

Global patterns in phytoplankton biomass and community size structure in relation to macronutrients in the open ocean

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Abstract

Owing to the dynamic nature of nutrient-phytoplankton interactions, ambient macronutrient concentrations reveal little about the impact of nutrient availability on phytoplankton biomass and community composition at any given point in time or space. Here, however, we examine a global dataset ($n = 262$) where phytoplankton community structure and biomass are related to ambient concentrations of dissolved inorganic nitrogen (DIN), phosphate (P), and silicate. The macroecological patterns emerging from the analysis suggests plausible causal relationships between nutrient availability and global phytoplankton community responses: When DIN and P are below ca. $5 \mu\text{M}$ and $0.5 \mu\text{M}$, respectively, increases in the concentration of either nutrient correlate with increases in both biomass and the contribution of large cells to the total phytoplankton biomass. At higher concentrations, increasing DIN or P concentrations do not correlate with increases in biomass. However, the fraction of large phytoplankton continues to increase with increasing concentrations suggesting DIN and P availability to be important factors in controlling phytoplankton community composition over the entire spectrum of potential macronutrient availability in the open ocean. Ambient silicate concentrations were not strongly associated with phytoplankton community structure or biomass. Nor did the DIN/P ratio correlate with community structure indicating that this ratio is a poor descriptor of phytoplankton limitation at the community scale. No empirical evidence was found for commonly referred threshold values for nutrient limitation (i.e., $2 \mu\text{M}$, $0.2 \mu\text{M}$, and $2 \mu\text{M}$ for DIN, P, and silicate, respectively) suggesting that these threshold values may not contain any practical information.

Phytoplankton biomass and community structure influence ecosystem productivity and food web structure at all scales and are directly linked to global biogeochemical cycling of major elements such as nitrogen, phosphorus, silicon, and carbon (Falkowski et al. 1998; Arrigo 2005; Hilligsøe et al. 2011). Therefore, a considerable amount of effort is spent on detecting and quantifying factors controlling the distribution of phytoplankton in space and time. In general terms, phytoplankton biomass and community structure are constrained by the physical environment through factors such as temperature, turbulence, and light climate, as well as by a number of biotic interactions such as nutrient competition and grazing (Kiørboe 1998; Behrenfeld et al. 2006; Boyd et al. 2010; Mousing et al. 2014).

Empirical evidence suggests that among these factors, the availability of inorganic nutrients is one of the most important

in terms of influencing patterns in the global distribution of phytoplankton biomass, productivity, and size (Behrenfeld et al. 2006; Huston and Wolverton 2009; Acevedo-Trejos et al. 2013; Marañón et al. 2014). At the global scale, nitrogen and phosphorus are often considered to be the most important nutrients as they are required by all phytoplankton groups. In addition, most diatoms have an obligate requirement for silicon (in the form of silicate) for the formation of their frustules and the availability of silicate is, therefore, considered to be important for the distribution of this important phytoplankton group. These three nutrients are usually required in relatively high quantities and are, consequentially, often referred to as macronutrients. In addition, phytoplankton need a number of other elements such as iron and cobalt in trace quantities and these elements are, therefore, referred to as micronutrients or trace metals.

Traditionally, nitrogen has been identified as the proximally limiting nutrient for phytoplankton biomass in the contemporary ocean (Howarth 1988; Tyrrell 1999).

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However, while nitrogen may be *proximally* limiting at the global scale, there are clear regional differences in the importance of different macro- and micronutrients. Thus, in a relatively recent review of nutrient limitation in the open ocean, Moore et al. (2013) concluded that about 30% of the global ocean can be classified as being comprised of iron limited high-nutrient-low-chlorophyll regions. The rest of the ocean between 40°S and 40°N is primarily nitrogen limited with occasional co-limitation by phosphorus and other micronutrients. Downing et al. (1999) came to a similar conclusion based on a meta-analysis of enrichment experiments from primarily coastal regions where nitrogen limitation was widespread but with occasional limitation by phosphorus and/or iron.

As exemplified by these studies (*see* Table S2 in Moore et al. 2013 for references), however, the major part of our current understanding of the distribution of oceanic phytoplankton nutrient limitation is based on enrichment experiments which are conducted to determine which nutrient or nutrients are limiting phytoplankton biomass and/or productivity [i.e., “yield limitation” *sensu* von Liebig (1855)]. The enrichment approach is, however, not without weaknesses (*see* de Baar 1994 for a historical review) as such experiments are usually carried out under light, circulation, grazing, and nutrient remineralization conditions that are significantly different from those in the natural situation (Lane and Goldman 1984). Even when these methodological weaknesses are dealt with, there are still at least two fundamental problems with enrichment studies:

First, the nutrient(s) responsible for limiting phytoplankton total biomass (“yield limitation”) are not necessarily also those responsible for limiting phytoplankton growth rates [i.e., “rate limitation” *sensu* Blackman (1905)]. The distinction between yield and rate limitation is critical to understand how nutrient limitation controls phytoplankton biomass/community structure as it is only rate limitation that affects phytoplankton directly. Thus, as the concentration of a given nutrient approaches depletion, a single phytoplankton cell will not experience yield limitation. It will experience a decreasing ambient nutrient concentration which, everything being equal, will lead to decreased uptake of that nutrient and, therefore, a decrease in growth rate (Tilman et al. 1982).

It also follows that, as ambient nutrient concentrations increase to a point where they do not limit yield, they may still only support less than maximal growth rates of individual phytoplankton cells because different species are adapted to different nutrient environments and exhibit vastly different nutrient uptake traits (Eppley et al. 1969; Lomas and Gilbert 2000; Sarthou et al. 2005). Thus, while enrichment experiments can reveal the nutrients which limit total phytoplankton biomass (i.e., yield), they do not necessarily identify the nutrients which (co-)limit the composition and succession of the *in situ* phytoplankton community at a given location. By focusing on yield limitation, important

nutrient effects may be overlooked leading to incorrect conclusions as to which nutrients play significant roles in controlling community composition and productivity in the open ocean.

A second problem with enrichment experiments is that while phytoplankton biomass and productivity are undoubtedly important community traits, the ecological information contained in these bulk traits alone is probably much more limited than originally thought. In recent years, it has become increasingly clear that community structure and the associated distribution of functional traits play crucial roles in the functioning of ecosystems (San Martin et al. 2006; Barton et al. 2013; Guidi et al. 2016; Chust et al. 2017). For example, in terms of carbon draw-down, efficient sequestration of carbon into the deep ocean has also been shown to be strongly related to phytoplankton community structure, with some groups and species being much more efficient than others (Arrigo et al. 1999; Ragueneau et al. 2006; De La Rocha and Passow 2007; Rynearson et al. 2013; Guidi et al. 2016). Thus, units of biomass and productivity are not necessarily comparable in space and time across different ocean regions and, by focusing on yield, we will probably fail in relating nutrient limitation to relevant ecological functions and how these may respond to changing ocean conditions.

From these considerations, a theoretical understanding of how nutrient limitation may contribute to the regulation of phytoplankton productivity and community composition, as well as ecosystem function, evolves. In this context, a better understanding of nutrient rate limitation in phytoplankton is necessary to fully understand phytoplankton-nutrient interactions. In natural samples, it is, of course, not possible to identify rate limitation of individual phytoplankton cells/species. In terms of nutrient control, the composition of the phytoplankton community will be a product of the relative growth rates of the species present, where increases in one species or group following nutrient increases will reflect increases in the growth rate of that group/species in response to the changing nutrient conditions (Sommer 1989; Marañón 2015).

In this study, therefore, we explore an empirical approach for determining the relationship between nutrient availability and phytoplankton community characteristics in the open ocean. Measurements of nitrogen, phosphorus, and silicon have been routinely collected on biological oceanographic cruises for decades and such data are, therefore, plentiful. As recognized by Goldman et al. (1979), however, it is challenging to use measurements of ambient nutrient concentrations together with measurements of biomass and/or productivity to inform about the nutrient status of any specific phytoplankton community. This is because low ambient concentrations might simply reflect recent uptake and, thereby, nutrient replete phytoplankton. Alternatively, low ambient concentrations might reflect a long-term low nutrients condition and a resultant nutrient depleted local phytoplankton community. Because of this lack of a clear

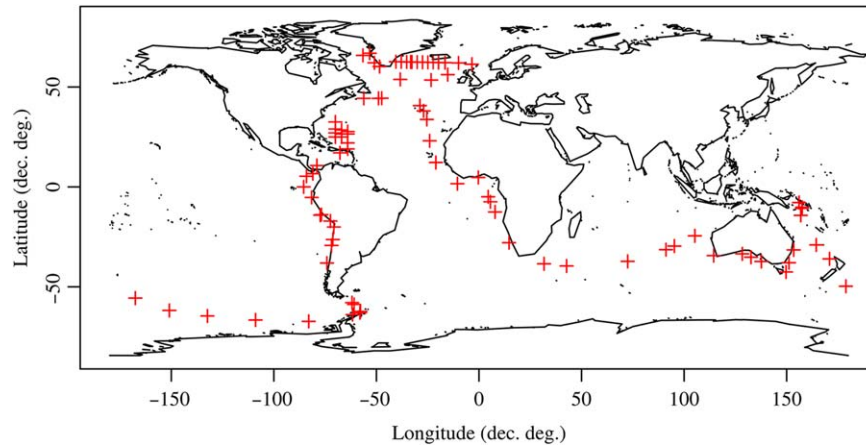


Fig. 1. World map showing the locations of the sample stations. [Color figure can be viewed at wileyonlinelibrary.com]

relationship between nutrient concentrations and phytoplankton biomass, biological oceanographers have generally shied away from using ambient nutrient concentrations as a tool for increasing the understanding of the relationship between nutrient availability and phytoplankton community characteristics.

Nevertheless, we argue that measurements of ambient inorganic nutrient concentrations, when available in large quantities and with sufficient environmental coverage, can indeed be used to develop a better understanding of nutrient control of phytoplankton (see also Marañón et al. 2014). While it is controversial to relate biomass to ambient nutrient concentrations, the relationships between size and nutrient concentrations are better known and formalized within resource competition theory (Tilman 1982; Grover 1997). Competition for nutrients is an important mechanism for controlling phytoplankton community size structure because a large surface-to-volume ratio and a small diffusive boundary layer lends smaller species a competitive advantage over larger species when nutrients are limiting (Tilman et al. 1982; Kiørboe 1993). Specifically, the diffusion-limited specific nutrient uptake rate is directly proportional to the external nutrient concentration as well as the inverse of the squared cell radius.

As vital metabolic rates are proportional to the inverse of the cell radius, it follows that it is beneficial to be small at low nutrient concentrations (Kiørboe 1993). However, as external nutrient concentrations increase, this would release large phytoplankton from the diffusion constraint, allowing larger species to increase their relative contribution to total biomass. The essential argument is that diffusion constraint on nutrient uptake will be present regardless of the history of the water mass. Water masses with low ambient nutrient concentrations will, therefore, continuously select for small phytoplankton whereas water masses with high nutrient concentration will to a higher degree select for large phytoplankton. Naturally, there will be a time lag in the relationship as communities adjust their composition toward steady state. However, we

argue that as the number of samples increases and the size distribution at every ambient nutrient concentration is determined, then the average pattern will circumvent the bias attributed by individual samples.

Through statistical analysis of data collected on a globally circumnavigating cruise in open ocean waters, we identify macroecological patterns in the relationships between global contemporary phytoplankton community size structure and biomass and ambient macronutrient concentrations. We then consider these patterns within the framework of resource competition and assess the evidence of nutrient limitation of phytoplankton communities in the open ocean.

Methods

Sampling and laboratory analyses

In total, 262 samples from 88 globally distributed stations were analyzed. In each sample, total chlorophyll *a* (Chl *a*) and size fractionated Chl *a* (used to derive phytoplankton community size structure) were paired with observations of inorganic nutrient concentrations. A detailed description of the sampling and laboratory procedures has been presented earlier (Hilligsøe et al. 2011; Mousing et al. 2014) and only a short summary is given here.

Sampling was done on the circumnavigating Galathea 3 expedition on HDMS *Vædderen* in 2006–2007 and on a cruise in the North Atlantic on RV *Dana* in August 2008 (Fig. 1). Conductivity, temperature, and depth were measured using a Seabird Instruments 911 CTD system mounted on a rosette with 12 Niskin water collection bottles. Total water depths were greater than 400 m at all sampling stations which should minimize potential anthropogenic effects such as extensive nutrient enrichment or pollution on phytoplankton biomass and community structure which can occur at near coastal locations.

Water samples were collected at the surface and at the depth of the Chl *a* maximum (if different from surface). On

selected stations, water was also collected at 30 m depth and at a standard sampling depth below the Chl *a* maximum. Collected water was filtered through Whatman GF/F and Millipore 10 μm filters and the Chl *a* retained was quantified following method 445.0 of the United States Environmental Protection Agency but using 96% ethanol as the solute instead of acetone. The fraction of Chl *a* originating from phytoplankton larger than 10 μm (in the following also referred to as the fraction of large phytoplankton) was then calculated as the concentration of Chl *a* retained on a 10 μm filter divided by the concentration retained on a GF/F filter. An increase in this fraction indicates an increase in the relative importance of large cells in the phytoplankton community.

Samples for the determination of inorganic nutrient concentrations were frozen and subsequently analyzed by wet-chemistry methods according to Grasshoff et al. (1999) with a SANPLUS System Scalar auto-analyzer. The detection limits were 0.1 μM , 0.04 μM , 0.3 μM , 0.06 μM , and 0.2 μM for nitrate (NO_3), nitrite (NO_2), ammonium (NH_4), phosphate (P), and silicate (Si), respectively. Dissolved inorganic nitrogen (DIN) was calculated as the sum of NO_3 , NO_2 , and NH_4 .

Statistical analyses

Statistical analyses were performed using R version 3.2.3 (R Core Team 2015). All variables, except for temperature and salinity, were \log_{10} -transformed to meet model specific assumptions of homogeneity and normality. Offsets used in the transformations were 0.04, 0.06, and 0.2 for DIN, P, and Si, respectively (Hilligsøe et al. 2011).

General patterns in the distribution of collected samples, as well as the measured biological and environmental variables, were investigated through a principal components analysis (PCA). Prior to the PCA, all variables were centered and scaled by subtracting the mean and dividing by the standard deviation. In addition to the PCA, we explored simple bivariate relationships visually using locally weighted polynomial regressions to indicate general trends in the data (Cleveland 1981).

The exploratory data analysis indicated differences in the relationship between phytoplankton community size structure and ambient nutrient concentrations when these concentrations were high and low. With respect to DIN, the relationship changed at around 5 μM and for P at around 0.5 μM . Therefore, concentrations higher than 5 μM for DIN and, for P, higher than 0.5 μM were considered as being "high." For the modeling analyses, the data were split into three datasets: (1) a dataset containing all 262 samples; (2) a "low DIN and P" dataset with 154 samples; and (3) a "high DIN and P" dataset with 93 samples.

The relationships between phytoplankton community size structure and ambient nutrient concentration were then investigated using generalized least square (GLS) modeling as:

$$\log_{10}(Y_i) = \log_{10}(x_i^1) + \dots + \log_{10}(x_i^n) + b + e_i$$

Where i represents the observations, Y the response variable, x are the explanatory variables from 1 to n , b is the intercept, and e represents the residuals specified as:

$$e \sim N(0, d)$$

Where 0 is the mean and d is the error-covariance matrix. Spatial autocorrelation was detected and, to explicitly take this into account, d was assumed to be spatially dependent and modeled as a spatial correlation structure where correlations were assumed to decrease exponentially with increasing spatial separation (Cressie 1993). Coefficients were estimated using maximum likelihood estimation to allow model comparison. In all cases, model specific assumptions of linearity, homogeneity, and residual normality were checked visually by investigating residual patterns.

The importance of individual variables was assessed through a model selection procedure using the second order Akaike information criterion (AICc; Burnham and Anderson 1998). Available variables were DIN, P, Si, and depth and the selection procedure involved creating a set of increasingly complex models all explaining the fraction of large phytoplankton. When comparing these models based on AICc, evidence of different explanations (i.e., combinations of explanatory variables) can be assessed simultaneously. While the model with the lowest AICc will be the best model with the minimum loss of information, slightly inferior models with an AICc difference compared to the best model (ΔAICc) of less than 2, can be seen as alternative explanations that are almost as good as the best model and can, therefore, not be ruled out (Burnham and Anderson 1998).

The potential influence of the DIN/P ratio on phytoplankton community size structure was investigated by allowing an interaction between DIN and P in the model specification. If the DIN/P ratio is an important factor influencing phytoplankton community size structure, then we would expect the relationship between the fraction of large phytoplankton and the concentration of DIN to be dependent on the concentration of P, and vice versa.

Results

PCA

The first and second axes in the PCA explained 57.7% and 17.1% of the variation, respectively (Fig. 2). All environmental variables except sampling depth were primarily associated with the first axis. All nutrient concentrations exhibited large positive scores and the ordination bi-plot suggested that they were all positively correlated. Temperature and salinity exhibited large negative scores and were positively correlated with each other. In addition, nutrient concentrations and temperature plotted directly opposite in the ordination space suggesting a strong negative correlation. Sampling depth was primarily

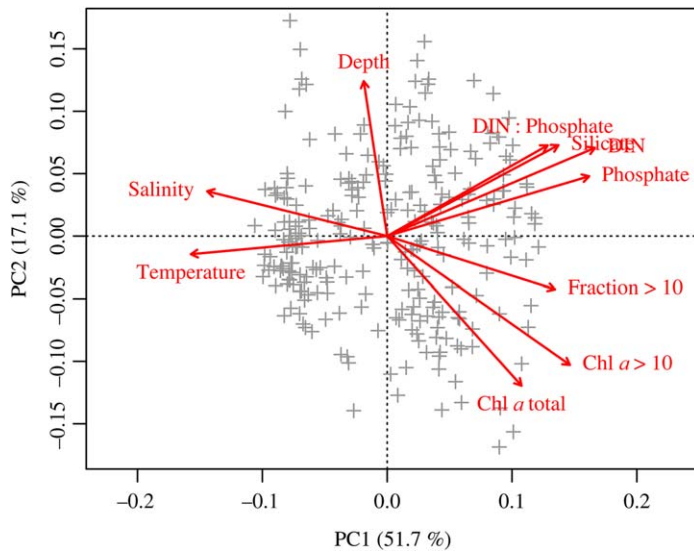


Fig. 2. Bi-plot of the first two axes of a PCA showing patterns in the distribution of samples and the overall relationship between all environmental and biological variables. [Color figure can be viewed at wileyonlinelibrary.com]

associated with the second axis and did not appear to be strongly related to any of the other environmental variables.

The biological variables were more or less positively correlated with each other but differed in their associations with the environment. Total Chl *a* scored higher (in absolute terms) on axis 2 than on axis 1, whereas the fraction of large phytoplankton scored higher on axis 1 compared to axis 2. The concentration of Chl *a* originating from cells larger than 10 μm exhibited scores in between. Thus, while high concentrations of total Chl *a* appeared to be primarily associated with shallow sampling depth, a high fraction of large phytoplankton was stronger associated with high nutrient concentrations and low temperature.

Ambient nutrient concentrations

As suggested by the PCA, all nutrients measured, as well as the DIN/P ratio, were strongly correlated with each other (Fig. 3). The strengths of the relationships and variability around the smoothed regression slopes were, however, not the same over the entire range of nutrients measured. In samples from regions with relatively low DIN concentration ($< 5 \mu\text{M}$) and relatively low DIN/P ratios (< 8), the relationship between DIN and P was variable and weak (Fig. 3a), whereas the relationship between DIN and DIN/P was strong (Fig. 3d). In samples from regions with relatively high concentration of DIN and high DIN/P ratios (8–16), the opposite patterns were found, i.e., a strong correlation between DIN and P (Fig. 3a) and little variation in P and DIN/P around the smoothed regression line for DIN (Fig. 3a,d). In addition, at DIN and P concentrations higher than $5 \mu\text{M}$ and $0.5 \mu\text{M}$, the smoothed regression line ran parallel to the line of the Redfield ratio of DIN/P = 16 : 1.

The relationship between Si and DIN (Fig. 3b) and Si and P (Fig. 3c) demonstrated the same overall patterns as that found between DIN and P (Fig. 3a) but differed in the respect that they showed about the same amount of variation around the smoothed regression line over the entire range. In addition, the relationships showed increasing strength at higher concentrations of DIN ($> 5 \mu\text{M}$) and P ($> 0.5 \mu\text{M}$). At low concentrations of DIN and P, the Si concentrations were predominantly below $2 \mu\text{M}$.

Chl *a*

The relationship between total Chl *a* and ambient nutrient concentrations of DIN and P showed an increasing and saturating pattern with maximum Chl *a* concentrations being associated with intermediate concentrations of DIN and P (i.e., the region where the curve breaks and we set our distinction between low and high nutrient concentrations; Fig. 4a,c). Hence, while the PCA showed that total Chl *a* and ambient nutrient concentrations are not generally correlated in the global ocean, the bivariate relationship revealed increasing Chl *a* concentrations to be associated with increasing DIN and P concentrations when these are relatively low.

Bivariate plots of Chl *a* concentrations for phytoplankton smaller and larger than 10 μm (Fig. 4b,d) exhibited patterns similar to that of total Chl *a*: the Chl *a* concentrations for both size groups increased with increasing DIN (when ambient concentrations were lower than $5 \mu\text{M}$) and P ($< 0.5 \mu\text{M}$) but with a steeper slope for large phytoplankton than for small phytoplankton. Changes in the fraction of large phytoplankton at DIN and P concentrations lower than $5 \mu\text{M}$ and $0.5 \mu\text{M}$ (Fig. 5a,b) were, thus, driven by a relatively greater increase in the Chl *a* concentration of large phytoplankton compared to small phytoplankton. At higher DIN and P concentrations, on the other hand, the Chl *a* concentration contributed by phytoplankton smaller than 10 μm exhibited a decrease, while the concentration of Chl *a* originating from phytoplankton larger than 10 μm showed a slightly increasing trend with increasing DIN and P. Thus, changes in the fraction of large phytoplankton at relatively high DIN and P concentrations (Fig. 5a,b) were primarily driven by a decrease in the contribution of small phytoplankton to total Chl *a*.

Fractionated Chl *a*

As suggested by the PCA (Fig. 2), the bivariate plots showed the fraction of large phytoplankton to be positively correlated with DIN and P and to a lesser extent with Si and the DIN/P ratio (Fig. 5). Hence, the fraction of large phytoplankton exhibited an increase with increasing ambient concentrations of DIN and P over the entire range of concentrations measured (Fig. 5a,b). The relationship between phytoplankton community size structure and Si was, however, not linear, showing an increase in the fraction of large phytoplankton at intermediate Si concentrations but not at very low ($< 1 \mu\text{M}$) and very high

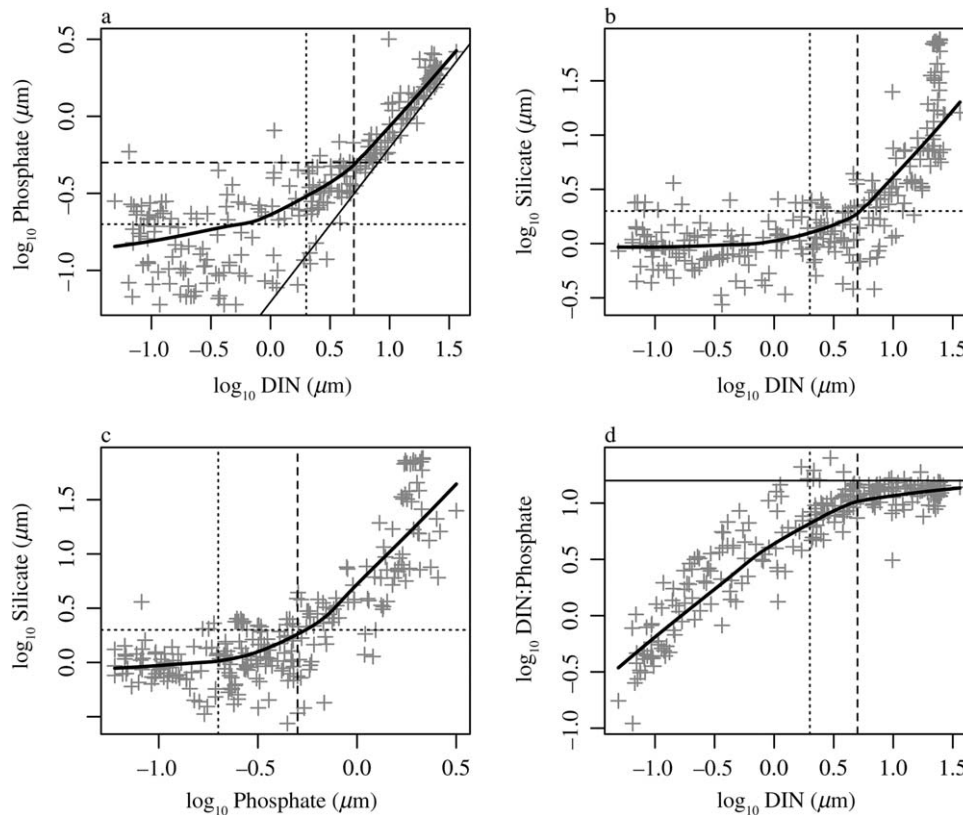


Fig. 3. Global patterns in the distribution of macronutrients: relationship between (a) phosphate and DIN, (b) silicate and DIN, (c) silicate and phosphate, and (d) the DIN : Phosphate ratio and DIN. The thick black lines represent LOWESS polynomial regressions. The dotted lines represent nutrient concentrations of $2 \mu\text{M}$ for (a–c) DIN and (b, c) silicate, and $0.2 \mu\text{M}$ for (a, c) phosphate. The dashed lines represent nutrient concentrations of $5 \mu\text{M}$ for (a–c) DIN and $0.5 \mu\text{M}$ for (a, c) phosphate. The thin black line in (d) represents the Redfield ratio (DIN/P = 16).

(> $10 \mu\text{M}$) concentrations (Fig. 5c). The relationship between the fraction of large phytoplankton and the DIN/P ratio was found to be unimodal with an increase in the fraction of large phytoplankton with increase DIN/P and ratios lower than ca. 8 and a large increase in the fraction of large phytoplankton at DIN/P ratios between ca. 8 and 16. Although there are only a few observations where DIN/P was higher than 16, the available data suggest a decrease in the fraction of large phytoplankton at very high DIN/P ratios (Fig. 5d).

GLS and model selection

The differences in the distribution of Chl *a* of different sized phytoplankton, above and below DIN and P concentrations of $5 \mu\text{M}$ and $0.5 \mu\text{M}$, indicate that the mechanisms driving the increase in the fraction of large phytoplankton may be different between ocean regions with high and low ambient nutrient concentrations. Therefore, in addition to examining the entire dataset as a whole, we also investigated each of these regions individually.

For the entire dataset, the model selection procedure identified three model candidates (variable sets) that were considerably better than the rest for explaining the variation in the fraction of large phytoplankton (Table 1). The model

with the lowest AICc included the variables DIN, P, depth as well as an interaction between DIN and P (Table 1, model 1.1). However, the second best model, including DIN, P, and an interaction between DIN and P (Table 1, model 1.2) exhibited a ΔAICc of 0.43 and was thus almost as good as the best model. According to the principles of Ockham's razor (Burnham and Anderson 1998), the less complex (1.2) should be chosen over the more complex model (1.1) and we, therefore, conclude that on the global scale, both DIN and P were positively correlated with the fraction of large phytoplankton. However, the negative interaction implies that the effects of DIN and P are dependent on each other such that an increase in one nutrient negatively influences the effect of the other nutrient on community size structure. In other words, in regions with high concentrations of DIN and P, the fraction of large phytoplankton is smaller than we would have expected if both nutrients had exhibited independent positive effects.

When the low nutrient regions were investigated (Table 2), the model selection procedure again identified three models as being significantly better than the rest. Here, the best model, according to the AICc (Table 2, model 2.1), included DIN, P, depth as well as an interaction between

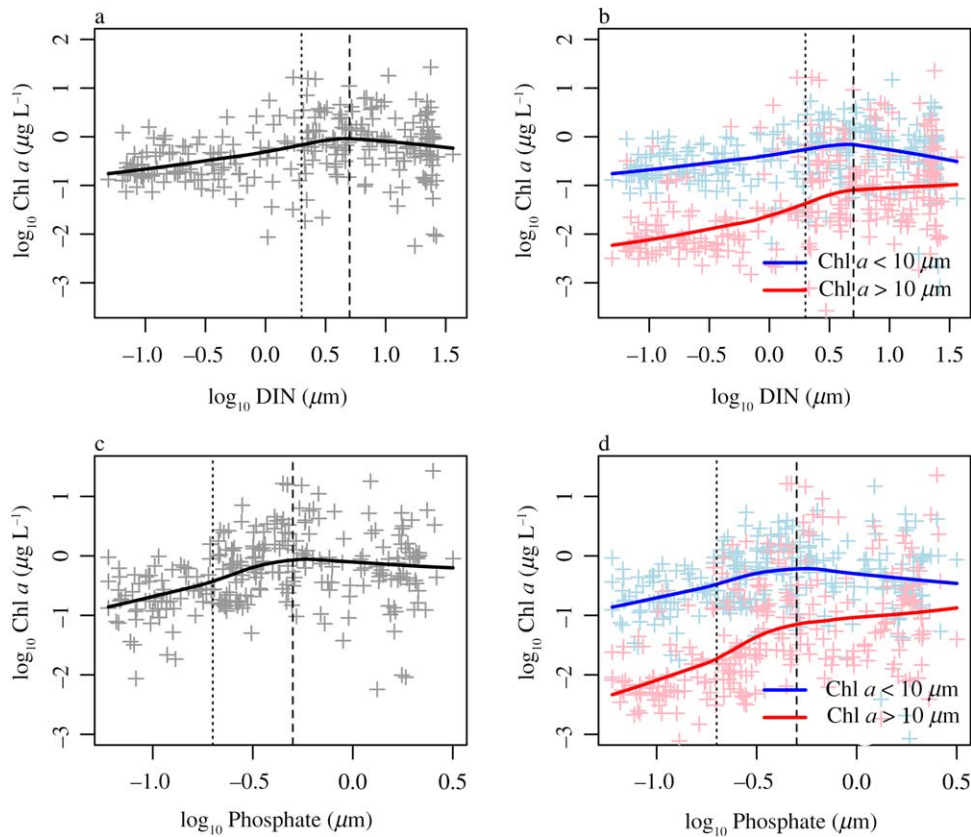


Fig. 4. Global patterns in the distribution of phytoplankton biomass: relationships between the Chl *a* concentration and (a) DIN and (c) phosphate. Relationship between Chl *a* originating from cells smaller (blue) and larger (red) than 10 μm and (b) DIN and (d) phosphate. The thick lines represent LOWESS polynomial regressions. The dotted lines represent nutrient concentrations of 2 μM for (a, b) DIN and 0.2 μM for (c, d) phosphate. The dashed lines represent nutrient concentrations of 5 μM for (a, b) DIN and 0.5 μM for (c, d) phosphate.

DIN and P. However, the second best model (Table 2, model 2.2) which did not identify an interaction between DIN and P to be important, was almost as good as the best model (ΔAICc of 0.71). Hence, again according to the principle of Ockham's razor (Burnham and Anderson 1998), model 2.2 was chosen as the most parsimonious model in favor of the more complex model, 2.1. A plot of the standardized vs. fitted residuals exhibited no violation of the assumption of linearity (data not shown) and, thus, supported our model choice in that we would expect deviation from linearity if a strong interaction between DIN and P was present. We conclude, then, that at DIN and P concentrations up to 5 μM and 0.5 μM , an increasing fraction of large phytoplankton was associated with relatively shallow sampling depths as well as independent increases in both DIN and P.

In the high nutrient regions (Table 3), the modeling selection procedure identified two model candidates exhibiting ΔAICc lower than 2. The best model (Table 3, model 3.1) suggested the fraction of large phytoplankton to be positively correlated with P and negatively correlated with depth. The second best model (Table 3, model 3.2) was equally complex as the best model and suggested the

fraction of large phytoplankton to be positively correlated with DIN and negatively correlated with depth. It is thus not possible to choose one model over the other. In regions with DIN and P concentrations higher than 5 μM and 0.5 μM , DIN and P were strongly correlated (Fig. 3a) which would make it hard to distinguish the influences statistically. We conclude that in regions with relatively high nutrient concentrations, an increasing fraction of large phytoplankton was associated with increasing nutrient concentrations of DIN and P, but due to co-correlation, it is not possible to discern any potential individual effects.

Distribution of nitrogen species

DIN was calculated as the sum of NO_3 , NO_2 , and NH_4 , and while NO_3 in general comprised the largest fraction of total DIN, there was a clear pattern in the relative distribution of the different nitrogen species with increasing DIN (Fig. 6a). Thus, at very low DIN concentrations ($< 0.1 \mu\text{M}$), NO_2 and NH_4 were the primary contributors to DIN and, together, they constituted about 70% of total DIN. With increasing DIN, the fractions of NO_2 and NH_4 rapidly decreased. The opposite pattern was observed for NO_3 which

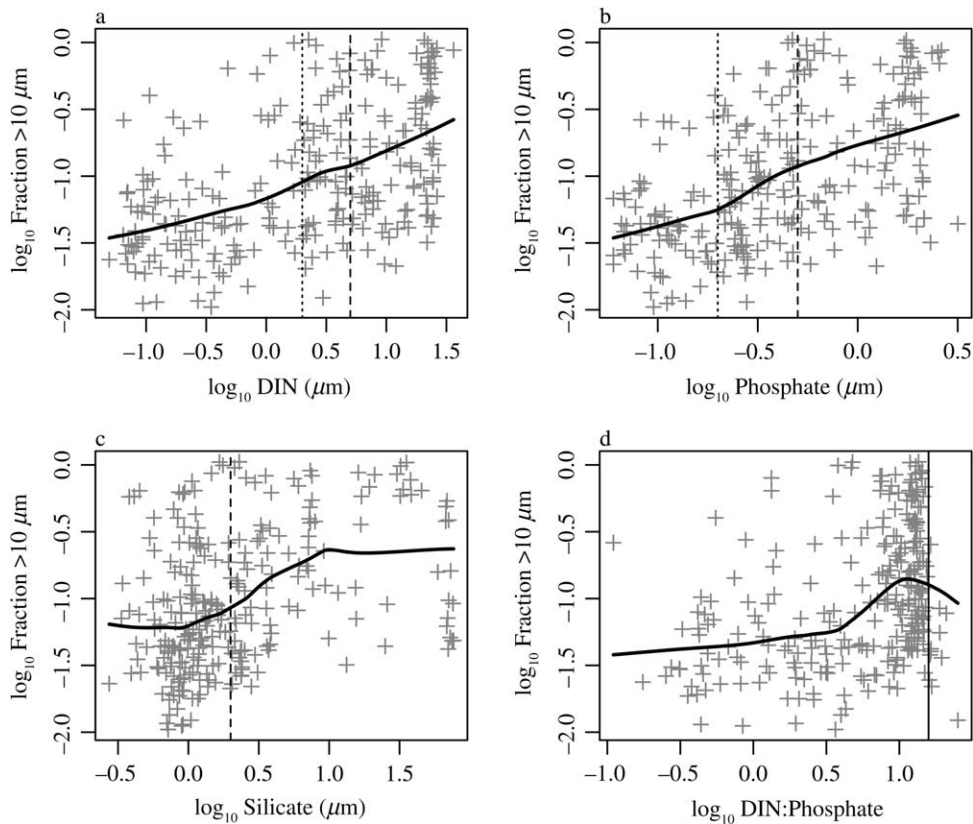


Fig. 5. Global patterns in phytoplankton community size structure: relationship between the fraction of large phytoplankton and (a) DIN, (b) phosphate, (c) silicate, and (d) the DIN :Phosphate ratio. The thick black lines represent LOWESS polynomial regressions. The dotted lines represent nutrient concentrations of 2 μM for (a) DIN and (c) silicate and 0.2 μM for (b) phosphate. The dashed lines represent nutrient concentrations of 5 μM for (a) DIN and 0.5 μM for (b) phosphate. The thin black line in (d) represents Redfield ratio (DIN/P = 16).

Table 1. Model coefficient and selection results using ΔAICc for determining the best combination of nutrient variables for explaining the fraction of large phytoplankton in the entire dataset. Only the top five models are shown.

Model	P	Si	DIN	Depth	DIN/P	AICc	ΔAICc
1.1	0.370	—	0.032	-0.071	-0.260	298.0	0
1.2	0.384	—	0.015	—	-0.248	298.4	0.43
1.3	0.338	0.081	-0.007	-0.068	-0.321	299.5	1.50
1.4	0.347	0.090	-0.026	—	-0.313	299.8	1.78
1.5	0.292	—	0.179	-0.141	—	302.2	4.23

Table 2. Model coefficients and selection results using ΔAICc for determining the best combination of nutrient variables for explaining the fraction of large phytoplankton in samples with relatively low nutrient concentrations (DIN < 5 μM and P < 0.5 μM). Only the top five models are shown.

Model	P	Si	DIN	Depth	DIN/P	AICc	ΔAICc
2.1	0.491	—	0.514	-0.156	0.448	150.8	0
2.2	0.378	—	0.229	-0.169	—	151.5	0.71
2.3	0.514	-0.145	0.548	-0.156	0.485	152.1	1.32
2.4	0.388	-0.104	0.237	-0.170	—	153.2	2.45
2.5	0.556	—	0.547	—	0.515	154.9	4.06

Table 3. Model coefficients and selection results using $\Delta AICc$ for determining the best combination of nutrient variables for explaining the fraction of large phytoplankton in samples with relatively high nutrient concentrations ($DIN > 5 \mu M$ and $P > 0.5 \mu M$). Only the top five models are shown.

Model	P	Si	DIN	Depth	DIN/P	AICc	$\Delta AICc$
3.1	—	—	0.656	-0.241	—	87.7	0
3.2	0.721	—	—	-0.231	—	88.9	1.25
3.3	—	0.076	0.540	-0.238	—	89.7	2.02
3.4	0.213	—	0.491	-0.240	—	89.8	2.08
3.5	-1.612	—	0.346	-0.231	1.626	90.2	2.48

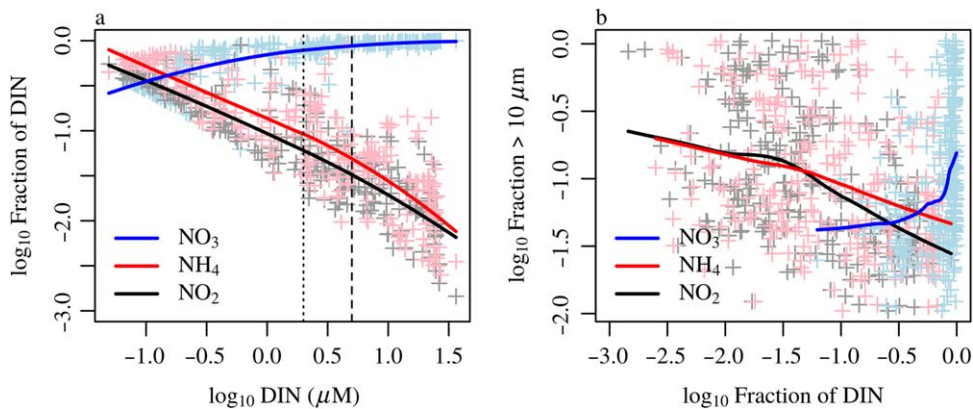


Fig. 6. Global patterns in the distribution of inorganic nitrogen species: fractions of nitrate (blue), nitrite (black), and ammonium (red) of DIN plotted against (a) DIN and the fraction of large phytoplankton plotted against (b) the fractions of nitrate, nitrite, and ammonium to DIN. The black lines represent LOWESS polynomial regressions. Dotted and dashed lines in (a) represent concentrations of $2 \mu M$ and $5 \mu M$ DIN.

showed an asymptotic increase toward 100% with increasing DIN and with NO_3 constituting more than 95% of total DIN at $5 \mu M$ DIN.

The relationship between the fraction of large phytoplankton and the relative distribution of nitrogen species (Fig. 6b) showed a large degree of variation. Nevertheless, the polynomial regression lines revealed clear patterns in the relative distribution of the three nitrogen species with increasing dominance of large phytoplankton. Thus, an increase in the fraction of large phytoplankton was generally associated with an increasing contribution of NO_3 to total DIN and a concomitant decrease in the contribution of NO_2 and NH_4 . Conversely, the fraction of large phytoplankton was generally low in regions where a large fraction of DIN was comprised on NO_2 and NH_4 .

Discussion

Macroecological patterns in phytoplankton community size structure

Individual effects of DIN and phosphate as limiting nutrients

The analyses conducted here show that phytoplankton community size structure in the open ocean is statistically dependent on both ambient DIN and P concentrations when

these are lower than $5 \mu M$ and $0.5 \mu M$, respectively. Specifically, the fraction of large phytoplankton was positively correlated with both DIN and P, even when the effect of one nutrient was controlled for in relation to the other.

As noted in the introduction, a positive relationship between ambient nutrient concentrations and phytoplankton community mean cell size would indicate nutrient limitation according to nutrient competition theory (Tilman 1982; Grover 1997). That is, as ambient nutrient concentrations decrease and approach zero, then small phytoplankton will out-compete larger ones primarily due to the intensification of diffusion limitation (Kiørboe 1993). The interpretation that the negative relationships between phytoplankton size and decreasing ambient concentrations of DIN and P are being caused by nutrient limitation is supported within the data by the distribution of the nitrogen species. DIN is comprised of both inorganic (NO_3) and organically derived species of nitrogen (NO_2 and NH_4). While NO_3 is the primary inorganic form delivered from terrestrial runoff and from the deep ocean, NO_2 and NH_4 are biologically derived through microbial degradation of organic matter (Azam et al. 1983; Arrigo 2005; Gruber 2008). Concentrations of NO_2 and NH_4 are shown here to be positively correlated and the distribution of the different species of nitrogen showed clear patterns with changes

in DIN, i.e., increasing relative importance of NO_2 and NH_4 as DIN decreased. Thus, at very low DIN concentration, NO_2 and NH_4 were the primary forms of nitrogen available to the phytoplankton indicating that phytoplankton activity under these conditions was primarily driven by nitrogen supplied at low concentrations through regeneration [i.e., “regenerated production,” *sensu* Dugdale and Goering (1967)].

Samples with high relative contributions of NO_2 and NH_4 to DIN were generally dominated by small phytoplankton. Being small under these conditions would be an adaptation to the low concentrations of gradually supplied nutrients from the regeneration process which is in support of the hypothesis that competition for limiting nutrients is an important factor shaping the patterns observed here. An interesting implication of this pattern is the potential possibility of using the ratio between NO_3 and NO_2 and/or NH_4 as a proxy for phytoplankton community size structure.

Thus, our results support the general knowledge that small phytoplankton are associated with low nutrient concentrations such as in the oligotrophic gyres, and large phytoplankton are associated with conditions of high nutrient availability such as during spring blooms or in upwelling regions (Chisholm 1992; Kjørboe 1993; Acevedo-Trejos et al. 2013; Roy et al. 2013). However, our analysis adds two novel pieces of information to this understanding. The independence of DIN and P suggests that if the concentration of one nutrient is increased, phytoplankton mean size will increase even if the concentration of the other nutrient remains very low and potentially limiting for total phytoplankton biomass. Second, DIN and P concentrations of $5 \mu\text{M}$ and $0.5 \mu\text{M}$ are considerably higher than those usually considered limiting for phytoplankton activity (i.e., less than $\sim 2 \mu\text{M}$ and $\sim 0.2 \mu\text{M}$; Reynolds 2006), indicating that these rules-of-thumb for nutrient limitation of phytoplankton need to be revisited and possibly discarded all together (discussed in the section “General threshold concentrations for nutrient limitation” below).

The effects of DIN and P on community size structure were here derived from a nearly global dataset. While the effects may not quantitatively be exactly the same everywhere, the fact that these relationships emerge at this spatial scale suggests that the patterns are general and we would, therefore, expect the effects of increasing DIN and/or P to lead to an increase in the relative contribution of large cells everywhere. Coupled with evidence that the amount of regenerated nitrogen decreased with an increase in the fraction of large phytoplankton, we conclude that it is very likely that both DIN and P, through growth limitation, play significant roles in regulating phytoplankton community size structure in the open ocean where concentrations of DIN and P are below $5 \mu\text{M}$ and $0.5 \mu\text{M}$, respectively.

Interactive effects and the DIN/P ratio

We were also interested in investigating potential interactive effects of DIN and P. In a meta-analysis of enrichment

experiments, Elser et al. (2007) reported that the addition of both N and P had a “strong synergistic” effect on phytoplankton biomass. While those authors did not explicitly specify what is meant by “synergistic,” a possible definition could be a multiplicative relationship between N and P where the effect of adding either nutrient would be positively affected by an increase in the other, and where this effect was larger than that of sequential addition assuming single nutrient limitation. While our analysis, as outlined in the introduction, is not directly analogous to the enrichment experiments reported in Elser et al. (2007), we would expect such a synergistic relationship to lead to a positive interaction between DIN and P. The results of our modeling exercise indicated, however, a negative interaction when all data were included. When the low and high nutrient regions were analyzed independently, the evidence for an important interaction was weak at best. We interpret the negative interaction between DIN and P at the global scale to be an indication of other confounding factors influencing community size structure at high nutrient concentrations. Such factors include light and iron which we know can limit biomass in nutrient rich regions (Cavender-Bares et al. 1999; Moore et al. 2013), leading to a smaller phytoplankton mean size than would have been predicted from DIN and P alone.

The lack of an important interaction between DIN and P implies that, while the external DIN/P ratio undoubtedly affects phytoplankton physiology (Geider and La Roche 2002; Finkel et al. 2010), it does not play an important and direct role in controlling phytoplankton community structure. Instead, our results indicate that, at ambient DIN and P concentrations below $5 \mu\text{M}$ and $0.5 \mu\text{M}$, changes in the availability of either DIN or P will elicit changes in the phytoplankton community so that it is able to take advantage of the more abundant nutrient regardless of external stoichiometry. Support for this hypothesis can be found in the relatively recent advances in the understanding of internal stoichiometry where Redfield’s canonical ratio of N/P = 16 : 1 is no longer considered the optimal internal ratio for phytoplankton groups in general (Geider and La Roche 2002; Klausmeier et al. 2004). Different phytoplankton groups express different N/P ratios as a result of evolutionary history, the amount of homeostatic regulation, and capacity of excess uptake and storage of abundant nutrients (Sterner and Elser 2003; Finkel et al. 2010). It is clear that, as DIN and P approach depletion, there will be an upper limit on the amount of biomass that can be supported. However, due to differences in internal N/P stoichiometry between phytoplankton groups, it is likely that changes in community composition can still be modulated by changes in the ambient concentration of nutrients that do not limit yield.

Effect of silicate as a limiting nutrient

In contrast to DIN and P, there was little or no support for a significant relationship between Si and community size

structure. High Si concentrations, therefore, do not appear to be necessary in order to have a high relative abundance of large phytoplankton. A plausible explanation for this lack of a relationship can be found in the fact that Si is only used by a few phytoplankton groups with diatoms being the ecologically most important. While large diatoms often dominate under high nutrient conditions such as intermediate-to-high-latitude spring blooms or in upwelling regions (Sieracki et al. 1993; Degerlund and Eilertsen 2010), other phytoplankton groups exhibiting large species (e.g., dinoflagellates and raphidophytes) can proliferate under high DIN and P and low Si conditions resulting in a potentially high contribution of large cells to total phytoplankton biomass, regardless of Si levels.

Variability and other confounding factors

The data presented here generally exhibit a large degree of variability, which is not surprising given the spatiotemporal scales at which the data was collected. However, even if spatiotemporal influences could be fully accounted for, we would still not expect that DIN, P, and Si availability alone would be able to explain all the variation in the global distribution of phytoplankton community size structure. Other potentially important factors include the availability of other resources such as light and various micronutrients (Mitchell et al. 1991; Coale et al. 1996; Saito et al. 2002), top-down processes such as grazing by zooplankton or viral lysis (Kjørboe 1998; Fuhrman 1999), as well as physical constraints such as turbulence and temperature (Kjørboe 1993; Marañoń et al. 2014; Mousing et al. 2014). We fully acknowledge that all of these factors play critical roles in regulating phytoplankton community structure and we cannot rule out that they may influence the patterns here interpreted as nutrient limitation effects.

Macroecological patterns in the distribution of phytoplankton biomass

Phytoplankton biomass, measured as total Chl *a*, increased with increasing concentrations of DIN and P up to around 5 μM and 0.5 μM . At higher nutrient concentrations, phytoplankton biomass levels off, with a tendency to a slight decrease with increasing DIN and P. In addition, phytoplankton biomass was less variable at DIN and P concentrations lower than 5 μM and 0.5 μM ($SD = 0.54$, $n = 154$) compared to phytoplankton biomass at higher concentrations ($SD = 0.65$, $n = 93$).

Thus, at the macroecological scale, phytoplankton biomass is generally higher and more variable in regions with higher nutrient availability. As an analogy, we can imagine the concentrations measured as the concentrations in a chemostat experiment where the nutrients are added in pulses. The biomasses measured represent then different states in a continuous change toward steady state which is represented by the average (tendency line in the plots). In this analogy, the variation around the average (steady state) can then be

interpreted as the amount of transience where a small amount of variation would indicate that biomass for that nutrient concentration is close to steady state. Conversely, a high amount of variation would indicate that the system is often far away from steady state and thus a more perturbed system.

Following this interpretation, the results in this study thus suggest that, at low DIN and P concentrations, phytoplankton biomass is generally low and close to steady state. As concentrations increase, both phytoplankton biomass and the amount of transience increase. At DIN and P concentrations higher than 5 μM and 0.5 μM , the amount of transience continues to increase indicating continuously more perturbed systems. Mean biomass, on the other hand, shows a slight decrease in this nutrient range. We interpret this as indicating that adding more DIN and P does not increase biomass, and thus that other factors than nutrient availability are more important for controlling phytoplankton biomass in regions with DIN and P concentrations higher than 5 μM and 0.5 μM .

General threshold concentrations for nutrient limitation

Threshold values for DIN and phosphate

In the ecological literature, it is not uncommon to see references to “rules of thumb” or “threshold values” suggesting that when DIN and P concentrations are below $\sim 2 \mu\text{M}$ and $\sim 0.2 \mu\text{M}$, respectively, phytoplankton activity will be limited (e.g., Fisher et al. 1992; Gallegos and Jordan 1997; Reynolds 2006). We found, however, no evidence that phytoplankton respond differently at these nutrient concentrations than at those immediately above or below these concentrations.

Instead, the patterns presented in this study suggest that both DIN and P play important roles in regulating phytoplankton activity, at least at ambient concentrations of up to 5 μM and 0.5 μM . At around these values, phytoplankton biomass ceased to increase with increasing DIN and P and the continued increase in the fraction of large phytoplankton changed from being primarily caused by an increase in the biomass of large phytoplankton to also being caused by a decrease in the biomass of small phytoplankton. We argue, however, that, while these values may represent a threshold value for when nutrient regulation by DIN and P breaks down to be substituted by other factors in the contemporary ocean, there is likely no canonical meaning associated with them.

If DIN and P concentrations of 5 μM and 0.5 μM were deterministic threshold values, then this would imply that nutrient limitation cannot occur at concentrations higher than these. While nutrient concentrations at these levels and higher do not appear to be limiting for total phytoplankton biomass, this does not imply that nutrient limitation of phytoplankton growth rates cannot occur at these concentrations and above. Indeed, the fact that the relative

proportion of large to small cells continues to increase with increasing ambient nutrient concentrations over the entire range of concentrations encountered indicates that community composition is constantly changing in response to nutrient availability regardless of the concentration.

One condition that would suggest nutrient regulation at high concentrations could occur, would be a “flattening” of the Monod growth curve with increasing cell size. This would translate into a higher half-saturation constant; i.e., an increase in the nutrient concentration at which half the maximum growth rate is reached (Grover 1997). There does appear to be empirical evidence of this in the literature: some phytoplankton species do, indeed, exhibit very high half-saturation constants for DIN, P, and Si of up to 10.2 μM , 8.9 μM , and 22 μM , respectively (Sarhou et al. 2005). Further support can be found in Hein et al. (1995) who, in a study encompassing both micro- and macro-algae, found a negative relationship between the half-saturation constant and the surface-to-volume ratio for both ammonium and nitrate. Last, Monod curves for diatoms in relation to Si often show no saturation or saturation at high concentrations (Tilman 1982), indicating that Si can limit the growth rate of some species of diatoms even at extremely high concentrations ($> 10 \mu\text{M}$). Thus, rather than representing absolute values for limitation, we suggest that 5 μM and 0.5 μM for DIN and P, respectively, may simply indicate a level where the importance of nutrient limitation is relatively less important relative to other drivers (light, trace metals, grazing, turbulence, etc.).

Threshold values for silicate

We also conclude that we cannot identify a “rule-of-thumb” concentration for diatom limitation by Si. Under experimental conditions, it has been shown that diatoms dominate when the Si concentration is above 2 μM (Egge and Aksnes 1992). Therefore, concentrations below this threshold are often cited as being limiting for diatom growth. Under natural conditions, however, diatoms rapidly take up Si when other macronutrients are available and diatoms have been shown to dominate phytoplankton biomass even at low Si concentrations (Mousing et al. 2016).

In the data presented here, Si concentrations were generally below 2 μM when DIN and P concentrations were lower than 5 μM and 0.5 μM . Diatoms are heavy and sink faster than non-ballasted phytoplankton groups and Si may, through sinking, therefore be quickly lost from the euphotic zone after its incorporation in the cells. This means that high values of Si ($> 2 \mu\text{M}$) are usually found in ocean regions where other resources than Si are limiting; i.e., iron in high-nutrients-low-chlorophyll regions (Cavender-Bares et al. 1999; Moore et al. 2013) or light at intermediate-to-high latitude regions before the onset of the spring bloom (Mitchell et al. 1991; Barlow et al. 1993). Thus, concentrations below 2 μM in our dataset may be associated with active Si uptake.

Furthermore, the fact that Si concentration, in general, only increases to above 2 μM when DIN and P concentrations are above 5 μM and 0.5 μM , suggests that such high Si concentrations are only seen when nutrient regulation breaks down.

Conclusion

Ambient nutrient concentrations at any given time and place reveal little, if anything, about the relationship between the physiological state or composition of the local phytoplankton community. This study suggests, however, that patterns derived from relating phytoplankton community characteristics to ambient nutrient concentrations in a sufficiently large data set can be used to improve our understanding of the overall role that nutrient availability plays in controlling phytoplankton biomass and in the shaping of phytoplankton communities. This macroecological approach is powerful in that it allows us to discern relationships that may be valid for the ocean as a whole. Nutrient availability has long been known to be important as a factor potentially limiting phytoplankton biomass (and/or primary production which is a function of biomass). The patterns emerging from this study confirm this potential role and indicate that, at low (below $\sim 5 \mu\text{M}$ DIN and $\sim 0.5 \mu\text{M}$ P) ambient nutrient concentrations, any increase in either nutrient correlates with an increase in biomass. While this result does not allow us to conclude that phytoplankton biomass will not be limited above these concentrations, it does suggest that the relative (vis à vis light and/or grazing, etc.) importance of nutrients as a factor limiting phytoplankton biomass may decrease above these levels of ambient nutrient concentrations. More interestingly, this study indicates that changes in phytoplankton community composition (as determined from community size structure) correlate with changes in ambient nutrient concentrations over the entire range of nutrients concentrations encountered in the dataset. This implies that phytoplankton diversity and community structure are at all times under nutrient control. We would expect this result from laboratory studies showing the large range in nutrient affinities between species even from the same taxonomic groups. However, it may require a study such as this to identify the response under natural conditions. Obtaining a more nuanced understanding of the potential role of nutrients in controlling phytoplankton community structure is important as it is becoming increasingly recognized that specific species and groups may differentially impact energy flow (food webs) and biogeochemical cycling of carbon and other nutrients in the ocean (see Introduction). Thus, identifying a relationship between nutrient availability and phytoplankton biomass or primary production is not sufficient to fully understand the role of nutrients in controlling the contribution of phytoplankton to ocean processes.

Author Contribution Statement

EAM carried out all statistical analyses and wrote the first draft of the manuscript; KR was responsible for the data collection. KR and ME contributed to both the planning of the study and editing the draft manuscript.

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Conflict of Interest

None declared.

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