Interplay between *r*- and *K*-strategists leads to phytoplankton underyielding under pulsed resource supply

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Received: 19 March 2017 / Accepted: 16 December 2017

Abstract

Fluctuations in nutrient ratios over seasonal scales in aquatic ecosystems can result in overyielding, a condition arising when complementary life-history

traits of coexisting phytoplankton species enables more complete use of resources. However, when nutrient concentrations fluctuate under short-period pulsed resource supply, the role of complementarity is less understood. We explore this using the framework of Resource Saturation Limitation Theory (*r*-strategists vs. *K*-strategists) to interpret findings from laboratory experiments. For these experiments, we isolated dominant species from a natural assemblage, stabilized to a state of coexistence in the laboratory and determined life-history traits for each species, important to categorize its competition strategy. Then, using monocultures we determined maximum biomass density under pulsed resource supply. These same conditions of resource supply were used with polycultures comprised of combinations of the isolated species. Our focal species were consistent of either *r*- or *K*-strategies and the biomass production achieved in monocultures depended on their efficiency to convert resources to biomass. For these species, the *K*-strategists were less efficient resource users. This affected biomass production in polycultures, which were characteristic of underyielding. In polycultures, *K*-strategists sequestered more resources than the *r*-strategists. This likely occurred because the intermittent periods of nutrient limitation that would have occurred just prior to the next nutrient supply pulse would have favored the *K*-strategists, leading to overall less efficient use of resources by the polyculture. This study provides evidence that fluctuation in resource concentrations resulting from pulsed resource supplies in aquatic ecosystems can result in phytoplankton assemblages' underyielding.

AQ1

AQ2

AQ3

Keywords

Nutrient pulses
Competition
Species traits
Resource saturation
Nutrient limitation

Communicated by Ulrich Sommer.

Deceased: Daniel B. Danielidis.

Introduction

Biomass production in ecosystems can be related not only to species richness but also to coexisting species' life-history traits (Hector 1998). When a multi-species assemblage outperforms the best of the component monospecific cultures, this generates the known situation of overyielding (Tilman et al. 1996; Hector 1998; Tilman et al. 2001; Beckage and Gross 2006). Overyielding is a topic of ongoing research (Singh et al. 2015; O'Connor et al. 2016) due to its important implications on the provision of ecosystem goods and services (Cardinale et al. 2012) as well as novel industrial applications (Shurin et al. 2013). In terrestrial plant systems greater species richness can lead to overyielding, as it is expected to result in higher variation of complementary traits across species allowing for niche differentiation and thus higher biomass production (Rocher et al. 2008; Hector et al. 2010; Muller et al. 2013). In aquatic primary producers, recent works have also advanced our knowledge on the effect of species richness on biomass production, but there is still much controversy (Cardinale et al. 2011). Experimental studies on macro- and micro-algae have reported relationships that were weak or negative (e.g., Bruno et al. 2006; Schmidtke et al. 2010), while other studies—including field surveys and modeling approaches—revealed positive relationships. When positive associations between richness and biomass were observed, they were either without evidence of complementarity (e.g., Ptacnik et al. 2008; Weis et al. 2008) or with indication of complementarity (e.g., Concoran and Boeing 2012; Schabhüttl et al. 2013; Roelke and Spatharis 2015).

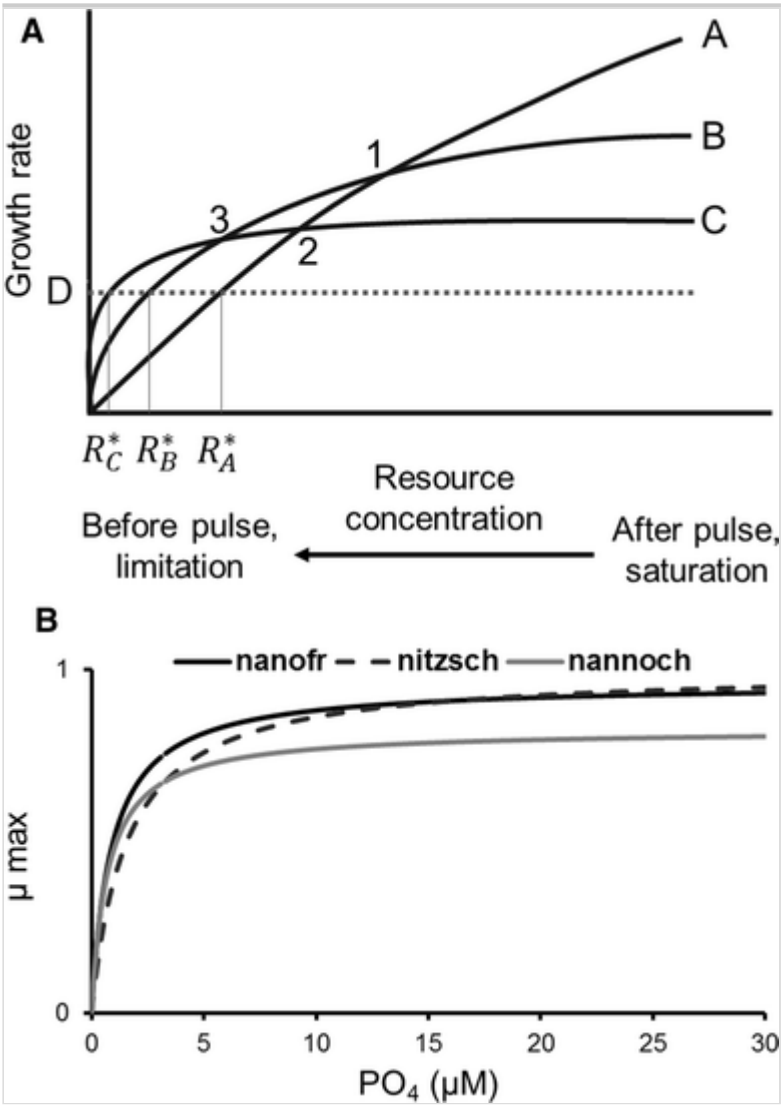
Niche differentiation and complementarity can only be enabled under some sort of environmental heterogeneity, temporal or spatial. Incorporating heterogeneity in experimental studies on phytoplankton overyielding has been recommended by previous investigators to render experimental systems more representative of natural environments (Gamfeldt and Hillebrand 2008; Duffy 2009; Power and Cardinale 2009). For marine phytoplankton in particular, a relevant form of heterogeneity is the temporal fluctuation of resources induced by nutrient pulses either from terrestrial runoff or upwelling events (Buyukates and Roelke 2005; Spatharis et al. 2007; Roelke and Spatharis 2015). These pulses are known to sustain diversity of phytoplankton assemblages in the field (Buyukates and Roelke 2005; Spatharis et al. 2007) and self-organized assemblages in the lab (Sommer 1989; Smeti et al. 2016). Despite the importance of pulsed nutrients on phytoplankton, to date, there has been little research regarding their effect on biomass production (but see Suttle et al. 1987).

A pulsed nutrient supply, where the interval between pulses is greater than the species generation times, can promote the coexistence of species with different growth strategies, even as the system approaches a quasi-steady state, as

evidenced from both theory and experimentations (Sommer 1989; Reynolds 1993). This occurs because the resource-saturated environment following a nutrient pulse will favor fast-growing species [*r*-selected with a higher μ_{max} or "opportunists" sensu Grover (1990)] whereas species which have higher affinity for the limiting nutrient [*K*-selected with higher ratios of μ_{max} to half saturation constant K_s or "gleaners" sensu Grover (1990)] will have the competitive advantage during the period of resource limitation that occurs prior to the next nutrient pulse (Kilham and Kilham 1980; Sommer 1989; Fig. 1a). In other words, the conceptual model (Fig. 1a) indicates that under resource-saturated conditions, which follow a nutrient pulse, the *r*-strategist (species A) has the competitive advantage as its growth rate is higher than the rest when nutrients are high. As phosphorus concentration decreases because of consumption by species A, B and C (Fig. 1a), and specifically when concentration reaches the intersection point 3, nutrients are low and the *k*-strategist (species C) takes the competitive advantage, since its growth rate at this nutrient concentration becomes higher compared to the *r*-strategist's. During this period, species C sequesters resources from species A. Thus, in a polyculture, species A presents a lower biomass than expected from its monoculture. Within the pulse interval, species C is able to reduce phosphorus concentrations as low as R_C^*; however, its biomass is lower than expected from its monoculture, since species A already consumed a significant amount of the available resource. Coined the Resource Saturation Limitation model (RSL) (Kilham and Kilham 1980; Sommer 1989), this theory provides a framework of understanding for exploring phytoplankton biomass production under pulsed nutrient supply.

Fig. 1

Growth rate of species as a function of ambient nutrient concentration. **a** A conceptualisation of the resource saturation limitation model (Kilham and Kilham 1980; Sommer 1989) describing the succession of species A, B, and C along a resource concentration gradient under a given flushing rate D. Concentrations R_A^*, R_B^*, R_C^* are indicative of the species affinity for the limiting nutrient. The resource-saturated conditions following a pulse favor *r*-strategists that tend to achieve greater biomass, whereas resource limitation just before a pulse, favors *K*-strategists that achieve lower biomass. Intersection points 1, 2, 3 indicate the points that the competitive advantage shifts from one species to the other along the resource gradient. **b** The estimated growth rates of species with increasing phosphate concentration. These estimates were based on the half saturation coefficient for *P* that was determined for each of our three focal phytoplankton species: nitzsch—*Nitzschia* cf *frustulum*, and nanofr—*Nanofrustulum* sp., nannoch—*Nannochloropsis granulata*



In the present study, we explore the incidence of over- or underyielding under a pulsed resource supply. To this aim, we apply an experimental procedure whereby a natural assemblage self-organized under pulsed resource supplies and experiment on the species that coexisted in quasi-steady state. We then used the RSL model to infer competitive interactions during the period between pulses from the species' growth strategies by measuring relevant life-history traits (i.e., K_s, μ_{max}). This information combined with the efficiency of different strategists to convert resources to biomass enabled us to provide a mechanistic understanding of observed biomass production patterns in our polycultures compared to the respective species' monocultures.

Methodology

Outline of methodology

We investigated the effect of species richness on biomass expressed as biovolume using an experimental approach consisting of three parts. First, a natural marine phytoplankton assemblage was allowed to self-organize under a

fixed nutrient pulsing interval (6 days) and the three dominant species coexisting at quasi-steady state were isolated and monocultures established. The second part involved experimentations under the same pulsed nutrient supply whereby the three isolated species were grown in polycultures of all pairwise combinations and in a three-species polyculture. The effect of species composition on biomass was tested with overyielding indices and the measurement of the proportionate participation of each species in the corresponding mixture. The third part of the experimental procedure involved the measurement of life-history traits related to growth, affinity and intracellular content for phosphorus as this was found to be the limiting nutrient in all of our treatments (molar N:P ratio was always higher than 50). In this study, we focus on the second and third part of the experiment. Details of the first one can be found in Smeti et al. (2016).

Natural assemblage self-organization and species isolation

Surface water from the Aegean Sea was collected into 10L Nalgene carboys and transferred to the laboratory shortly after. Upon arrival, a portion of the water was filtered through 47 mm Whatman GF/F glass fiber filters, and used for the preparation of *f*/2 medium (Guillard and Ryther 1962) which was autoclaved (at 121 °C and 1.3 bar for 17 min). Water to be used as inoculum for three polycultures was pre-filtered through a 100-μm mesh-size plankton net to exclude mesozooplankton (Katechakis et al. 2002). Assemblage self-organization was conducted using semi-continuous cultures in three 1-L vessels. Nutrient supplies were pulsed to the polycultures every 6 days with an averaged flushing rate of $D = 0.1\ \text{day}^{-1}$. This was achieved by removing a polyculture volume of 600 ml every 6 days and replacing it with an equivalent volume of fresh *f*/2 medium.

The assemblage collected from the natural environment that was used to initiate the three polyculture vessels contained approximately 100 phytoplankton species from different phylogenetic groups. The three vessels were allowed to self-organize and reach quasi-steady state over a period of 3 months. At the end of the self-organization process, assemblages in the three vessels comprised of 7–9 species (Smeti et al. 2016) as most species were competitively excluded. One of the three polycultures was selected for follow-on species isolations. From this polyculture, the most abundant species were targeted for isolation, which made up over 99% of the total biomass. Our isolation technique involved 6–7 successive transfers, each employing a dilution of 1:10. Three phytoplankton species were successfully isolated and cultured, which were *Nitzschia* cf. *frustulum* and *Nanofrustulum* sp. (Bacillariophyta) and *Nannochloropsis*

granulata (Ochrophyta). These cultures were then used in the experiments described below.

Biomass production experiments

We compared species' biomass production in polycultures to the respective biomass production in monocultures by performing a laboratory experiment involving treatments using individual cultures (three monocultures) and all combinations of the cultures (three polycultures using two-species combinations and one polyculture using all three species). All treatments were conducted in triplicate and employed a semi-continuous culture technique in volumes of 1 L. All treatments were initiated with equal total biovolume (~ 52.000 $\mu m^3\ L^{-1}$) and equal biovolumes among species (Schmidtke et al. 2010; Schabhüttl et al. 2013). As in the initial stage of self-organization, nutrients in the cultures (*f*/2 medium of a PO_4 concentration of 36.2 µM) were pulsed every 6 days with an averaged flushing rate of $D = 0.1\ day^{-1}$.

Quasi-steady state was achieved when the species composition and relative abundances, measured just before a new nutrient pulse, were invariable for more than 18 days. This invariability state was occurring after the ~ 18th day of the experiment's initiation. Thus, the total duration of the experiment was ~ 36 days. At quasi-steady state, three consecutive samplings were carried out every 6 days whereby the 600 ml sample was taken and filtered using GF/F filters. The filtrates were stored at − 18 °C for subsequent analysis of residual phosphate and nitrate according to Parsons et al. (1984). A 5 ml sample fixed with Lugol's iodine solution was used for cell counting using inverted light microscopy (Motic AE31, 400X) according to Utermöhl (1958).

Biomass production was measured as biovolume (cell numbers × average cell volume) which is the suggested biomass proxy in experimental studies testing the effect of richness on phytoplankton biomass (e.g., Schmidtke et al. 2010). Cell volume was based on measurements of cell dimensions of ~ 30 cells per species using the formulas of Hillebrand et al. (1999) and was measured in each of the 7 treatments to account for differences in the cell size of each species. Treatment did not have an effect on the cell volume of any of the three species (ANOVA, *Nitzschia* cf *frustulum*: $F = 2.23$, p value = 0.19, *Nannochloropsis granulata*: $F = 0.33$, p value = 0.73, *Nanofrustulum* sp: $F = 0.086$, p value = 0.91).

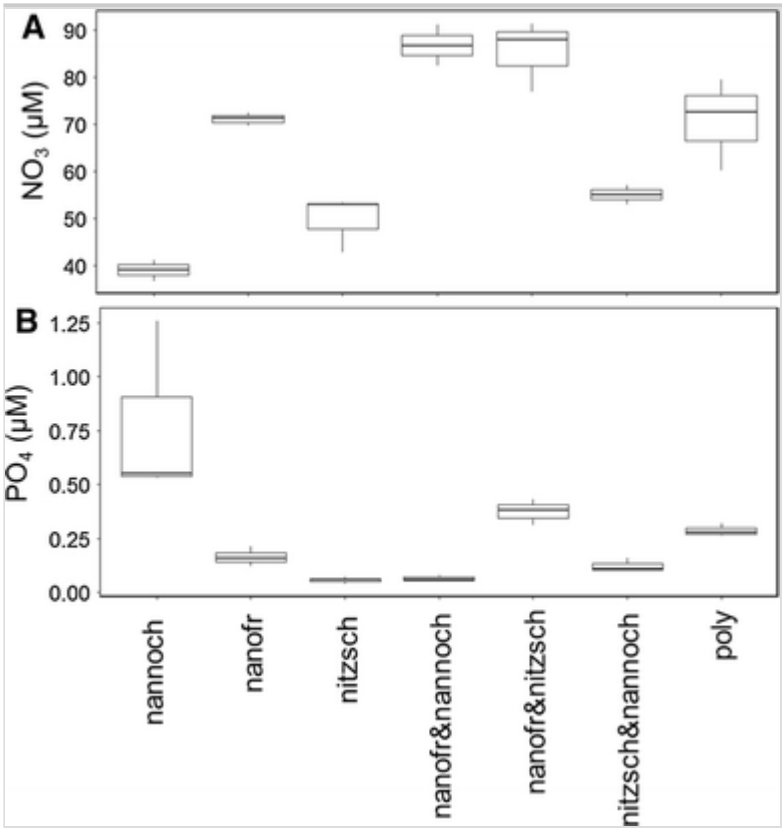
The experiments were performed in a climate-controlled chamber, where temperature was held constant at 20 °C and photoperiod at a 12-h light:dark cycle. Cool white fluorescent bulbs were used as a light source and irradiance

was set at 200 μmol/s^2, which is in the range of typical light saturated photosynthesis rates for many phytoplankton species (Kirk 1994). Since the species that were used in the experiment were the most abundant in an assemblage that self-organized from a natural assemblage over a period of 3 months under the same light conditions employed in these experiments, we assume that species' critical light intensities were not significantly different. Stirring was fixed at 300 rpm using magnetic stirrers, which resulted in well-mixed experimental units. Thus, individual cells experienced a gradient of light intensities from saturation to lower levels as they circulated from outer to interior positions within experimental units.

Across the three monocultures and four polycultures, phosphorus (measured just before a nutrient pulse at day 6) was always the limiting nutrient as the molar N:P ratio was greater than 50 across the seven treatments. More specifically soluble reactive phosphorus (SRP) concentrations were in the range of 0.05–0.55 μM (with the exception of one outlier 1.26 μM) whereas nitrate concentration was in the range of 36.7–91.1 μM (see Fig. 2). Phosphorus commonly limits production in coastal environments (Moore et al. 2013) and these concentrations are consistent with many aquatic habitats where SRP concentrations are considered low (Reynolds 2006). The measurement of parameters related to the competitive ability of the species (see below) was thus focused on phosphorus.

Fig. 2

Nitrate (NO_3) and phosphate (PO_4) concentrations at steady state measured at day 3 (just before the nutrient pulse) across the different treatments. Key for abbreviated names: nitzsch—*Nitzschia* cf *frustulum*, and nanofr—*Nanofrustulum* sp., nannoch—*Nannochloropsis granulata*



Quantification of species traits

We used batch cultures in duplicates supplied with *f*/2 medium to determine the maximum growth rate ($\mu_{i\ \max}$) of each species. The maximum growth rate was then estimated from the slope of ln (N/N_0) versus time (t) in the exponential phase, based on the exponential growth model $N(t) = N_0 e^{(\mu_{i\max} t)}$, where N_0 is the number of cells at the beginning of the exponential growth phase and $N(t)$ the number of cells at time t (Hill and Robinson 1974).

The parameter R^* (the resource availability at steady state) and cell quota, Q for P were measured using 360 ml flow-through vessels (chemostats) in duplicate. The flushing rate was constant (D = 0.2 day^{-1}) throughout this experiment. The medium supplied was P-deficient (i.e., 12 µM P), eventually leading to P-limitation. The total number of chemostat cultures was thus six (three species x two replicates). When cell number was invariable over a period of 5 days, chemostat cultures were considered at steady state and the concentration of the ambient limiting nutrient R_i^* was measured. The half saturation coefficient (K_{S_i}) which summarizes, in part, the competitive ability of the species for a limiting resource was then calculated using the R^* equation (Tilman 1982) solved for $K_{S_i} = R_i^* \left(\frac{\mu_{i\max} - D}{D}\right)$ where $\mu_{i\ \max}$ is the maximum growth rate of the species i and D is the dilution rate (0.2 day^{-1}). Nutrient affinity, i.e., the initial slope of the Monod curve ($\mu_{i_{\max}} / K_{S_i}$), was also calculated. Cell quota (Q for P) was determined by measuring the intracellular total phosphorus according to

Pujo-Pay and Raimbault (1994) and then dividing by the corresponding cell density and biovolume to determine quota per cell $Q_{\mathrm{p(cell)}}$ and per unit biomass $Q_{\mathrm{p(biovolume)}}$, respectively.

AQ4

Overyielding indices and composition of mixtures

Here we employed overyielding indices whereby the biomass production of polycultures was compared to the average of the respective monocultures. Specifically, the incidence of overyielding was calculated using the relative yield total (RYT) (Fridley 2001) as $\mathrm{RYT} = \sum_{i=1}^{S} \mathrm{RY}_i$, where S is the total number of species in the polyculture and RY_i is the relative yield of species i calculated as $\mathrm{RY}_i = \frac{O_i}{M_i}$, where O_i is the biovolume of species i observed in the polyculture and M_{i} is its biovolume in monoculture (Hector 1998). When *RYT* exceeds the value of 1, polycultures are over-yielded (but see Loreau 1998). Over- or underyielding can be further broken down to the yield exponents y_i to estimate whether specific species over- or under-yields in the polyculture. This is calculated as $y_i = \log_{\mathrm{S}}\left(\frac{O_i}{M_i}\right)$ (Lambers et al. 2004). Yield exponents greater than -1 indicate that the species i overyields.

To obtain insights into the mechanisms leading to over- or underyielding, we used a formula that partitions the net biodiversity effect on biovolume (ΔY) into three statistically additive components as follows: $\Delta Y = \mathrm{TIC} + \mathrm{TDC} + \mathrm{DE},$ where TIC is the 'trait-independent complementarity', TDC is the 'trait-dependent complementarity' and DE is the 'dominance effect' (Loreau and Hector 2001; Fox 2005; Schmidtke et al. 2010). The equations for the calculation of the three components follow the annotation in Schmidtke et al. (2010).

$$\mathrm{TIC} = S \times \bar{M} \times \overline{\mathrm{RF}}, \tag{1}$$

where S is the total number of species in the polyculture, $\bar{M}$ is the average monoculture volume and $\mathrm{RF} = \frac{1}{S} \times \sum_{i=1}^{S} \mathrm{RY}_{\mathrm{i}}$ is the averaged relative yield of the species in the mixture.

$$\mathrm{TDC} = S \times \mathrm{Cov}\left(M_i, \mathrm{RY}_i - \frac{\mathrm{RY}_i}{\mathrm{RYT}}\right), \tag{2}$$

where S is the total number of species in the polyculture, $\mathrm{RYT} = \sum_{i=1}^{S} \mathrm{RY}_i$ is the relative yield total, $\mathrm{RY}_i = \frac{O_i}{M_i}$ is the relative yield of species i, O_i is the

biovolume of species *i* observed in the polyculture and M_i is its biovolume in monoculture.

$$\mathrm{DE} = S \times \mathrm{Cov}\left(M_i, \frac{\mathrm{RY}_i}{\mathrm{RYT}} - \mathrm{RY}_{E,i}\right) \quad (3)$$

where $\mathrm{RY}_{E,i} = \frac{1}{S}$ is the expected relative yield of species i in mixture and the rest as described in equations above.

Trait-independent complementarity is positive when overall biomass production of the polyculture is greater than expected based on monoculture performances when species occupy distinct niches or facilitate each other. When TIC is negative this indicates interspecific interference competition. Trait-dependent complementarity only benefits species with certain traits. Positive TD suggests that species occupy 'nested niches' (Fox 2005) whereby species with wider niche produce more biomass in monocultures and also present high relative yield in the polyculture but not at the expense of species with narrower niches. On the other hand, the dominance effect (DE) suggests that species occupy similar niches and a species performs better in polyculture than expected based on monoculture biovolume at the expense of other species. DE takes a positive value when species with high monoculture biomasses are also the dominant species in the polyculture and negative values when the polyculture is dominated by a species with low monoculture biomass (Fox 2005).

We also calculated the proportional composition of each species biovolume in every polyculture (Table 2).

Results

Measured traits

From monocultures, *Nitzschia* cf *frustulum* and *Nanofrustulum* sp. had the highest maximum specific growth rates (Fig. 1b; Table 1), which suggests that they might have been the fastest growing species in our polyculture experiments during periods immediately following nutrient pulses when phosphorus concentrations would have been high (i.e., 21.72 µM soluble reactive phosphorus). The maximum specific growth rate of *N.* cf *frustulum* was slightly greater than the maximum specific growth rate of *Nanofrustulum* sp. *Nannochloropsis granulata* had the highest μ_{max}/K_s from monoculture (Fig. 1b; Table 1), which suggests that it might have been the fastest growing species in our polyculture experiments during periods prior to nutrient pulses when phosphorus concentrations were low (Fig. 2). The μ_{max}/K_s of *Nanofrustulum* sp. was the second highest, taken together with it also having the second highest

maximum specific growth rate, suggests that over a range of intermediate phosphorus concentrations, it would have been the fastest growing algae in polyculture. According to the *r*- and *K*-selection theory for phytoplankton (Kilham and Kilham 1980) [also known as "growth" and "affinity" strategy in Sommer 1989 and "opportunist" and "gleaner" in Grover (1990)], *N.* cf *frustulum* follows the description of an *r*-strategist and *N. granulata* follows the description of a *K*-strategist. *Nanofrustulum sp.* is difficult to classify as either *r*- or *K*-strategist in these experiments, as its growth rate is almost as high as that of *N.* cf *frustulum* and its affinity close to that of *N. granulata.*

Table 1

Mean and standard deviation of measured parameters of our three phytoplankton species maximum specific growth rate μ_{max} (day^{-1}), phosphorus concentration where net growth (μM-P), half saturation constant K_s for phosphorus (μM-P), intracellular phosphorus cont $cell^{-1}$ and fM μm^{-3} biovolume), biomass that species achieve under pulsed conditions in mo measured as biovolume (mm^3/L), and the resource affinity determined by μ_{max}/K_s-P (day^{-1} μ

Species	μ_{max} **(*n* = 2)**	***R*-P* (*n* = 2)**	K_s***-P***	$Q_{P(cell)}$ **(*n* = 2)**	$Q_{P(biovolume)}$ **(*n* = 2)**	**Monoculture biomass (*n* = 3)**
Nanofrustulum sp.	0.960 (0.020)	0.235 (0.091)	0.893	63.265 (2.702)	0.494 (0.021)	490 (61.3)
Nitzschia cf *frustulum*	0.997 (0.017)	0.398 (0.032)	1.586	68.618 (16.335)	1.345 (0.218)	155 (13.6)
Nannochloropsis granulata	0.823 (0.004)	0.235 (0.033)	0.732	13.349 (3.477)	3.337 (0.869)	72 (8.9)

Because our experimental conditions were phosphorus-limiting, the biomass production of species in monocultures depended on each species' requirements for phosphorus per unit biomass. This is expressed by the inverse relationship between minimum intracellular nutrient quota $Q_{P(biovolume)}$ and the biomass that species achieved in monoculture (Fig. 3; Table 1). *N. granulata* has the greatest requirements for phosphorus thus it is the least efficient in converting nutrients to biomass reflected by its significantly lower monoculture biomass (Fig. 3d; Table 1). On the other extreme, *Nanofrustulum* sp. has the least requirements for phosphorus being the most efficient in converting nutrients to biomass and thus having the highest monoculture biomass (Fig. 3a, b, d; Table 1). This difference in biomass production between monocultures of the three species was statistically significant (Table 2).

Fig. 3

Biomass expressed as biovolume with standard deviation bars. Each panel shows the biomass production expressed as biovolume of species in monocultures (nannoch—*Nannochloropsis granulata*, nitzsch—*Nitzschia* cf *frustulum*, and nanofr—*Nanofrustulum* sp.) together with their expected biomass (exp) and the realised biomass in their polyculture (poly)

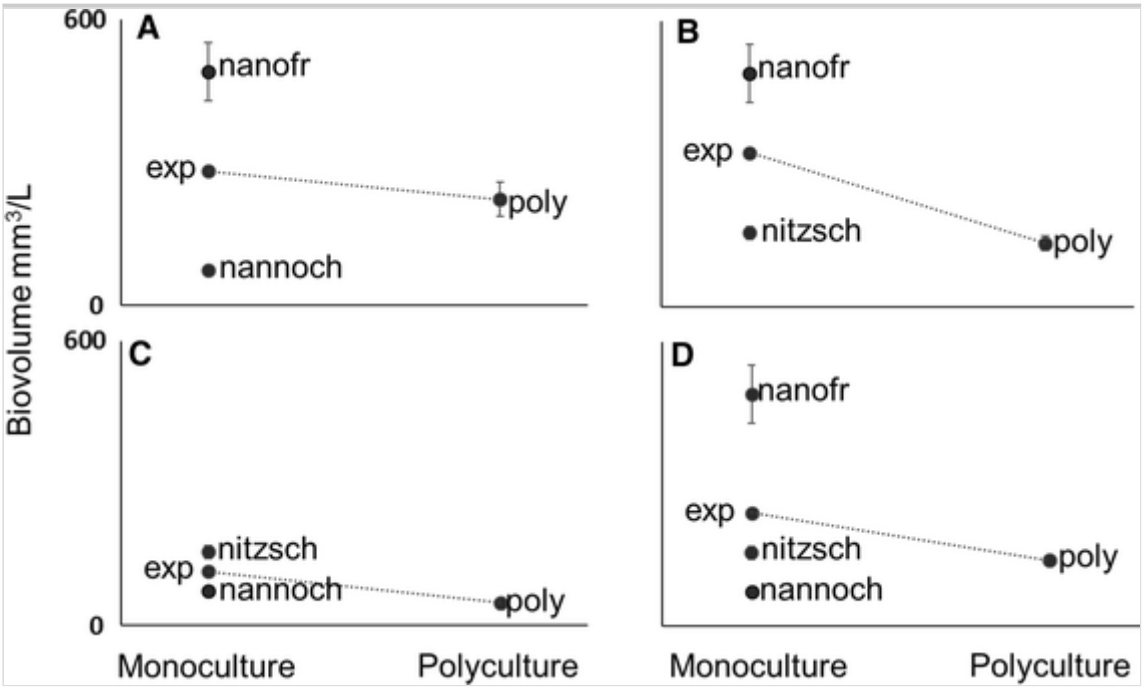


Table 2

The mean and standard deviation of the proportion of the total biovolume for each species' in the corresponding polycultures

	nanofr %	**nitzsch %**	**nannoch %**
nitzsch + nannoch		93.79 (3.54)	6.21 (3.54)
nanofr + nitzsch	40.47 (7.10)	59.53 (7.10)	
nanofr + nannoch	94.35 (3.89)		5.65 (3.89)
nanofr + nitzsch + nannoch	10.72 (3.03)	88.75 (3.01)	0.53 (0.04)

nannoch—*Nannochloropsis granulata*, nitzsch—*Nitzschia* cf *frustulum*, nanofr—*Nanofrustulum* sp

Biomass comparisons and overyielding indices

Dominance within polycultures favored species with higher μ_{max}. For example, when *N. cf frustulum* was present it was always the most abundant species. When *N. cf frustulum* was absent, the second fastest growing species, *Nanofrustulum* sp., was the most abundant species (Table 2). Interestingly,

Nanofrustulum sp. and *N. granulata* had the same R^* values and similar affinity for phosphorus (Table 1), yet *Nanofrustulum* sp. was dominant when these two species competed in the absence of *N.* cf *frustulum*. This suggests that under our conditions of pulsed nutrient supply (6-day pulsing period with nutrient additions at *f*/2 concentrations), phosphorus concentrations were not limiting growth for a greater proportion of the 6-day period compared to the amount of time phosphorus was limiting. This would have favored species with higher μ_{max} (i.e., *Nanofrustulum* sp.) over species with higher μ_{max}/K_s (i.e., *N. granulata*). The greater growth of *Nanofrustulum* sp. under such conditions would have deprived nutrients from the less productive *N. granulata*, a result also suggested by the *DE* index for these species (Table 3).

Table 3

Overyielding indices calculated across phytoplankton polycultures, which include biodiversity effect (ΔY), trait-dependent complementarity (TDC), effect size of trait-independent complementarity (TIC), dominance effect (DE), relative yield total (RYT), and the species-specific yielding (y)

Species involved	Index							
	ΔY	**TDC**	**TIC**	**DE**	**RYT**	**y_{nit}**	**y_{ext}**	**y_{nan}**
Nitzschia, *Nannochloropsis*	− 66.0	− 20.3	− 76.4	30.2	0.3	− 1.8		− 4.7
Nanofrustulum, *Nitzschia*	− 189.4	42.5	− 123.5	− 108.4	0.6	− 0.9	− 3.2	
Nanofrustulum, *Nannochloropsis*	− 59.0	− 31.2	− 108.0	81.0	0.6		− 1.2	− 2.7
Nitzschia, *Nanofrustulum*, *Nannochloropsis*	− 100.0	11.6	− 39.0	− 72.5	0.8	− 0.2	− 3.2	− 4.2
All indices were calculated using averaged phytoplankton biovolume across three consecutive samplings at quasi-steady state								

All polycultures produced less biomass than the sum of the ratios of the species' biomass in a mixture over their monocultures' biomass (Fig. 3). In other words, the polycultures were underyielding (i.e., RYT < 1, Table 3). In seven out of nine cases, species were having relative yields (y_i) below − 1 (Table 3), indicating that these species performed worse in polycultures based on what was expected from their performance in the monocultures. When in polycultures where both *N. cf frustulum* and *Nanofrustulum* sp. were included, *N. cf frustulum* was able to overyield (Table 3, $y_{nit} > -1$) even though the assemblage underyielded. The dominance effect *(DE)* was positive in the case that a pure *K*-strategist and an *r*-

strategist coexisted leading to the dominance by the fast-growing species (*r*-strategist). This was the case of *Nanofrustulum* sp. and *N. granulata* that occupied similar niches (share the same *R**) since the species best adapted to the niche (i.e., *Nanofrustulum sp.*) performed better in monoculture and was a strong competitor in the mixture (DE = 81.0). The DE was negative when *Nanofrustulum sp.* and *N. cf frustulum* coexisted. This was because *N. cf frustulum*, as the fastest growing species, dominated these polycultures while being less productive than *Nanofrustulum* in monoculture. The negative biodiversity effect (ΔY) was mainly driven by the trait-independent complementarity (TIC). The trait-dependent complementarity (TDC) was positive when *N. frustulum* was attaining high relative yields and thus it was overyielding. The TIC was negative in all of our polycultures indicating strong interspecific competition (Table 3).

Discussion

The RSL model proved useful for mechanistically interpreting our experimental results. The temporally variable resource supply employed here led to intermittent periods of growth limitation by phosphorous separated by periods of nutrient-replete conditions. In turn, this promoted the coexistence of *r*- and *K*-strategists. The traits of our species provide an insight into the observed succession patterns under conditions of pulsed nutrient supply. There is a direct link between the theoretical species growth curves (Fig. 1a) and our cultured species growth curves (Fig. 1b), whereby the competitive advantage shifts from the *r*-strategist *N.* cf *frustulum,* to *Nanofrustulum* sp. and then to the *K*-strategist *N. granulata*. The biomass production of species in monocultures was driven by the strategy they followed and specifically their growth rate and affinity for the limiting phosphorus (however, note uncertainly due to only two replicates for the growth rate measurements). Specifically, the two fast-growing diatom species following the *r*-strategy were also the most productive in monoculture, whereas *N. granulata* which produced less biomass was characterized by life-history traits congruent with the *K*-strategy.

Biomass comparisons across our cultures as well as complementary information from the overyielding indices show that all polycultures and most of the constituent species underyield relative to what is expected from the monocultures. Interestingly, the total resource used in a polyculture is the same as the one used by the most demanding species in monoculture (i.e., the *K*-strategist in our case). But in the polycultures, however, phosphorus is being allocated less efficiently than in the monocultures. This results in lower total biomass. The *K*-strategist, *N. granulata*, deprives resources from its more efficient competitors when in polyculture (either *N.* cf *frustulum* or

Nanofrustulum sp.) and it converts resources to biomass less efficiently, i.e., higher $Q_{P(biovolume)}$. Similarly, when the coexisting species are both stronger competitors at higher nutrient concentrations, this will again result in lower total biomass because one of the two species was less efficient in converting resource into biomass (*N. cf frustulum*). Following from this, a polyculture is expected to produce as much biomass as expected from monocultures only when the coexisting species are equally efficient in converting resources to biomass.

Previous studies, using either batch cultures (Weis et al. 2007) or semi-continuous cultures, (Schmidtke et al. 2010) have attributed phytoplankton assemblage underyielding to a 'negative dominance' effect whereby polycultures were dominated by a species of low biomass production. However, this does not seem to be the mechanism for underyielding in all our polycultures. In contrast to the predicted outcome of sampling effect (e.g., Huston 1997; Hector et al. 1999; Wardle 1999) our fully factorial experimental design allowed us to demonstrate that polycultures underyielded even when they were dominated by *Nanofrustulum* sp. which was the most productive species in monoculture. Moreover, our findings do not support previous suggestions that overyielding would be expected in phytoplankton assemblages when fast-growing but highly productive species are dominant (Schmidtke et al. 2010). In our study, the two most productive species were also the faster growing species (*N. cf frustulum* and *Nanofrustulum* sp.); however, no overyielding was observed when they were present in polycultures. This could only be explained by the species-specific competitive abilities at high- and low-resource concentrations in an environment with fluctuating periods of nutrient limitation and replete nutrients.

In plant systems, differences in rooting architecture and depth, as well as facilitation allow for spatial complementarity in resource use among species leading to assemblage overyielding (Dimitrakopoulos and Schmid 2004; Cardinale et al. 2012). For aquatic systems, it has been suggested, in studies that used both batch and semi-continuous cultures, that the complementarity potential is limited due to the lack of spatially structured resource distributions (Weis et al. 2008; Power and Cardinale 2009; Schmidtke et al. 2010). However, due to the rapid life cycles of phytoplankton, temporal resource variability of the limiting nutrient might be particularly relevant to promoting niche partitioning and the emergence of complementarity. Indeed, recent theoretical studies (Roelke and Spatharis 2015) have shown that when two limiting resources are supplied in a fluctuating manner causing the resource ratio to shift in a periodic manner, overyielding occurs due to complementary use of resources. Shifts of the resource ratio in time open windows of opportunity to species with a range of different competitive abilities allowing a more efficient use of resources and thus the production of higher biomass (Roelke and Spatharis 2015). However,

based on our present findings, it seems that not all types of temporal variability in the resource supply can promote phytoplankton overyielding, since the shift of resource magnitude over time tested here resulted in underyielding in phytoplankton assemblages.

Acknowledgements

The authors would like to thank Ulrich Sommer, Jason Matthiopoulos, Georg Pohnert and three anonymous reviewers for their essential comments on this research work.

Author contribution statement

DLR and SS originally formulated the idea. SS and ES designed the experiments. LAP performed the experiments. DBD, ES, LAP measured the species traits. SS, ES, GDK, LAP performed statistical analysis. ES, SS, PGD, DLR provided the ecological and mechanistic interpretation of experimental results. SS, ES, GDK, LAP wrote the manuscript and DLR provided editorial advice. LAP, ES, SS and DLR revised the manuscript.

References

Bruno JF, Lee SC, Kertesz JS, Carpenter RC, Long ZT, Duffy JE (2006) Partitioning the effects of algal species identity and richness on benthic marine primary production. Oikos 115:170–178. https://doi.org/10.1111/j.2006.0030-1299.14927.x

Buyukates Y, Roelke D (2005) Influence of pulsed inflows and nutrient loading on zooplankton and phytoplankton community structure and biomass in microcosm experiments using estuarine assemblages. Hydrobiologia 548:233–249. https://doi.org/10.1007/s10750-005-5195-x

Cardinale BJ, Matulich KL, Hooper DU, Byrnes JE, Duffy E, Gamfeldt L, Balvanera P, O'Connor MI, Gonzalez A (2011) The functional role of producer diversity in ecosystems. Am J Bot 98:1–21. https://doi.org/10.3732/ajb.1000364

Cardinale BJ, Duffy JE, Gonzalez A, Hooper DU, Perrings C, Venail P, Narwani A, Mace GM, Tilman D, Wardle DA, Kinzig AP, Daily GC, Loreau M, Grace JB, Larigauderie A, Srivastava DS, Naeem S (2012) Biodiversity loss and its impact on humanity. Nature 486:59–67. https://doi.org/10.1038/nature11148

David AW, Bonner KI, Barker GM, Yeates GW, Nicholson KS, Bardgett RD, Watson RN, Ghani A (1999) Plants removals in perennial grassland: vegetation dynamics, decomposers, soil biodiversity and ecosystem properties. Ecol Monogr 69:535–568. doi: 10.1890/0012-9615(1999)069[0535:PRIPGV]2.0.CO;2
AQ5

Dimitrakopoulos PG, Schmid B (2004) Biodiversity effects increase linearly with biotope space. Ecol Lett 7:574–583. https://doi.org/10.1111/j.1461-0248.2004.00607.x

Duffy JE (2009) Why biodiversity is important to the functioning of real-world ecosystems. Front Ecol Envir 7:437–444. https://doi.org/10.1890/070195

Fox JW (2005) Interpreting the 'selection effect' of biodiversity on ecosystem function. Ecol Lett 8:846–856. https://doi.org/10.1111/j.1461-0248.2005.00795.x

Fridley JD (2001) The influence of species diversity on ecosystem productivity: how, where, and why? Oikos 93:514–526. https://doi.org/10.1034/j.1600-0706.2001.930318.x

Gamfeldt L, Hillebrand H (2008) Biodiversity effects on aquatic ecosystem functioning: maturation of a new paradigm. Int Rev Hydrobiol 93:550–564. https://doi.org/10.1002/iroh.200711022

Grover JP (1990) Resource competition in a variable environment: phytoplankton growing according to Monod's model. Am Nat 136:771–789. https://doi.org/10.1086/285131

Guillard RRL, Ryther JH (1962) Studies of marine planktonic diatoms. I. Cyclotella nana Hustedt and Detonula confervacea Cleve. Can J Microbiol 8:229–239. https://doi.org/10.1139/m62-029

Hector A (1998) The effect of diversity on productivity: detecting the role of species complementarity. Oikos 82:597–599. https://doi.org/10.2307/3546380

Hector A, Schmid B, Beierkuhnlein C, Caldeira MC, Diemer M, Dimitrakopoulos PG, Finn JA, Freitas H, Giller PS, Good J, Harris R, Hogberg P, Huss-Danell K, Joshi J, Jumpponen A, Korner C, Leadley PW, Loreau M, Minns A, Mulder CPH, O'Donovan G, Otway SJ, Pereira JS, Prinz

A, Read DJ, Scherer-Lorenzen M, Schulze ED, Siamantziouras ASD, Spehn EM, Terry AC, Troumbis AY, Woodward FI, Yachi S, Lawton JH (1999) Plant diversity and productivity experiments in European grasslands. Science 286:1123–1127. https://doi.org/10.1126/science.286.5442.1123

Hector A, Hautier Y, Saner P, Wacker L, Bagchi R, Joshi J, Scherer-Lorenzen M, Spehn EM, Bazeley-White E, Weilenmann M, Caldeira MC, Dimitrakopoulos PG, Finn JA, Huss-Danell K, Jumpponen A, Mulder CPH, Palmborg C, Pereira JS, Siamantziouras ASD, Terry AC, Troumbis AY, Schmid B, Loreau M (2010) General stabilizing effects of plant diversity on grassland productivity through population asynchrony and overyielding. Ecology 91:2213–2220. https://doi.org/10.1890/09-1162.1

Hill GA, Robinson CW (1974) Measurement of aerobic batch culture maximum specific growth rate and respiration coefficient using a dissolved oxygen probe. Biotechnol Bioeng 16:531–538. https://doi.org/10.1002/bit.260160409

Hillebrand H, Durselen CD, Kirschtel D, Pollingher U, Zohary T (1999) Biovolume calculation for pelagic and benthic microalgae. J Phycol 35:403–424. https://doi.org/10.1046/j.1529-8817.1999.3520403.x

Huston A (1997) Hidden treatments in ecological experiments: re-evaluating the ecosystem function of biodiversity. Oecologia 110:449–460. https://doi.org/10.1007/s004420050180

Katechakis A, Stibor H, Sommer U, Hansen T (2002) Changes in the phytoplankton community and microbial food web of Blanes Bay (Catalan Sea, NW Mediterranean) under prolonged grazing pressure by doliolids (Tunicata), cladocerans or copepods (Crustacea). Mar Ecol Prog Ser 234:55–69. https://doi.org/10.3354/meps234055

Kilham P, Kilham SS (1980) The evolutionary ecology of phytoplankton. In: Morris I (ed) The physiological ecology of phytoplankton. University California Press, Berkley, pp 571–597

Kirk JTO (1994) Light and photosynthesis in aquatic ecosystems. Cambridge University Press, Cambridge

Lambers JHR, Harpole WS, Tilman D, Knops J, Reich PB (2004) Mechanisms responsible for the positive diversity—productivity relationship

in Minnesota grasslands. Ecol Lett 7:661–668. https://doi.org/10.1111/j.1461-0248.2004.00623.x

Loreau M (1998) Separating sampling and other effects in biodiversity experiments. Oikos 82:600–602. https://doi.org/10.2307/3546381

Loreau M, Hector A (2001) Partitioning selection and complementarity in biodiversity experiment. Nature 412:72–76. https://doi.org/10.1038/35097128

Moore CM, Mills MM, Arrigo KR, Berman-Frank I, Bopp L, Boyd PW, Galbraith ED, Geider RJ, Guieu C, Jaccard SL, Jickells TD, La Roche J, Lenton TM, Mahowald NM, Marañón E, Marinov I, Moore JK, Nakatsuka T, Oschlies A, Saito MA, Thingstad TF, Tsuda A, Ulloa O (2013) Processes and patterns of oceanic nutrient limitation. Nat Geosci 6:701–710. https://doi.org/10.1038/NGEO1765

O'Connor MI, Gonzalez A, Byrnes JEK, Bradley J, Cardinale J, Duffy E, Gamfeldt L, Griffin JN, Hooper D, Hungate BA, Paquette A, Thompson PL, Dee LE, Dolan KL (2016) A general biodiversity—function relationship is mediated by trophic level. Oikos 000:001–014. https://doi.org/10.1111/oik.03652

Parsons TR, Maita Y, Lalli CM (1984) A manual of chemical and biological methods for seawater analysis. Pergamon Press, Oxford

Power LD, Cardinale BJ (2009) Species richness enhances both algal biomass and rates of oxygen production in aquatic microcosms. Oikos 118:1703–1711. https://doi.org/10.1111/j.1600-0706.2009.17585.x

Ptacnik R, Solimini AG, Andersen T, Tamminen T, Brettum P, Lepistö L, Willén E, Rekolainen S (2008) Diversity predicts stability and resource use efficiency in natural phytoplankton communities. PNAS 105:5134–5138. https://doi.org/10.1073/pnas.0708328105

Pujo-Pay M, Raimbault P (1994) Improvement of the wet oxidation procedure for simultaneous determination of particulate organic nitrogen and phosphorus collected on filters. Mar Ecol Prog Ser 105:203–207. https://doi.org/10.3354/meps105203

Reynolds CS (1993) Scales of disturbance and their role in plankton ecology. Hydrobiologia 249:157–171. https://doi.org/10.1007/BF00008851

Reynolds C (2006) The ecology of phytoplankton. Cambridge Univ. Press, New York

Roelke DL, Spatharis S (2015) Phytoplankton succession in recurrently fluctuating environments. PLoS One 10:e0121392. https://doi.org/10.1371/journal.pone.0121392

Schabhüttl S, Hingsamer P, Weigelhofer G, Hein T, Weigert A, Striebel M (2013) Temperature and species richness effects in phytoplankton communities. Oecologia 171:527–536. https://doi.org/10.1007/s00442-012-2419-4

Schmidtke A, Gaedke U, Weithoff G (2010) A mechanistic basis for underyielding in phytoplankton communities. Ecology 91:212–221. https://doi.org/10.1890/08-2370.1

Shurin JB, Abbott RL, Deal MS, Kwan GT, Litchman E, McBride RC, Mandal S, Smith VH (2013) Industrial-strength ecology: trade-offs and opportunities in algal biofuel production. Ecol Lett 16:1393–1404. https://doi.org/10.1111/ele.12176

Singh M, Awasthi A, Soni SK, Singh R, Verma RK, Kalra A (2015) Complementarity among plant growth promoting traits in rhizospheric bacterial communities promotes plant growth. Sci Rep 5:15500. https://doi.org/10.1038/srep15500

Smeti E, Roelke DL, Spatharis DL (2016) Spatial averaging and disturbance lead to high productivity in aquatic metacommunities. Oikos 125:812–820. https://doi.org/10.1111/oik.02684

Sommer U (1989) The role of competition for resources in phytoplankton ecology. In: Sommer U (ed) Plankton ecology: succession in plankton communities. Springer-Verlag, Berlin, pp 57–106

Spatharis S, Tsirtsis G, Danielidis DB, Chi TD, Mouillot D (2007) Effects of pulsed nutrient inputs on phytoplankton assemblage structure and blooms in an enclosed coastal area. Estuar Coas Shelf Sci 73:807–815. https://doi.org/10.1016/j.ecss.2007.03.016

Suttle CA, Stockner GJ, Harrison PJ (1987) Effects of nutrient pulses on community structure and cell-size of a freshwater phytoplankton assemblage

in culture. Can J Fish Aquat Sci 44:1768–1774. https://doi.org/10.1139/f87-217

Tilman D (1982) Resource competition and community structure. Princeton Univ. Press, Princeton

AQ6

Utermöhl H (1958) Zur vervollkommnung der quantitativen phytoplankton-methodik. Verh Int Ver Theor Angew Limnol 9:1–38

Weis JJ, Cardinale BJ, Forshay KJ, Ives AR (2007) Effects of species diversity on community biomass production change over the course of succession. Ecology 88:929–939. https://doi.org/10.1890/06-0943

Weis JJ, Madrigal DS, Cardinale BJ (2008) Effects of algal diversity on the production of biomass in homogeneous and heterogeneous nutrient environment: a microcosm experiment. PLoS One 3:e2825. https://doi.org/10.1371/journal.pone.0002825