of potentially growth-limiting resources (e.g. light, carbon dioxide and the essential mineral nutrients N and P). These resources act as ''bottom-up'' regulators of the potential microalgal biomass that can be produced. Once harvested, the cellular lipids in this microalgal biomass can be extracted and processed to create biodiesel fuels. The lipid content of microalgal biomass is not constant, however, and can be influenced by many factors, including nitrogen:phosphorus supply ratios, light, CO_2 and the hydraulic residence time of the bioreactor. Moreover, natural assemblages of microalgae are taxonomically diverse: some species are small and can easily be consumed by herbivorous zooplankton. Undesirable grazing losses of edible microalgae (and their cellular lipids) to large-bodied zooplankton can be reduced by adding zooplanktivorous fish, which can greatly restrict large-bodied zooplankton growth via size-selective predation (''top-down'' regulation).

pagules of microalgae, protozoa and zooplankton, and will therefore typically contain multispecies communities. High microalgal diversity in pond bioreactors potentially confers two important ecological advantages, relative to single-species photobioreactors. First, ecological diversity-productivity theory proposes that multispecies pond ecosystems should produce much higher algal biomass yields (and thus solar energy fixation) than simpler, single-species PBRs. Stimulation of microalgal biomass by increased supplies of nutrients and CO_2 thus should be significantly greater in species-rich than in species-poor systems (e.g. [65–67]). Moreover, high-diversity microalgal

communities have been observed to contain a higher content of carbon per unit of limiting nutrient in their biomass [68], reflecting an increase in their potential ability to store solar energy as harvestable oils. Second, because of resource complementarity, retention of other plant nutrients by high-diversity microalgal assemblages should be much more efficient than single-species systems [32,65–67], thereby enhancing the ability of pond bioreactors to remove nutrient pollutants from incoming wastewater feeds.

However, pond bioreactors will not contain communities of microalgae alone. They will be susceptible to invasions

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Box 3. Effects of food web structure on algal biomass

The effects of food web structure on algal biomass in ponds and lakes have been key subjects of study for aquatic ecologists for more than half a century, and predator–prey interactions are now thought to be a key determinant of ecosystem processes and stability [86]. In particular, it has been found that differences in the abundance and community structure of fish communities can dramatically influence the biomass of microalgae by modifying the intensity of cell mortality due to zooplankton grazing. Herbivorous cladocerans such as *Daphia pulex* and *Daphnia pulicaria* typically become abundant in lakes having few or no zooplanktivorous (zooplankton-eating) fish, and these large-bodied herbivores are extremely effective herbivores. For example, Hrbáček *et al.* [87] first noted that fish-free ponds tended to exhibit much lower standing crops of phytoplankton than those containing fish. Work on the effects of food web structure expanded rapidly after the synthetic papers of Shapiro *et al.* [88,89], leading to the concepts of trophic cascades and top-down versus bottom-up control of primary productivity in aquatic ecosystems (e.g. [90]). This research also led to the proposed use of deliberate food web manipulations to reduce nuisance phytoplankton biomass in nutrient-enriched lakes (biomanipulation: [88,91]).

Aquatic ecosystems dominated by zooplanktivorous fish typically produce a significantly higher algal biomass per unit phosphorus than lakes dominated by piscivorous (fish-eating) fish [92–95], because the intensity of zooplankton grazing on algae is typically lowest in planktivore-dominated systems. Fishless microalgal bioreactors can be expected to develop large populations of large-bodied herbivorous zooplankton, and thus to exhibit significantly lower algal biomass per unit total phosphorus than would be predicted from their phosphorus supply, relative to bioreactors that contained zooplanktivorous fish and small, less-efficient grazers. Because sustainable and economically viable biodiesel production requires the constant production of high levels of microalgal biomass, the deliberate establishment of large and persistent populations of highly efficient zooplanktivores (e.g, the mosquitofish *Gambusia affinis*, or similar fish species) should increase the probability that consistently large and temporally stable crops of microalgae can be produced by nutrient-enriched outdoor pond bioreactors (see Figure 2). For example, a meta-analysis of the effects of food web structure on microalgal standing crops in experimental enclosures and ponds revealed that the addition of zooplanktivorous fish could result in as much as a 32-fold increase in chlorophyll *a*, relative to fishless systems [96].

by herbivorous zooplankton (protozoa, rotifers and micro-crustaceans; see Figure 2), creating a relatively simple, 2-trophic level food web. However, a 2-level food web structure can potentially exhibit strong day-to-day variance in microalgal biomass due to temporal variations in the grazing activities of large-bodied herbivores. For example, fishless, 2-trophic level systems tend to become dominated by large-bodied herbivores such as *Daphnia*, which can graze microalgal biomass down to extremely low levels (Box 3). Similar food web dynamics occur in HRAPs and sewage oxidation ponds: strong oscillations have been observed between *Daphnia* and microalgae in aerated sewage lagoons, HRAPs, and other wastewater-fed systems, with algal biomass concentrations that can exceed 1 mg L^{-1} as chlorophyll *a* [64,69–71].

From a biofuel production standpoint, high amplitude predator–prey oscillations can lead to algal biomass "crashes," causing large and unpredictable reductions in biodiesel production. For example, microalgal biomass peaks as high as 0.33 mg L^{-1} chlorophyll a were observed in a Luxembourg lagoon when *Daphnia* were rare in the water column; in contrast, microalgal biomass declined by more than two orders of magnitude to only 0.001–0.002 mg L^{-1} during periods of maximum *Daphnia* abundance and grazing intensity [70].

Here, we suggest that the ecological principles of top-down control (Box 3) can be applied to dampen these undesirable biomass oscillations: introducing one or more species of zooplankton-consuming fish into these systems should help to reduce microalgal losses to zooplankton grazers, and thereby should help to maximize the magnitude of microalgal biomass that can be obtained at a given concentration of total phosphorus in the pond bioreactor (Figure 2). However, it is clear that the effects of top-down interactions in freshwaters can be modified by many factors, including the absolute nutrient content of the water and the morphometry of the system itself [72]. We also note that because outdoor bioreactors will exhibit even more strongly compressed aquatic food webs than most natural lakes, they could be extremely sensitive to dynamic instabilities [73]. Confirmation that food web manipulations can be used to help assure stable, high-yield algal biodiesel production thus awaits explicit experimental tests.

Concluding remarks

Increases in atmospheric CO_2 concentrations and rapid declines in global oil reserves have made it imperative that we move more rapidly towards the development of carbon-neutral, cost-effective, renewable substitutes for fossil energy sources [10]. High energy prices, increasing energy imports, concerns about the sustainability of existing petroleum supplies, and a growing recognition of the environmental consequences of fossil fuel use have served to drive strong interest in the development of new transportation biofuels [24]. Biodiesel produced from the mass cultivation of microalgae potentially offers a highly attractive and ecologically-friendly biofuel, but after almost half a century of research the full promise of algae as a feedstock for biofuel production has remained largely unfulfilled.

We recognize that numerous barriers remain to be overcome before the large-scale production of microalgae-derived biofuels can become a commercial reality ([11; see also Refs. [4,10,74]), and we have addressed several important scientific and technical barriers in this review. We have summarized three of the major environmental benefits of using microalgae as a biofuel feedstock, and identified key ecological factors that we believe will be essential to consider if microalgae grown in outdoor pond bioreactors are to become a partial solution to the world's energy needs. Combining ecological principles with insights from the disciplines of algal physiological ecology, limnology, and ecological stoichiometry can provide important new guidance for the design and successful operation of microalgae-based biofuel production ecosystems.

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