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Trait diversity enhances yield in algal biofuel assemblages

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Summary

1. Phytoplankton offer great potential as a bioenergy crop; however, technological advances are needed to intensify their yield and reduce their footprints for water, nutrients and land. One approach to enhance productivity is to grow polycultures of mixed species, which convert abiotic resources into biomass more efficiently than any single taxon.

2. We measured traits related to nutrient and light use, growth rate, biomass production, stoichiometry and neutral lipid concentration in 16 diverse microalgal taxa. Species with large cells (primarily Chlorophyta) showed rapid growth, high asymptotic biomass, low minimum nutrient demands, and high cellular C : N and C : P ratios. These same species also exhibited high minimum demands for light and low lipid concentrations. We grew all 119 possible species pairs and found that biomass yield exceeded the component monocultures in polycultures consisting of species with highly divergent traits. However, underyielding occurred frequently as many pairs produced less biomass than either the mean or the maximum of the two component monocultures.

3. In terms of ecological trade-offs, competitive ability for N and P were positively correlated, but negatively related to ability to grow at low light. In terms of bioenergy production, the species with high cellular lipid concentrations showed both slow growth and high demands for nutrients.

4. *Synthesis and applications*. Our results identify trade-offs among functional traits that determine the suitability of different algal species as biofuel feedstocks and narrow the search for productive and robust species combinations to maximize bioenergy productivity. An approach based on the ecology of species traits will be more effective in optimizing yield in bioenergy communities than promoting high species diversity *per se*.

Key-words: biodiversity, bioenergy, ecosystem function, phytoplankton, stoichiometry, trade-offs

Introduction

The cultivation of photosynthetic organisms to replace fossil fuels as sources of energy for society is an alluring prospect (Service 2011; Georgianna & Mayfield 2012). However, all crops have substantial footprints for land, water and fertilizers, raising the question of whether bioenergy can actually reduce environmental damage relative to conventional fuels (Hill *et al.* 2006; Fargione *et al.* 2008; Searchinger *et al.* 2008). Unicellular algae are far more productive than vascular plants (Cebrian 1999; Chisti 2007); however, their demands for nutrients and water as well as their susceptibility to a range of pathogens and consumers limit their potential for industrial-scale cultivation (Clarens *et al.* 2010). Efforts aimed at increasing yield and minimizing inputs of fertilizers, water and pesticides have largely focused on manipulating the genetics and cellular metabolism of various algal species (Radakovits *et al.* 2010). Ecological approaches to maximizing production have been proposed, but rarely tested (Smith *et al.* 2010; Stockenreiter *et al.* 2012; Shurin *et al.* 2013).

One generality, which has emerged in the ecological literature, is a positive effect of species diversity on the rate of ecosystem biomass production (Hooper *et al.* 2005; Cardinale *et al.* 2011). A diverse community may acquire

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and convert abiotic elements into biomass more efficiently than monocultures if species are complimentary in their use of resources (Loreau 1998; Ptacnik *et al*. 2008). This principle has been tested and supported in bioenergy production by both algae (Stockenreiter *et al*. 2012) and grassland plants (Tilman, Hill & Lehman 2006). Although studies have shown greater productivity in more diverse communities, the effect of diversity on productivity varies tremendously within and among systems (Hooper *et al*. 2005; Cardinale *et al*. 2006; Hillebrand & Matthiessen 2009), indicating that growing diverse species *per se* is unlikely to be a reliable approach to increasing bioenergy yield. In fact, negative selection effects or reduction in community production or function at high diversity has been documented in a number of communities (Jiang, Pu & Nemergut 2008; Schmidtke, Gaedke & Weithoff 2010; Becker *et al*. 2012). Identifying the most productive combinations of taxa and developing a predictive model relating species composition to productivity are critical to applying ecological principles to industrial bioenergy (Hillebrand & Matthiessen 2009).

We employed laboratory microcosm experiments in a trait-based approach (McGill *et al*. 2006) to understand the effects of algal diversity on productivity. First, we measured a suite of traits related to life history (exponential biomass growth rate r, and asymptotic density K), biochemistry (cellular neutral lipid concentration), stoichiometry (cellular C : N : P ratios) and minimum requirements for N, P and light (N*, P* and L*, the critical resource levels below which growth cannot be maintained, Tilman 1981) among 16 species. These included nine Chlorophyta, four Cyanophyta, one each of Bacillariophyta, Heterokontophyta and Glaucophyta (Table 1). Species were chosen to represent a broad taxonomic diversity as well as several candidate bioenergy crops (Rodolfi *et al*. 2009). Traits were selected to be relevant to both bioenergy production and competitive interactions, including minimum resource requirements, growth rates, asymptotic density and lipid content. Correlations among traits indicate functional associations or trade-offs in the abilities of species to perform various processes related to fitness (Wright *et al*. 2004; Litchman & Klausmeier 2008;

Table 1. Species used in the experiment with their sources and the codes shown in Fig. 1. The regression parameters (Intercept and Slope) are for their fitted relationships between optical density (OD) and dry weight (DW), shown in Fig. S1, Supporting information

Code	Species	Collection code	Group	K (μg chla L^{-1})	r (day^{-1})	Cell volume (μm^3)
E	*Aphanothece* sp.	CCMP 2529	*Cyanophyta*	21·0	0·3	10·7
K	*BLO 902*	Isolated strain	*Cyanophyta*	14·7	0·4	7·3
N	*BLO 910*	Isolated strain	*Chlorophyta*	1860·4	5·2	12·4
D	*Chlamydomonas reinhardtii*	Isolated strain	*Chlorophyta*	1871·7	26·3	299·8
L	*Chlorella minutissima*	UTEX 2219	*Chlorophyta*	2384·8	14·6	21·9
M	*Chlorella vulgaris*	UTEX 395	*Chlorophyta*	871·5	2·0	42·2
H	*Chlorococcoum* sp.	UTEX 105	*Chlorophyta*	1979·5	13·9	781·7
O	*Cyanophora biloba*	UTEX LB 2767	*Glaucophyta*	35·1	1·0	171·0
F	*Nannochloropsis oculata*	UTEX LB 2164	*Heterokontophyta*	93·6	2·5	16·4
J	*Navicula* sp.	Isolated strain	*Bacillariophyta*	847·9	1·4	49·3
G	*Neochloris oleoabundans*	UTEX 1185	*Chlorophyta*	1547·7	8·6	77·3
P	*Scenedesmus dimorphus*	UTEX 1237	*Chlorophyta*	1945·6	10·0	140·5
A	*Scenedesmus obliquus*	UTEX 393	*Chlorophyta*	2220·0	6·5	110·1
B	*Synechococcus elongatus*	PCC 7942	*Cyanophyta*	27·4	0·5	2·6
C	*Synechocystis* sp.	PCC 6803	*Cyanophyta*	9·9	0·6	4·1
I	*Tetraselmis* sp.	UTEX LB 2767	*Chlorophyta*	2063·8	15·2	89·7

Code	Lipid %DW	L* (μM m^{-2} s^{-1} PAR)	N* (M)	P* (M)	C : N	C : P	Intercept	Slope
E	NA	1·66	9·1E-04	5·6E-06	1·7	234·0	NA	NA
K	6·9	1·56	9·5E-04	3·6E-06	2·7	281·8	8·7	606·3
N	10·7	0·40	5·2E-06	1·5E-07	13·0	920·0	3·0	861·3
D	4·3	0·25	1·9E-05	1·4E-05	7·6	475·2	−9·6	895·4
L	10·5	0·04	4·4E-05	1·9E-07	7·6	421·8	−7·7	538·9
M	3·6	0·67	4·5E-04	7·6E-05	5·2	242·8	9·1	740·6
H	1·8	0·02	9·7E-05	6·3E-06	8·1	605·3	−61·3	2041·3
O	6·9	0·41	1·4E-04	1·4E-06	4·1	163·5	−8·3	942·6
F	5·3	0·59	1·9E-03	5·2E-05	3·8	301·4	−10·9	793·1
J	14·2	0·00	6·1E-06	5·5E-06	3·3	368·9	−20·6	1081·5
G	6·4	0·14	6·2E-05	3·3E-06	5·9	96·9	5·0	516·1
P	5·3	5·22	1·2E-05	2·1E-06	7·9	759·3	−2·9	818·0
A	6·6	0·67	1·6E-06	3·1E-07	8·5	346·1	−11·4	638·1
B	6·7	1·03	2·3E-04	9·3E-06	2·5	159·8	2·5	687·8
C	26·1	0·95	9·8E-05	6·1E-07	2·8	438·8	−14·6	565·7
I	5·2	0·13	1·5E-05	1·7E-06	4·2	173·1	2·7	527·7

PAR, photosynthetically active radiation.

Edwards, Klausmeier & Litchman 2011). Secondly, we grew all 119 pairs of these species in combination and measured $\log_e$ of the ratios of biomass produced in polyculture to that of both the average [the net biodiversity effect (NBE), Loreau & Hector 2001] and the maximum [overyielding (OY)] of their two component monocultures. We used the measured trait values to predict NBE and OY of the species pairs in order to gain a mechanistic understanding of the factors giving rise to positive effects of diversity on production.

Materials and methods

TRAIT EXPERIMENTS

Each species was grown in monoculture at four levels of N and P, and five light levels with five replicates per treatment. The same nutrient concentrations were used for every species; however, different light levels were used for prokaryotes vs. eukaryotes because cyanobacteria generally grew poorly at high light levels. Nutrient supply was manipulated by varying the concentration of N (as $NaNO_3$) between 1×10^{-6} and 1×10^{-3} M in factors of 10, and of P (as K_2HPO_4) between 5×10^{-8} and 5×10^{-5}M. Light supply was manipulated by covering the cultures with layers of shade cloth. The light supply levels were 70, 24, 14, 10 and 8 μmol m^{-2} s^{-1} of photosynthetically active radiation (PAR) for eukaryotes and 70, 14, 8, 3 and 2 μmol m^{-2} s^{-1} PAR for prokaryotes. In the light experiments, the nutrients were supplied at their highest levels, while light in the nutrient experiments was set at 70 μmol m^{-2} s^{-1} PAR for eukaryotes and 14 μmol m^{-2} s^{-1} PAR for prokaryotes. These light and nutrient levels were chosen to span the range from resource limitation to saturation for the majority of species (Interlandi & Kilham 2001). The trait experiments were performed in 50-mL Erlenmeyer flasks with a volume of 20 mL using WC medium (Guillard & Lorenzen 1972) and a dilution rate of 20% every two days (10% per day). Growth was measured every other day as chlorophyll-*a* concentration using *in vivo* fluorescence in a Turner Trilogy fluorometer and the experiments lasted until the species reached steady state in a majority of treatments (18–34 days). A mean temperature of 21·3 °C was maintained in a growth chamber with a 12 : 12 photoperiod. Because we measured traits for 16 species, the trait experiments were performed for groups of three to six species at a time, and the N, P and light experiments were run simultaneously for each species.

Growth curves were used to estimate asymptotic density at steady state (K, in μg chlorophyll-*a**L^{-1}), maximum exponential growth rate (μ_{max}, in day^{-1}) and critical minimum limiting thresholds (R*) for N, P and light (N*, P* in moles L^{-1} and L* in μmol m^{-2}*s). We used the 'grofit' package in R (R 2010) to fit growth functions that estimate the lag phase before growth is initiated, the maximum growth rate (μ_{max}) and the asymptotic density. The grofit function estimates these parameters using four different models (logistic, Richards, Gompertz and modified Gompertz) and then selects the best model using AIC. We adopted this approach because the growth curves took many forms and could not all be well-approximated with the same function. However, the different models generally gave very similar estimates of μ_{max} and K. We estimated μ_{max} and K for every replicate in the experiment and used the mean K of the treatment with the highest average value as the estimate for the species' trait. We chose this value as it indicates the maximum density that the species could achieve under optimal growing conditions.

We then plotted μ_{max} for each replicate against resources level (light or nutrient, S) to estimate R*. For light, where most species showed positive growth at most treatment levels, we used a modified Michaelis–Menten equation to estimate maximum growth rate (r) and minimum resource requirements as:

$$\mu_{max} = \frac{V_{max}(S + a)}{K_m + S + a}$$

V_{max} is the maximum growth rate at resource saturation, K_m is the half-saturation constant, and a is the y-intercept. We used the fitted value of V_{max} as our estimate of μ_{max} for each species, and $-a$ as our R*, the value of S where μ_{max} is zero. Because all species failed to grow at one or two of the lowest levels of nutrients, we did not have enough data to estimate parameters using the above equation for the nutrient experiments. For N and P, we used the mid-point on a $\log_e$ scale between the lowest nutrient level where positive growth was observed and the next lowest level as the estimate of R*. For example, if growth was observed at $8{\cdot}5 \times 10^{-3}$ μmol N*L^{-1}, but not at $8{\cdot}5 \times 10^{-4}$ μmol N*L^{-1}, we defined N^* as $2{\cdot}7 \times 10^{-3}$. For this reason, each species was estimated to have one of only three different values of P* and one of two values of N* (Fig. 1).

Samples were collected at the end of each growth experiment to analyse neutral lipid and C, N and P contents of the algal biomass. The Nile red method was used to measure neutral lipid content according to Chen *et al.* (2009). Neutral lipids include triglycerides, the precursor molecules of biodiesel via transesterification (Chisti 2007), and are therefore of the greatest relevance for bioenergy. C and N contents were measured by filtering 4–6 ml of the culture onto pre-combusted Whatman GF/F filters and using an elemental analyser at the UC Davis Stable Isotope Facility. P content was measured according to Menzel & Corwin (1965) using the PO_4 module in a Turner Trilogy fluorometer. Species-specific values for C : N, C : P and percentage lipid content were calculated by averaging across all of the nutrient and light treatments for which sufficient biomass was present to permit measurement. Although the nutrient and light treatments affected the stoichiometry and lipid content of the algae, the differences among species were generally substantially greater than those between growing conditions. The variation in C : N, C : P and percentage lipid content explained by the species' identity in factorial ANOVAS (the partial R^2) was between 1·5 and 50× greater than that explained by the light, N or P treatment, with a mean of 15·6× greater explanatory power. We therefore use the average values across nutrient and light treatments as the species mean values of nutrient and lipid contents.

BIODIVERSITY EXPERIMENTS

All 16 species were grown singly and in pairwise combination in WC Medium (Guillard & Lorenzen 1972) in 96-well plates (well volume = 0·3 mL) incubated at 26 °C on an orbital shaker of 120 rpm with a 24-hour photoperiod of 60 μmol m^{-2} s^{-1} PAR. Inocula were diluted with medium to create the species mixtures, so that monocultures and species pairs were initiated at optical density at 750 mn of 0·02 [i.e. each species' initial optical density (OD) was 0·01; therefore, the initial OD of the mixtures was

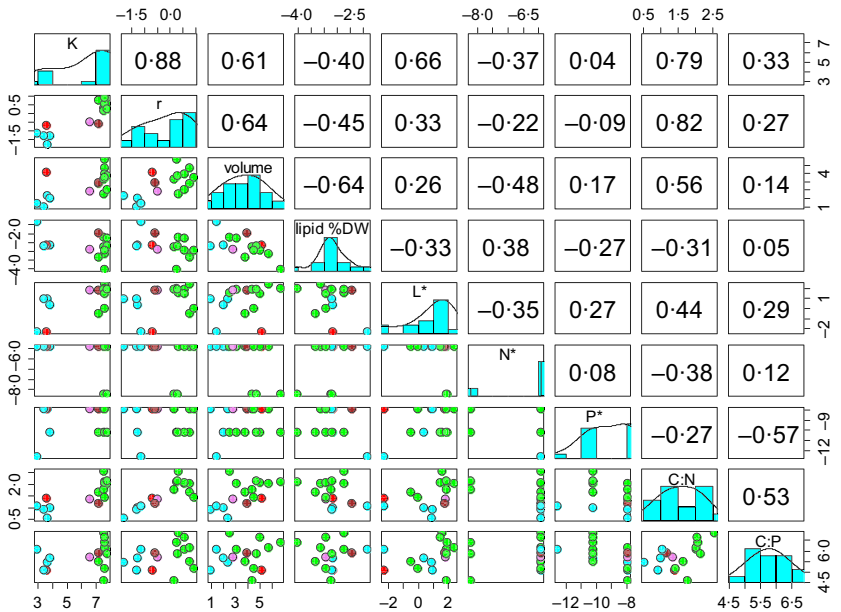

Fig. 1. Correlations among the nine traits measured for the sixteen species of algae. Each point represents a species, and the colour indicates the taxonomic affiliation as in Fig. 2. All variables are plotted on $\log_e$ scales, and the units are ug chla L^{-1} (*K*), day^{-1} (*r*), μm^3 (cell volume), per cent of dry weight (lipid), $\mu M^* m^{-2} * s^{-1}$ PAR (L*) and moles (N* and P*). The upper panel indicates the Pearson correlation for each pair of variables, the lower panel is the raw data plots, and the diagonal shows the histogram for each variable.

0·02, the same as that of the monocultures]. All treatments were replicated five times. Growth was measured as OD at 750 nm every other day using a Thermo Scientific® Multiskan FC microplate spectrophotometer after 30 s of shaking. OD approached steady state after 8 days for a majority of species (Fig. S3, Supporting information); therefore, we used OD on day eight as a measure of final biomass yield. Curves relating OD to dry weight for each species are shown in Fig. S1 (Supporting information). Because of the large number of species combinations, the diversity treatments were performed over four separate experiments. Each species was grown alone and in combination, and growth in pairs was compared with monocultures from the same experiment for calculating NBE and OY. NBE and OY measure the difference in biomass yield between the polyculture and the mean and the maximum of its two component species in monoculture, respectively. Figure S3 (Supporting information) shows examples of calculations of NBE and OY. We tested the abilities of the trait values of the species making up the pairs to predict values of NBE and OY by summarizing the trait correlations using principal components analysis (PCA). NBE and OY were regressed on the mean and range of the values of the first two principal component axes to test for their effects on the productivity of species pairs relative to their component monocultures.

Results

We found strong patterns of association among the nine traits measured for the 16 species. Figure 1 shows the raw correlations among the traits, and Fig. 2 shows the first two axes of a principal components analysis (PCA) summarizing their patterns of association. Large-celled species (primarily Chlorophyta) grew rapidly (high μ_{max}), reached high asymptotic biomass (*K*), had high C : N and C : P ratios in their cells, low N* and P*, high L*, and low

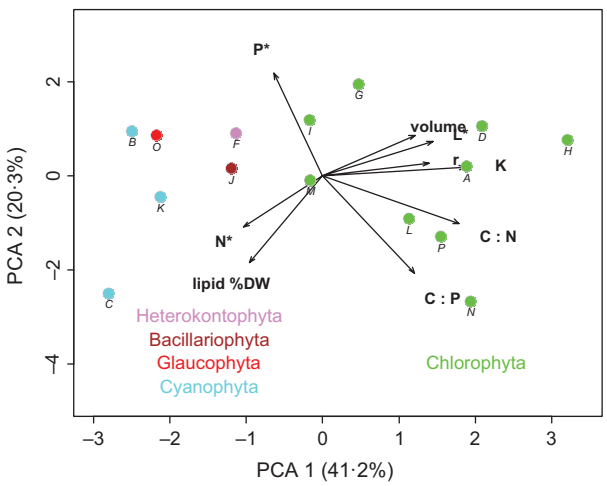

Fig. 2. A principal components analysis (PCA) summarizing the correlations among the seven traits measured for all species in the experiments except *Aphanothece* (Table 1) for which lipids could not be measured due to poor growth. The red arrows show the correlation between the trait values and each principal axis. For instance, *r*, *K*, L*, cell volume, C : N and C : P are all positively correlated with PC1 (and with each other, see Fig. 1), while % lipid, N* and P* are negatively correlated with PC1. The points show the position of the species; for instance, species H (*Chlorococcum* sp.) has high values of all traits correlated with PC1, while species B (*Synechococcus elongatus*) has low values on this axis. The colour of the points indicates taxonomic group of each algal species as indicated on the plot, and the letter marking each point corresponds to the species codes in Table 1. PCA1 and PCA2 explain 41·2% and 20·3% of the variation among species, respectively.

cellular neutral lipid concentrations. The most lipid-rich species were mostly small and slow-growing cyanobacteria. Demands for N and P were closely associated and

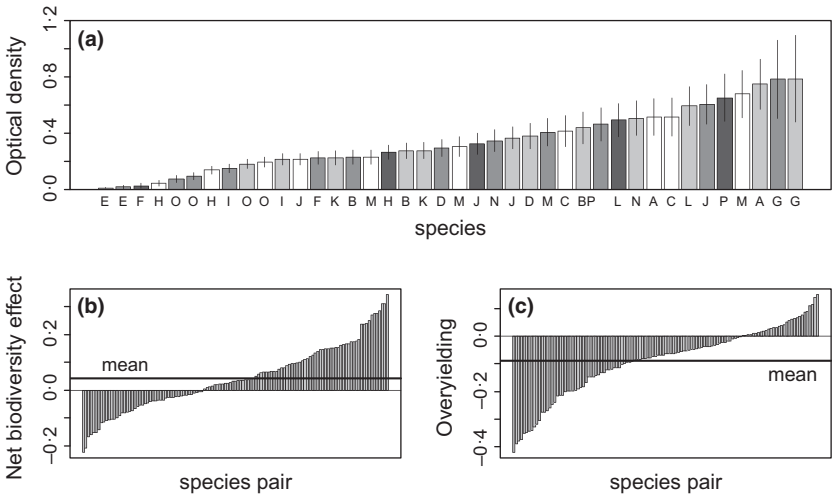

Fig. 3. The distribution of (a) final optical density (OD) after eight days of growth, (b) the net biodiversity effect ($\log_e$ NBE) and (c) overyielding ($\log_e$ OY) for all 119 pairs of species grown together. The letters on the *x*-axis of (a) indicate the species from Table 1. Growth in monoculture and polyculture was measured over four separate experiments. As all species were included in multiple experiments, OD is shown for each experiment in panel a, and the shading of the bar indicates the experiment [darkest is Experiment 1 shown in Fig. S2 (Supporting information), lightest is Experiment 4].

negatively correlated with minimum light requirements. Species with low N* and P* had high C : N and C : P contents in their cells and also showed fast growth and high final biomass density (Fig. 1). The first two principal components axes explained 41·2% and 20·3% of the variation among species.

The species showed a wide range of performance when grown in pairwise combinations. Figure 3 shows the biomass of each species alone, as well as the distributions of NBE and OY for the 119 pairwise combinations. Species pairs could produce much more or much less than either the average or the maximum of the two monocultures (i.e. NBE and OY ranged from much greater to much less than zero). On average across all combinations, the polycultures produced 12·8% more biomass than the average monoculture, but 18·3% less than the maximum of the two constituent taxa. In addition, the single most productive monoculture yielded 2·7% more biomass than the single best species pair. OY was positive (polyculture yield > the maximum monoculture) in 30 out of 119 species pairs.

Some of the effect of biodiversity on production was predictable based on the combination of traits making up the species pairs. Both NBE and OY increased when the two species were more divergent along the first principle axis (PC1) of multivariate trait space (Fig. 4b,d), although means and differences in PC1 explained only 11·9% and 9·7% of the variation in NBE and OY, respectively (Table 2). NBE and OY both declined with greater mean PC1 of the species pair (Fig. 4, Table 2), indicating weaker biodiversity effects when two highly productive species were grown together. Functional dispersion (FDis, Laliberte & Legendre 2010), an alternative measure of trait diversity within the species pairs, was strongly correlated with the dispersion in PC1 in the species pairs ($r = 0{\cdot}78$, Spearman rank correlation, $P < 0{\cdot}0001$), but uncorrelated with either OY or NBE ($P > 0{\cdot}1$). Multiple regression models including the individual traits with stepwise AIC minimization procedures found that OY increased with greater difference in C : N between the two species ($P = 0{\cdot}0007$) with a smaller difference in volume ($P = 0{\cdot}02$), and with high average values of volume ($P = 0{\cdot}03$) and C : P ($P = 0{\cdot}01$) and lower average values of C : N ($P < 0{\cdot}0001$). NBE increased with the difference in C : N ($P = 0{\cdot}0002$), the average volume ($P = 0{\cdot}001$) and average P* (P=), and declined with increases in the difference in L* ($P = 0{\cdot}0005$) and volume ($P = 0{\cdot}002$) and the mean of C : N ($P = 0{\cdot}008$).

Discussion

Our results indicate that basic functional trade-offs constrain the lipid yield from candidate bioenergy feedstock species of microalgae. The traits that contribute to fitness were largely positively correlated among 16 highly diverse taxa. The first principal axis was related strongly to nutrient competitive abilities (N* and P*), maximum growth rates, maximum densities, cellular concentrations of N and P, and light requirements (Fig. 1). The species with positive values of PC1 are fast growers that reach high density and are able to grow under low nutrient supply, but not at low light levels. However, these species also showed the lowest neutral lipid concentrations in their cells and may therefore be less valuable as bioenergy crops. In addition, we found that polycultures of multiple taxa may be useful as a strategy to enhance yield of bioenergy, but that polycultures varied greatly in biomass production relative to component monocultures. The mean and dispersion of trait values among species grown in combination showed some limited power to predict the performance of polycultures relative to their component monocultures. The effects of biodiversity on yield increased when species were more divergent in their traits, indicating that a trait-based approach may contribute to the search for productive and resilient bioenergy communities. However, the majority of variation in NBE and OY was left unexplained by any of the traits measured in our experiment, leaving unanswered the question of what traits determine the effects of algal polyculture on biomass production.

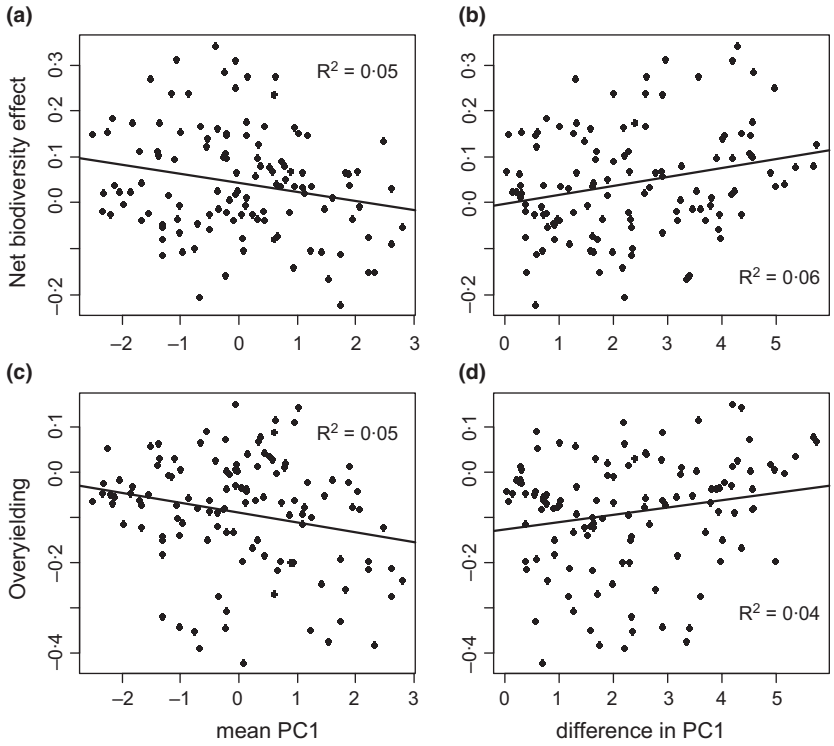

Fig. 4. Regression plots for the model to explain variation in net biodiversity effect (NBE) (a,b) and overyielding (OY) (c,d) based on the average and difference in values of PC1 summarizing the nine traits for each pair of species. The regression statistics for the models are shown in Table 2.

Table 2. Regression model to predict OY and NBE corresponding to the data shown in Fig. 4

	OY	R^2 = 0·097		NBE	R^2 = 0·119	
	Estimate ± SE	*t*	*P*	Estimate	*t*	*P*
Intercept	−0·13 ± 0·02	−6·45	<0·0001	−0·01 ± 0·02	−0·28	0·78
Mean PC1	−0·02 ± 0·01	−2·75	0·0007	−0·02 ± 0·01	−2·77	0·007
Difference PC1	0·02 ± 0·01	2·46	0·015	0·02 ± 0·01	3·09	0·002

NBE, net biodiversity effect; OY, overyielding.

The main trade-off among different algal traits related to fitness was between competitive abilities for light and nutrients. Positive correlations were observed between abilities to grow at low nutrient levels, exponential growth at low density and biomass at carrying capacity. Species with large cell volume (primarily *Chlorophyta*) grew rapidly, reached greater final biomass density, had low N and P contents in their cells and grew at low minimum concentrations of mineral nutrients. However, these species grew poorly at low light levels, as indicated by their high measured values of L*. The species with the lowest R*'s for light are cyanobacteria, which grow at low light levels and are also often inhibited at higher irradiance (Richardson, Beardall & Raven 1983). As far as we are aware, this is the first direct evidence for a trade-off between competitive abilities for light vs. nutrients among phytoplankton species.

Our results contrast with a number of studies showing faster growth of small taxa (Banse 1976; Edwards *et al.* 2012). This disparity may reflect the relatively narrow size range among our species (2·6–781·7 μm^2, as opposed to <1 to >10^7 μm^3 in Edwards *et al.* 2012). Cell volume showed very little relation to exponential growth rate within this size range (see fig. 3 in Edwards *et al.* 2012). Our results agree with Marañon *et al.* (2013), who showed that growth rate increased with cell volume from 10^{-1} to 10^2 μm^3 and declined from 10^2 to 10^6 μm^3, and also that C : N increased with cell size. The positive association between size and growth largely results from the faster growth rates of Chlorophyta relative to the other groups (Fig. 1), particularly the cyanobacteria. The correlation was not significant within the Chlorophyta ($P > 0·05$), the group containing the most species (Table 1). Our results also agree with Edwards, Klausmeier & Litchman (2011) who found that ability to acquire N and P was positively correlated among species that varied greatly in cell size. A trade-off in the ability to acquire different mineral nutrients is the basis for the resource ratio theory of species coexistence (Tilman 1981). However, larger cells are likely to have lower volume- or mass-specific nutrient uptake rates due to lower surface-to-volume ratios and therefore be poorer competitors for all dissolved nutrients

(Edwards, Klausmeier & Litchman 2011). The relationship between nutrient uptake rate and minimum requirements (R*) depends on cellular demands for nutrients. Our results show that larger species can have lower N* and P* despite lower surface areas for exchange with the environment (relative to cell volume) as a result of lower cellular nutrient concentrations (Fig. 1). These results agree with patterns among grassland plants where the species with the lowest R* for nitrate also had the lowest tissue N concentrations (Tilman 1990). These patterns suggest that trade-offs between competitive abilities for different essential nutrients may not promote coexistence in phytoplankton communities.

In addition to trade-offs among ecological traits, we also found a strong constraint related to the utility of species as bioenergy crops. The species that achieved the fastest growth reached the highest biomass density and grew at the lowest nutrient concentrations also had the lowest cellular lipid and nutrient contents. These results have implications for selecting and engineering bioenergy crop taxa. Within taxa, lipid pathways are often expressed mainly under conditions of N limitation and growth arrest (Mandal & Mallick 2009; Rodolfi *et al.* 2009), leading to an intraspecific trade-off between conditions for maximizing growth and cellular lipid synthesis. That is, high N concentrations lead to rapid growth but low lipid content in many species because lipids are mainly synthesized to store energy under conditions of nutrient scarcity (Chisti 2007). Cellular nutrient concentrations and biochemical composition (lipid, carbohydrate and protein concentrations) vary considerably among phytoplankton species (Geider & La Roche 2002). However, the relationship between stoichiometric and biochemical make-up among species or along environmental gradients is largely unknown. Our results indicate a broad interspecific trade-off between a suite of traits related to growth and resource use, and the production of energy-rich lipids, the main target of bioenergy production.

The results of our diversity experiment growing all 119 pairwise combinations of 16 species showed tremendous variability in polyculture biomass yield relative to the mean (NBE) and maximum (OY) of the component monocultures. OY and NBE both ranged from positive to negative, indicating that pairs of species could produce much more or less biomass than either one alone. The mean species pair produced 12·7% more biomass than the average of the two species in monoculture, but 18·3% less than the most productive species alone. Our results indicate less tendency for overyielding than found by Stockenreiter *et al.* (2012) who showed increasing lipid yield up to four species and greater yield in polyculture than in any monoculture. Our results indicate that underyielding is a relatively common outcome of growing phytoplankton species in combination, in agreement with Schmidtke, Gaedke & Weithoff (2010) who found a tendency for underyielding among phytoplankton species. Underyielding is likely the outcome when the dominant competitor between the two species is less productive on its own. Schmidtke, Gaedke & Weithoff (2010) argued that underyielding occurred as a result of a negative association between exponential growth rate and biomass at asymptotic density whereby the fastest growing species monopolizes resources but does not yield much biomass at steady state. We observed the opposite association between *r* and K in that the fastest growing species achieved the greatest final density at steady state (Fig. 1).

The highly variable effect of growing species in polyculture on biomass yield relative to monoculture (Fig. 3) indicates that diversity is unlikely to be a reliable guide to assembling productive bioenergy communities. We found similar results in experiments manipulating higher levels of diversity (1, 2, 5 or 10 species, Shurin *et al.* 2013) in that combinations of species rarely produced more biomass (estimated as biovolume), and often produced less biomass, than the single best species in the mixture on its own. Whether these results scale to the more variable and unpredictable conditions of outdoor cultivation, where phytoplankton are subject to weather as well as invasion by grazers, pathogens and wild competitors over longer periods than our laboratory experiments, remains to be tested. Our results are also limited by the use of OD to estimate biomass in species mixtures (see Supporting Information). The relationship between OD and biomass varies among species; therefore, OD of mixtures reflects both the combined biomass and the relative abundances of the species making up the mixture. However, the results of the diversity experiments agreed broadly with those in Shurin *et al.* (2013), which measured biovolume in mixtures from cell counts. Together, the results of the two experiments show that some species pairs greatly outperformed their respective monocultures, indicating that identifying the most productive combinations of species is critical to adopting ecological approaches to bioenergy productivity (Smith *et al.* 2010; Stockenreiter *et al.* 2012).

Knowledge of organismal traits can help explain the tremendous variability observed in the response of biomass productivity to the diversity of organisms in communities (Hooper *et al.* 2005; Cardinale *et al.* 2006). Positive effects of diversity occurred most often with pairs of species with more divergent traits, likely indicating weakened competitive effects and greater complementarity among taxa with very different organismal properties. Our results also agree with Griffin *et al.* (2009) who found that high functional diversity among macro-algae was associated with greater tendency for over-yielding. Similar patterns have also been shown for detritivorous invertebrates in soils (Heemsbergen *et al.* 2004) and bacterial communities (Jousset *et al.* 2011). Species combinations with more divergent traits may give rise to greater complementarity effects. For instance, species with high light requirements may induce shading that facilitates species such as cyanobacteria that are photo-inhibited under strong illumination. Identifying the traits responsible for positive diversity effects on biomass productivity of algae is one of

the most important challenges in applying ecological principles to industrial biofuel production. Our results indicate that growing pairs of species with greater divergence along the main axis describing variability in their competitive and growth-related traits yields the strongest benefits for productivity. Extensive field testing is required in order to translate these results from the laboratory to industrial settings because the performance of strains, and mixtures of strains, is often highly venue-specific. Laboratory studies allow us to screen many strains and combinations; however, the specific algal communities that perform best in outdoor conditions can only be validated by field experiments.

The associations between algal trait diversity and the effects of diversity on productivity in polyculture were highly significant, but explained only 11·9 and 9·7% of the variation in NBE and OY, respectively (Table 2). Thus, the power of trait diversity to predict the outcome of biodiversity experiments was low. The weakness of the relationship between the mean and dispersion of trait values and the effects of diversity on production is in some sense the most surprising outcome of our study. We measured nine traits that would be expected to have major impacts on the strength of competition between algae, including R* for light, N and P, maximum growth rates and asymptotic density, cell volume and cellular C : N and C : P ratios. These traits, together or in combination, failed to account for most of the variation in NBE and OY, which are manifestations of how strongly species compete. In the absence of competition, they should both be positive as the species' densities will be sum of their biomasses in monoculture. The weakness of the correlation suggests that the strength of interactions among algae is likely to be determined by a more complex combination of traits, by traits not measured in our study or by other forms of interaction such as allelopathy. Explaining how competition is shaped by species traits, and how traits determine the effects of diversity on productivity, remains a significant challenge for ecology and bioenergy.

Our results indicate that an approach rooted in understanding the ecology of species traits will be more effective in optimizing yield in bioenergy communities than promoting high diversity *per se*. We observed interspecific trade-offs between competitive abilities for light vs. nutrients, and between the production of biomass and the concentration of neutral lipids in the cells of phytoplankton. We also found that effects of diversity on productivity ranged from negative to positive, and that some of this variation could be explained by competitive, stoichiometric and life-history traits. However, most of the variation remained unaccounted for, indicating that the interactions among phytoplankton species depend on traits other than those measured in our experiments. In order for a positive effect of diversity on productivity to have application to industrial bioenergy production, it is necessary that we develop a predictive understanding of the biological and environmental factors that determine the outcome of interactions among species. Our results represent a first step towards this goal, but also indicate that much work remains to permit the engineering of productive phytoplankton communities for bioenergy.

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Author contributions

All three authors designed the experiments, SM and RLA performed the experiments and commented on the manuscript, and JBS analysed the data and wrote the paper.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Fig. S1. Relationship between dry weight and optical density at 750 nm for the species used in the experiment. (Supplementary information is available at ISMEJ's website)

Fig. S2. Growth curves for single species (left plots) and species pairs (right plots) in the four diversity experiments.

Fig. S3. Examples of final OD for three cases corresponding to species pairs showing (A) high, (B) average and (C) low values of NBE and OY.

Appendix S1. Using optical density to measure biomass of algae polycultures.