

Phytoplankton species richness, evenness, and production in relation to nutrient availability and imbalance

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Abstract

We used natural phytoplankton communities from four coastal regions to test diversity-functioning relationships, relations of N₂-fixing cyanobacteria to nutrient imbalance, and the importance of metacommunity dynamics. Resource availability was measured as total nitrogen and phosphorus. Resource imbalance was determined as (1) the ratio of dissolved inorganic nitrogen to total phosphorus and (2) an experimentally verified indicator quantified by modelling responses in ¹⁴C-based primary production to nutrient additions. Resource availability explained variance in biomass, productivity, and species richness, as expected by the Species Energy Theory, but not evenness. Linear mixed-effects models confirmed the overall relation between productivity and resource availability, whereas other resource availability relations showed also notable clustering by region. The Resource Ratio Theory predicting that diversity increases with the number of limiting resources was not supported. Nutrient imbalance had a weak effect on the biomass of N₂-fixing cyanobacteria, but not their share of total phytoplankton biomass. Contrary to many previous studies on biodiversity-ecosystem functioning relationships, we found a highly significant inverse relationship between evenness and biomass. This indicates that species-rich natural phytoplankton communities form the basis for opportunistic species to temporarily monopolize resources and create blooms. The result was consistent across regions, although their community composition differed. Metacommunity dynamics were important, since distance between the regions explained higher percentage of the variability than local resources together. As the species able to monopolize resources vary widely in their role for aquatic food webs and environmental consequences (e.g., food quality, toxicity), species-level trait data is essential to understand better diversity-productivity relationships.

Essential community properties, i.e., diversity, biomass, and productivity, and their relationships to available resources, have been actively studied especially in terrestrial ecosystems, as they are connected to agriculture and other food production for humans, but certainly also because aquatic communities present methodological challenges, e.g., in representative sampling in relation to community turnover. This is most pronounced in microbial planktonic systems, which are further complicated by the constant flux of both resources and organisms, and the demanding taxonomical resolution of the latter. Theories aiming to explain causalities behind resources and community properties include the Liebig's Law of the Minimum (Liebig 1840), the Species-Energy Theory (SET, Wright 1983), the Resource-Ratio Theory

(RRT, Tilman 1982), and the recently evolved paradigm "biodiversity-ecosystem functioning" (BEF, Naeem et al. 1994; Gross and Cardinale 2007; Hillebrand and Matthiessen 2009).

SET argues that available energy explains the variation in species richness by controlling population sizes and the probability of stochastic extinction (Wright 1983). Amount of available energy would ideally be measured in units of energy per time (joules per year), but any relative measure of available energy serves. RRT predicts that species richness is affected by the imbalance in the supply of two or more resources, due to increased possibilities for competitive replacements (Tilman 1982). The BEF paradigm has challenged the historical view of diversity merely reflecting resource-driven productivity by reversing the causality—with diversity suggested as a driver for ecosystem properties, including biomass production and resource use efficiency (Naeem et al. 1994; Gross and Cardinale 2007; Hillebrand and Matthiessen 2009). In an attempt to elaborate the connections between various ecosystem properties, Cardinale

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et al. (2009) outlined a reciprocal productivity–diversity hypothesis studying interactions among four distinct variables: (1) the overall quantity of limiting resources, (2) the stoichiometric ratios of different limiting resources, (3) the summed biomass produced by a group of potential competitors, and (4) the richness of co-occurring species in a local competitive community.

Empirical evidence for the alternative theories has stemmed largely from observational spatial data, meta-analyses, or long-term experimentation in terrestrial systems (e.g., Tilman et al. 1996). Later, small-scale experimentation with artificially assembled microbial communities has been increasingly applied to diversity-productivity studies, especially in aquatic environments (e.g., Striebel et al. 2009; Gamfeldt and Hillebrand 2011). The results of experimental studies have accumulated evidence for biodiversity acting as a driver of ecosystem processes and services (Cardinale et al. 2012). Still, a basic shortcoming for comparing analyses of diversity–productivity relationships—as well as a potential source of apparent conflicts—is the heterogeneity in definitions and available proxies both for “productivity” and “diversity” in widely different systems (habitats, trophic levels, turnover rates, natural vs. artificial), scales of observation, and data sets (Cardinale et al. 2009; Svensson et al. 2012). It has been consequently argued that it is unclear whether the outcomes of experimental BEF studies can be transferred to complex natural communities and ecosystems (Miller et al. 2005; Hautier et al. 2014; Hodapp et al. 2015).

Ptacnik et al. (2008) were the first to show that natural phytoplankton communities exhibit relations between resource use efficiency (RUE) and diversity, which correspond to those shown for higher organisms. However, recent studies by Filstrup et al. (2014) and Hodapp et al. (2015) demonstrated a strong inverse relationship between evenness and either phytoplankton biomass or RUE. Olli et al. (2014) showed with long-term analyses that phytoplankton genus diversity, as well as RUE, have increased in the past 40 yr in the Baltic Sea.

In this study, we analyze how community properties (species richness, evenness, biomass, production) are interconnected, and how they depend on resource (nitrogen and phosphorus) availability and imbalance in natural phytoplankton communities, with experimentally controlled and verified nutrient availability and limitation (imbalance). We make use of a dataset where nutrient limitation was experimentally assessed in laborious bioassays (Andersen et al. 2007; Tamminen and Andersen 2007). While other studies have relied on more derived information about nutrient limitation, our dataset enables us to link information on community parameters and environmental background directly with experimentally validated information on nutrient limitation.

The data used in this study originate from a 3-yr experimental campaign covering a 1000-km gradient of Finnish coastline, the northern Baltic Sea. Like most areas of the estuarine

brackish Baltic Sea (Hagström et al. 2001 and references within), the sub-basins Gulf of Finland and Archipelago Sea are mainly N-limited during summer (Kivi et al. 1993; Kirkkala et al. 1998; Tamminen and Andersen 2007), while the Bay of Bothnia is predominantly P-limited (Tamminen and Andersen 2007). In addition to its northern location, the latter differs from the other areas also by its lower salinity, and by the lowest concentrations of total nutrients (HELCOM 2009, 2014).

We also test if the biomass of N₂-fixing, i.e., diazotrophic cyanobacteria in the natural communities correlates with the experimentally verified N limitation. This represents a specific, yet essential effect of nutrient imbalance on community composition, as N₂-fixing cyanobacteria are an important component of the phytoplankton globally (Deutsch et al. 2007), as well as in the Baltic Sea, where the abundance of cyanobacterial blooms has intensified significantly in the past 50 yr, attributed to human-induced eutrophication (Funkey et al. 2014; Kahru and Elmgren 2014). It has been argued, in line with the original homeostasis scenario of Redfield (1934) that N limitation is necessarily transient in aquatic ecosystems, because N₂-fixers gain competitive advantage and reverse the system back to P limitation (Schindler 1977; Tyrrell 1999). This view has been, however, challenged both for freshwater and marine systems (Howarth and Marino 2006; Elser et al. 2007; Tamminen and Andersen 2007; Scott and McCarthy 2010). It is therefore of interest to analyze how the presence of N₂-fixers relates to observed nutrient imbalance, in a coastal system where N₂ fixation is prevalent.

We thus used natural phytoplankton communities originating from geographically distinct coastal regions, representing varying levels of trophic state and contrasting limitation patterns, in an experimental setting which made it possible both to quantify primary production (CO₂ fixation) and to verify nutrient availability and imbalance. We carried out the diversity analyses with two descriptors that have been considered independent dimensions of diversity (yet debated; see Smith and Wilson 1996; Jost 2010; Tuomisto 2012): species richness (S) and Pielou's evenness (J). Specifically, we tested how SET, RRT, and BEF-based hypotheses performed with natural phytoplankton communities:

1. Resource availability predicts total biomass and species richness. We use total nutrients (nitrogen and phosphorus) as relative measures of available resources.
2. Phytoplankton diversity is highest at combined N and P limitation. We verified limitation either experimentally (Tamminen and Andersen 2007), or employing a chemical limitation indicator (Ptacnik et al. 2010a).
3. Diversity predicts productivity. We tested how diversity explained initial biomass, productivity, RUE, or responses to experimental enrichment.

Inspired by the approach of Cardinale et al. (2009), we used path analysis by structural equation models (SEMs) to further examine the interactions between diversity, community

properties, and resource availability and imbalance. In addition, we studied how N_2 -fixing cyanobacteria (order Nostocales) relate to resource availability and nutrient limitation patterns, and to which extent the local phytoplankton community composition (species-level biomass composition) in geographically distinct coastal regions is influenced by regional metacommunities vs. local environmental variables, including resource availability and imbalance. Such information is currently in high demand also for applied purposes, e.g., to understand how phytoplankton monitoring data can be interpreted in environmental assessments.

Material and methods

We utilize data from experimental bioassays on 62 natural phytoplankton communities along a nutrient limitation gradient along the Finnish coast, northern Baltic Sea. Phytoplankton community composition was analyzed quantitatively at the start situation of the bioassay experiments. The northernmost study site was situated in the Bay of Bothnia ($65^\circ 13'N$, $24^\circ 60'E$), and other sites were in the Archipelago Sea ($60^\circ 25'N$, $21^\circ 95'E$), western Gulf of Finland ($60^\circ 14'N$, $25^\circ 14'E$) and eastern Gulf of Finland ($60^\circ 42'N$, $27^\circ 65'E$) (Fig. 1). Actual bloom situations were not covered in the experiments, as the chlorophyll *a* (Chl *a*) levels remained at moderate levels for the Baltic Sea (Table 1). The study was carried out in June–October during years 1992–1994. From the Bay of Bothnia, only samples taken in July–October were included because of the later spring bloom in the north. A detailed description of the study sites and the bioassay experiments is given in Tamminen and Andersen (2007), but the most relevant information is provided below.

The quantitative phytoplankton samples and water for the bioassay experiments were taken simultaneously, with some exceptions (1-d delay in nine sampling events, 2-d delay in two sampling events, and 3-d delay in three sampling events in the western Gulf of Finland). Total number of sampling events was 62. Phytoplankton samples represent the initial situation for the bioassay experiments. Phytoplankton samples were taken as integrated samples from 0 m to 2 m. The samples were preserved with acidic Lugol's solution and kept refrigerated in the dark until the analysis with a Leica Diavert or Leitz Fluovert FS (western Gulf of Finland) inverted light microscope using the Utermöhl (1958) method. Smaller than $2 \mu\text{m}$ —sized phytoplankton was not included into the study since it is not possible to count them reliably with a light microscope. In order to warrant a constant counting effort among samples, HELCOM recommendations were followed by selecting the settling volume based on abundance of cells in a sample (10 mL, 25 mL, or 50 mL) and counting at least 50 counting units of each dominating taxon (the total count should exceed 500 units per magnification) (HELCOM 2015). Two

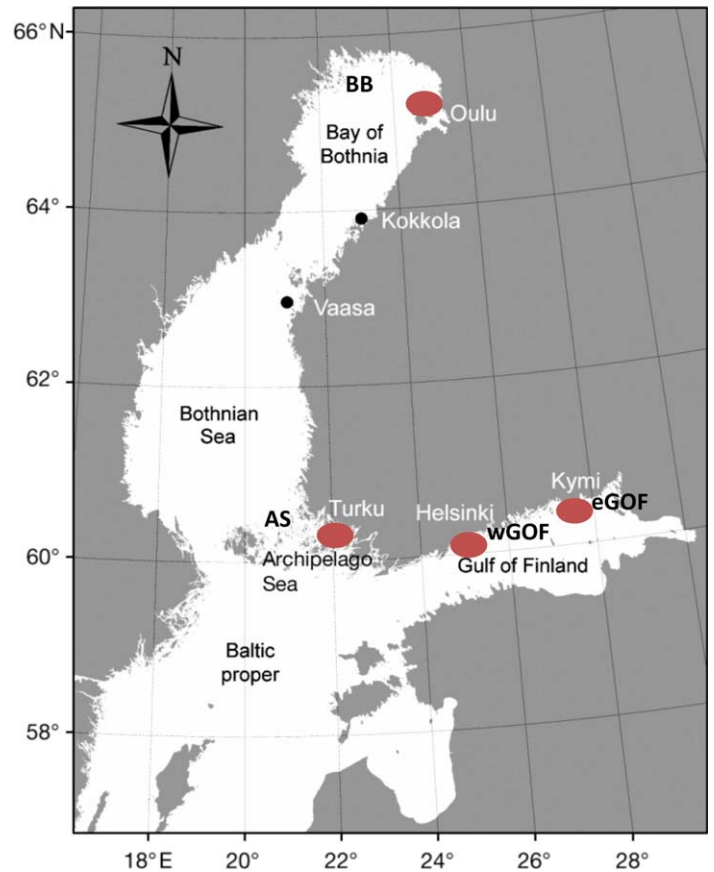


Fig. 1. Map showing the four coastal regions (red dots) along the Finnish coastline of the Baltic Sea: BB = Bay of Bothnia, AS = Archipelago Sea, wGOF = western Gulf of Finland, eGOF = eastern Gulf of Finland.

different magnifications have been used (ca. 100X magnification to count taxa $>20 \mu\text{m}$, ca. 500X magnification to count taxa $<20 \mu\text{m}$). The biovolumes (wet weight, $\mu\text{g L}^{-1}$) were calculated based on size measurements and cell shape approximations by simple solids (as in Hillebrand et al. 1999; Olenina et al. 2006). All samples were counted by the same person except samples from the western Gulf of Finland, minimizing variability in species identification. Pielou's evenness *J* (Pielou 1966) calculation was based on species-level biomass results.

For the bioassay experiments, a 50-L sample was taken from the euphotic surface layer by pooling casts taken at 1 m intervals down to twice the Secchi depth. The bioassay experiments were run in eight experimental 6-L units. No prescreening was applied, i.e., the ambient plankton community was present intact. The experiments started by adding phosphate ($20 \mu\text{g P L}^{-1}$) and/or ammonium ($80 \mu\text{g N L}^{-1}$) in a 2×2 factorial design with replicates. All experiments were carried out at in situ temperature in a thermostated water bath under the locally prevailing light–dark cycles, with daylight-spectrum fluorescent tubes (Philips TLD 965) producing an irradiance of $100 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$ inside the polycarbonate bottles to

Table 1. Basic information for the four coastal regions. The mean value \pm standard deviation is given. Species richness is calculated as number of species in a sample. Pielou's evenness is calculated using species-level biomass results. Limitation indicator L14C is based on primary production response on nutrient additions during the bioassay experiment: L14C: $-1 = \text{N-limited}$, $+1 = \text{P-limited}$, $0 = \text{combined or no limitation}$. Resource use efficiency $\text{RUE}_{\text{chl}} = \ln(\text{Chl } a/\text{TP})$. $\text{RUE}_{\text{pp}} = \ln(\text{Primary production}/\text{TP})$.

	Bay of Bothnia (BB)	Archipelago Sea (AS)	Western Gulf of Finland (wGOF)	Eastern Gulf of Finland (eGOF)
Coordinates	65° 13'N, 24° 60'E	60° 25'N, 21° 95'E	60° 14'N, 25° 14'E	60° 42'N, 27° 65'E
Number of samples	16	15	15	16
Salinity psu	2.6 \pm 0.4	6.0 \pm 0.2	5.1 \pm 0.3	3.7 \pm 0.4
Temperature °C	12.9 \pm 2.6	14.6 \pm 2.9	12.4 \pm 3.4	14.7 \pm 3.2
Species richness	21 \pm 9	19 \pm 4	37 \pm 8	26 \pm 8
Pielou's evenness	0.58 \pm 0.15	0.48 \pm 0.29	0.65 \pm 0.14	0.62 \pm 0.12
Phytoplankton biomass mg L ⁻¹	0.46 \pm 0.60	1.1 \pm 1.19	0.92 \pm 0.79	0.96 \pm 0.56
Chl <i>a</i> $\mu\text{g L}^{-1}$	2.7 \pm 1.7	3.2 \pm 0.9	4.7 \pm 2.5	5.1 \pm 1.3
Primary production $\mu\text{g C L}^{-1} \text{d}^{-1}$	64 \pm 41	156 \pm 100	157 \pm 74	183 \pm 98
RUE_{chl}	-1.10 \pm 0.48	-1.77 \pm 0.27	-1.75 \pm 0.52	-1.31 \pm 0.35
RUE_{pp}	2.06 \pm 0.47	2.04 \pm 0.42	1.78 \pm 0.59	2.13 \pm 0.46
DIN (NO ₂ + NO ₃ + NH ₄) $\mu\text{g N L}^{-1}$	56.1 \pm 20.1	12.7 \pm 7.6	36.8 \pm 32.3	19.9 \pm 15.8
DIP (PO ₄) $\mu\text{g P L}^{-1}$	0.7 \pm 0.3	3.1 \pm 2.2	7.0 \pm 6.9	2.2 \pm 2.5
DSi (SiO ₂) mg Si L ⁻¹	1.56 \pm 0.19	0.27 \pm 0.14	0.43 \pm 0.25	0.09 \pm 0.07
PON $\mu\text{g N L}^{-1}$	47 \pm 17	69 \pm 19	92 \pm 25	104 \pm 19
POP $\mu\text{g P L}^{-1}$	5 \pm 1	9 \pm 3	11 \pm 2	12 \pm 4
POC $\mu\text{g P L}^{-1}$	516 \pm 109	584 \pm 67	624 \pm 122	756 \pm 117
TN $\mu\text{g N L}^{-1}$	252.1 \pm 19.0	339.8 \pm 47.0	423.0 \pm 81.4	378.0 \pm 34.3
TP $\mu\text{g P L}^{-1}$	6.9 \pm 1.6	18.3 \pm 3.1	24.2 \pm 4.5	19.3 \pm 4.5
DIN : TP (mol : mol)	18.4 \pm 9.1	1.5 \pm 0.7	3.1 \pm 2.3	2.1 \pm 1.2
L14C	0.73 \pm 0.59	-0.14 \pm 0.80	-0.42 \pm 0.54	-0.37 \pm 0.66

DIN, dissolved inorganic nitrogen; DIP, dissolved inorganic phosphorus; DSi, dissolved silicate; PON, particulate organic nitrogen; POP, particulate organic phosphorus; POC, particulate organic carbon; TN, total nitrogen; TP, total phosphorus.

ensure that the natural communities were not light-limited during experiments.

Experimental units were sampled for primary productivity at the start, at 24 h, and at 48 h. Primary production was measured using the ¹⁴C-technique (for details of all measurements, see Tamminen and Andersen 2007). Chl *a* was sampled at the start, at 24 h, at 48 h, and at 72 h, and measured fluorometrically. To quantify phytoplankton total biomass, we use wet weight biomass results in analyses, but conclusions based on Chl *a* values were concordant (results not shown). Dissolved inorganic nitrogen (DIN), dissolved inorganic phosphorus (DIP), total nitrogen (TN), and total phosphorus (TP) were measured from the initial sample in duplicate according to standard methods. DIN was calculated as the sum of ammonium, nitrate, and nitrite. The ratio of dissolved inorganic nitrogen to total phosphorus (DIN : TP, mol : mol) was used to express the N : P limitation of initial communities (Ptacnik et al. 2010a).

Resource use efficiency (RUE) of a phytoplankton community was calculated by relating a biomass proxy (Chl *a*) or primary productivity to the amount of a potentially limiting resource (total phosphorus). RUE_{chl} was thus calculated as the natural logarithm of the ratio between Chl *a* and TP concentrations, both expressed in weight units ($\mu\text{g L}^{-1}$; Ptacnik et al. 2008), and RUE_{pp} accordingly, as the natural logarithm of the ratio between daily primary productivity ($\mu\text{g C L}^{-1} \text{d}^{-1}$) and TP concentration ($\mu\text{g L}^{-1}$). Short-term responses of primary productivity to enrichments were analyzed from experimental units which received both P and N addition during the bioassay experiment, as the difference between initial and 48-h samples.

Linear regression analysis (R software, www.r-project.org) was used to initially test (1) the effects of resource availability (TP, TN) on phytoplankton community parameters (biomass, productivity, species richness, evenness); (2) diversity-productivity relationships (species richness and evenness vs. biomass, productivity, RUE_{chl} , RUE_{pp}); and (3) the effects of community

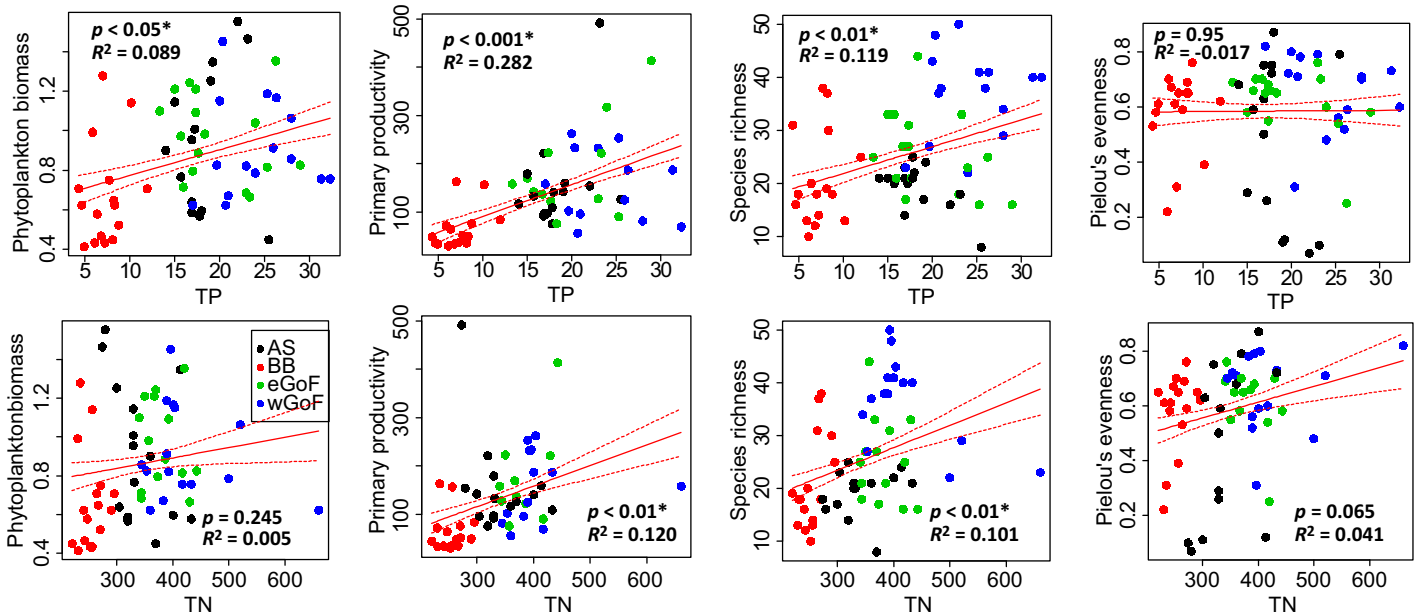


Fig. 2. Resource availability (total phosphorus (TP $\mu\text{g L}^{-1}$), upper row; total nitrogen (TN $\mu\text{g L}^{-1}$), lower row) explaining phytoplankton biomass (cubic root transformed), daily primary productivity ($\mu\text{g C L}^{-1} \text{d}^{-1}$), species richness, and Pielou's evenness in four coastal regions (color codes). Red dashed lines show the confidence interval of 95% for the linear regression analyses. The p -value and the adjusted R^2 are shown. * indicates statistically significant linear regression.

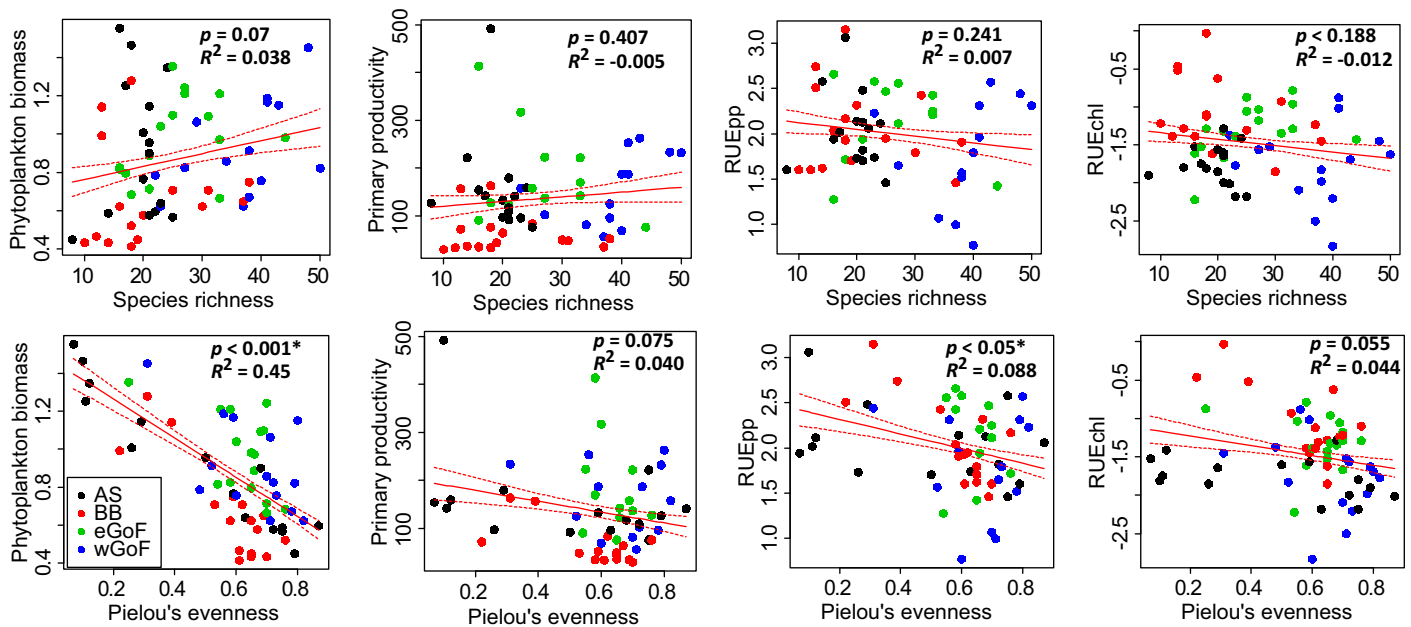


Fig. 3. Species richness (upper row) and Pielou's evenness (lower row) explaining phytoplankton biomass (cubic root transformed), daily primary productivity ($\mu\text{g C L}^{-1} \text{d}^{-1}$), and resource use efficiency calculated based on Chl *a* (RUE_{chl}) or daily primary productivity (RUE_{Epp}), in four coastal regions (color codes). Red dashed lines show the confidence interval of 95% for the linear regression analyses. The p -value and the adjusted R^2 are shown. * indicates statistically significant linear regression.

properties on responses to increased nutrient availability during the experiments (species richness, evenness, and RUE_{chl} vs. short-term productivity response). Total autotrophic biomass (wet weight) was cubic root transformed for the

regression analyses. Data points are color-coded by region in Figs. 2 and 3 for illustrative purpose.

Since our aim was to identify general trends in the diversity-functioning relationships across potential spatial

gradients/scales in environmental conditions and species composition, we complemented the ungrouped univariate regressions with linear mixed-effects models (LMEs), where the hierarchical structure of the data is represented as a random effect of region on regression intercepts. For LME, we used the nlme package for R (lme, Pinheiro and Bates 2000; Pinheiro et al. 2016).

The effects of nutrient imbalance (N, P, or combined N and P limitation, respectively) on parameters describing the structure or functioning of the phytoplankton community were tested after dividing the experiments into the three limitation categories according to two criteria: (1) experimentally verified limitation of primary productivity, *L14C*, and (2) a chemical limitation indicator, DIN : TP (Ptacnik et al. 2010a). Experimentally verified nutrient limitation was quantified by modeling the responses in primary production (¹⁴C-uptake) to nutrient additions, but a similar pattern emerged from corresponding Chl *a* responses (data not shown; see Tamminen and Andersen 2007). For details of limitation analyses, see Andersen et al. (2007) and Tamminen and Andersen (2007). By subtracting the probability of N limitation from the corresponding probability of P limitation, Ptacnik et al. (2010a) calculated a one-dimensional limitation indicator (*L*) which is also utilized in this study. The indicator value ranges from -1 to +1, where the endpoints represent pure N and P limitation, respectively, and the midpoint represents combined or no limitation. Communities were considered N-limited if the *L14C* was ≤ -0.33 , P-limited if the *L14C* was ≥ 0.33 , and NP-limited if the *L14C* was -0.33 to 0.33 . Two communities were non-limited based on primary production responses, and they were excluded from the analyses. For a specific analysis of the Resource Ratio Theory, an “imbalance index” was created by taking the absolute value of *L14C*, yielding values from 0 (perfect N : P balance) to 1 (exclusive N or P limitation).

Since nutrient limitation is usually not verified experimentally from phytoplankton responses in routine monitoring, we analyzed the data also by using the molar ratio of dissolved inorganic nitrogen to total phosphorus (DIN : TP, mol : mol), which is widely available in monitoring data, to divide the communities into corresponding three limitation categories. Communities were considered N-limited if the molar DIN : TP ratio was ≤ 2 , P-limited if the ratio was ≥ 5.1 , and NP-limited if the ratio was 2–5.1 (Ptacnik et al. 2010a).

While both limitation indices (*L14C*, DIN : TP) are expressed as univariate variables, there is no reason to assume monotonous responses over their gradients. Therefore, we applied ANOVA (*aov*, R software) and the Studentized range statistic, Tukey’s “Honest Significant Difference” method (*TukeyHSD*, R software) to detect statistically significant differences between the three limitation categories. The TukeyHSD created a set of confidence intervals on the differences between the means of the levels of the limitation categories with the 95% family-wise probability of coverage.

Multivariate relationships between variables were explored by structural equation modelling (SEM; e.g., Grace 2006) using the lavaan package for R (Rosseel 2012). Regression relationships in the models were chosen to represent the basic SET, RRT, and BEF hypotheses outlined in the introduction with total P (TP) and DIN : TP as exogenous variables and phytoplankton biomass, primary productivity, and either richness or evenness as endogenous variables. All variables used in SEM model fits were standardized to zero mean and unit standard deviation to simplify comparison of path weights and covariances. Model adequacies were inferred from non-significant chi-square goodness-of-fit tests and modification indices < 2 . SEM path structures and covariances were visualized with the SemPlot package (Epskamp 2015) using the “tree” layout, and with exogenous variances hidden since they are unity on standardized variables.

Non-metric Multidimensional Scaling (NMDS) (*metaMDS*, R software package “vegan”) was used to ordinate communities based on Bray–Curtis dissimilarities in their species-level phytoplankton community composition (Oksanen et al. 2015). The communities are positioned in the NMDS ordination space based on their species-specific biomass composition, and the species can likewise be ordinated. In the NMDS, we used cubic root transformation for phytoplankton biomass (wet weight).

Importance of local environmental variables vs. geographic location in explaining the phytoplankton species-level biomass (cubic root transformed) composition was tested with Permutational Multivariate Analysis of Variance Using Distance Matrices (PERMANOVA) (*adonis*, R software package “vegan”) (Oksanen et al. 2015). Local environmental parameters included in the Adonis test were salinity, temperature, TP, TN, DIN : TP ratio, and *L14C*. Geographic location was included in the analysis as relative distance between sampling locations (Fig. 1) using a 4-level numerical variable, 1 representing the most eastern location (eastern Gulf of Finland = 1; western Gulf of Finland = 2; Archipelago Sea = 3; Bay of Bothnia = 5). Distance for the Bay of Bothnia was doubled due to its distance compared to the other locations. As Adonis is sensitive to the sequence of parameters in the model, the selection of the entry order was done by first testing each parameter as the first variable on their turn, and selecting the final order of parameters based on their respective R^2 values. The non-significant parameters in the preliminary ranking round were omitted from the final Adonis test.

Results

Basic information for the four sampling regions is given in Table 1. Each region had some special characters. Bay of Bothnia differed from the other stations in freshwater influence and oligotrophy, as salinity, mean concentrations of DIP, PON, POP, POC, TN, TP, and Chl *a*, phytoplankton biomass, and primary productivity were lowest there, while DIN

Table 2. Results of linear mixed-effects model (LME) with phytoplankton biomass, primary productivity, species richness, and Pielou's evenness as dependent variables, total phosphorus (TP) and total nitrogen (TN) as fixed effects, and region as a random effect on intercepts. Degrees of freedom was 49.

Dependent variable	Fixed effect	Value	SE	t-value	p-value	Adjusted. p-value	Random effect SD	Residual SD
Phytoplankton biomass	TP	0.2155	0.1380	1.5618	0.1248	0.2806	0.6870	0.9141
	TN	-0.2738	0.1827	1.4988	0.1403	0.2806	0.4580	0.9259
Primary productivity	TP	0.5185	0.1206	4.298	0.0001*	0.0008*	0.3787	0.8244
	TN	0.1996	0.1639	1.2176	0.2292	0.3667	0.6560	0.8376
Species richness	TP	-0.0666	0.1890	-0.3522	0.7262	0.7534	0.8664	0.7127
	TN	-0.0602	0.1512	0.3983	0.6921	0.7534	0.5214	0.6730
Pielou's evenness	TP	-0.0622	0.1970	-0.3159	0.7534	0.7534	0.2373	0.9560
	TN	0.3478	0.24656	1.5586	0.1255	0.2806	0.3778	0.8957

* Indicates statistically significant p -value ($p < 0.05$).

and DSi concentrations and DIN : TP ratio were highest. Archipelago Sea was the most saline region. In the western Gulf of Finland, TN and TP concentrations and species richness were highest. RUE_{pp}, and Chl *a*, POC, PON, and POP concentrations were highest in the eastern Gulf of Finland.

Effects of resource availability on phytoplankton community parameters (biomass, productivity, species richness, evenness) were first tested by using total phosphorus (TP; Fig. 2, upper row) and total nitrogen (TN; Fig. 2, lower row) as explanatory variables in linear regression models. Species richness and primary productivity correlated significantly with both TP and TN, and biomass with TP. Still, amount of variance in species richness and primary productivity explained by TP (adj. $R^2 = 0.12$, $R^2 = 0.28$, respectively) and TN (adj. $R^2 = 0.10$, adj. $R^2 = 0.12$, respectively), and variance in biomass explained by TP (adj. $R^2 = 0.05$), was not very high. Correlations of nutrients with evenness were non-significant. In this study, microscopically obtained wet weight biomass is shown in figures, but results using Chl *a* were concordant (results not shown).

Since our data has a clear hierarchical structure, 62 community samples grouped by four regions, we conducted the corresponding analyses also with LME with region as a random effect, to account for spatial clustering of the response variables. In accordance with the results of the linear regression models, LME showed a significant fixed effect of TP on primary productivity ($p < 0.001$) (Table 2). Unlike the results of the linear regression models (Fig. 2), fixed effects of TP on phytoplankton biomass and species richness and of TN on primary productivity and species richness were all non-significant. Most of the explained variance was captured by the random effect of region, although the random effect variance was higher than the residual variance only in the LME model for species richness.

Diversity-productivity relationships were first tested with linear regression models by using species richness (Fig. 3, upper row) and Pielou's evenness measure (Fig. 3, lower row)

as explanatory variables for biomass, primary productivity, and productivity proxies (resource use efficiency based on Chl *a* (RUE_{chl}) or primary productivity (RUE_{pp})). Highest biomass ($p < 0.001$) and RUE_{pp} (< 0.05) co-occurred with lowest evenness levels. Other correlations were weak or negligible (Fig. 3). Evenness showed higher coefficients of determination than species richness, the highest describing the inverse relation between evenness and biomass. The highly significant negative relationship between evenness and biomass was shown also by the fixed effect of the corresponding LME model ($p < 0.001$) (Table 3). Even though standard deviation of the random effect was lower than the residual standard deviation in all LME models, neither biomass nor RUE_{pp} had significant fixed effects on evenness when adjusting p -values for multiple testing by the false discovery method (Benjamini and Hochberg 1995; R function *p.adjust*), as they did based on the linear regression models (Fig. 3).

Since regression analyses indicated that samples clustered based on regions for species richness (see color codes in Fig. 3), we analyzed this also with ANOVA. Species richness was significantly higher in the western Gulf of Finland compared to the three other coastal regions (ANOVA; $df = 58$, $F = 16.4$, $p < 0.0001$).

The effects of community properties on responses to increased nutrient availability during the bioassay experiments (primary productivity increase after 2 d) were tested for species richness, evenness, and RUE_{chl}. Based on both linear regression models and LMEs, neither evenness, species richness, nor RUE_{chl} showed association with the short-term productivity responses (results not shown).

The effects of nutrient imbalance (N, P, or combined N and P limitation, respectively) on phytoplankton communities were tested with ANOVA and TukeyHSD after dividing the communities into three limitation categories (N-limitation, P-limitation, combined N and P limitation) according to two criteria (experimentally verified limitation of primary productivity, L14C; chemical limitation indicator, DIN : TP;

Table 3. Results of linear mixed-effects model (LME) with phytoplankton biomass, primary productivity, and resource use efficiency calculated based on Chl *a* (RUE_{chl}) or daily primary productivity (RUE_{pp}) as dependent variables, and species richness and Pielou's evenness as fixed effects, and region as a random effect on intercepts. Degrees of freedom was 49.

Dependent variable	Fixed effect	Value	SE	t-value	p-value	Adjusted. p-value	Random effect SD	Residual SD
Phytoplankton biomass	Richness	0.1402	0.1549	0.9049	0.3700	0.4933	0.0697	0.9732
	Evenness	-0.8377	0.0939	-8.9214	<0.0001*	<0.0001*	0.0698	0.5671
Primary productivity	Richness	-0.0316	0.2511	-0.1257	0.9005	0.9751	0.3882	0.8690
	Evenness	-0.3018	0.1219	-2.4745	0.0169*	0.0676	0.0355	0.8412
RUE _{chl}	Richness	-0.0054	0.1730	-0.0314	0.9751	0.9751	0.1641	0.8224
	Evenness	-0.4287	0.1902	-2.2534	0.0287*	0.0765	0.2826	0.7511
RUE _{pp}	Richness	-0.1614	0.1369	-1.1790	0.2441	0.3906	<0.0001	<0.0001
	Evenness	-0.4125	0.2174	-1.8971	0.0637	0.1274	0.2833	0.9261

* indicates statistically significant p-value ($p < 0.05$).

Table 4. ANOVA results for parameters which significantly differed between the N-, NP, and P-limited communities. RUE_{chl} = resource use efficiency calculated based on Chl *a*. Parameters that did not show significant variation between the N-, NP-, and P-limited communities were Pielou's evenness, resource use efficiency calculated based on primary productivity (RUE_{pp}), short term primary productivity response, and share of N₂-fixing cyanobacteria of total phytoplankton biomass (results not shown). Dividing of communities into limitation classes based on L14C values: N-limited if the L14C was < -0.33 , P-limited if the L14C was > 0.33 , and NP-limited if the L14C was -0.33 to 0.33 . Dividing of communities into limitation classes based on DIN : TP ratios; N-limited if the molar DIN : TP ratio was < 2 , P-limited if the ratio was > 5.1 , and NP-limited if the ratio was $2-5.1$. Degrees of freedom was 59.

	F	p-value	Tukey HSD 95% family-wise conf. level
<i>Limitation based on L14C</i>			
Phytoplankton biomass	10.14	<0.001*	Higher in N-lim than in P-lim
Daily primary productivity	8.32	<0.001*	Higher in N-lim than in P-lim
Species richness	5.565	<0.01*	Higher in N-lim than in P-lim
RUE _{chl}	3.165	<0.05*	Higher in P-lim than in NP-lim
Biomass of N ₂ -fixing cyanobacteria	3.161	<0.05*	Higher in N-lim than in P-lim
<i>Limitation based on DIN : TP</i>			
Phytoplankton biomass	14.15	<0.001*	Higher in N-lim than in P-lim
Daily primary productivity	11.15	<0.001*	Higher in N-lim than in P-lim
Biomass of N ₂ -fixing cyanobacteria	3.373	0.05	Higher in N-lim than in P-lim

* Indicates statistically significant p-value ($p < 0.05$).

see "Material and methods" section). Both limitation indices showed that total biomass and primary productivity were very significantly ($p < 0.001$) higher in the N-limited than in the P-limited communities (Table 4). The biomass of N₂-fixing cyanobacteria showed the same pattern, albeit with less significance ($p < 0.05$; Table 4). Additional significant differences between limitation groups were detected only with L14C as the limitation indicator (Table 4): the number of species was higher in N-limited communities than in P-limited ones ($p < 0.01$), whereas RUE_{chl} was higher in P-limited communities than in NP-limited ones ($p < 0.05$). Species richness, evenness, RUE_{pp}, short-term primary productivity response, and the share of N₂-fixing cyanobacteria of total biomass failed to show significant relations to

limitation patterns determined with either indicator (data not shown).

When the nutrient limitation categories (N- or P-limitation) were collapsed into a single imbalance index, ranging from perfect balance to exclusive single nutrient limitation, no association with species richness or evenness was observed ($\text{adj.}R^2 < 0.02$, data not shown).

For path analysis, we constructed identical SEMs for richness and evenness (Fig. 4), accounting for exogenous effects of resource availability (here TP) on primary productivity and richness/evenness, and of resource ratios (here DIN : TP) on biomass and richness/evenness. Endogenous paths related richness/evenness to biomass, and biomass to primary productivity. Both models were adequate representations of the

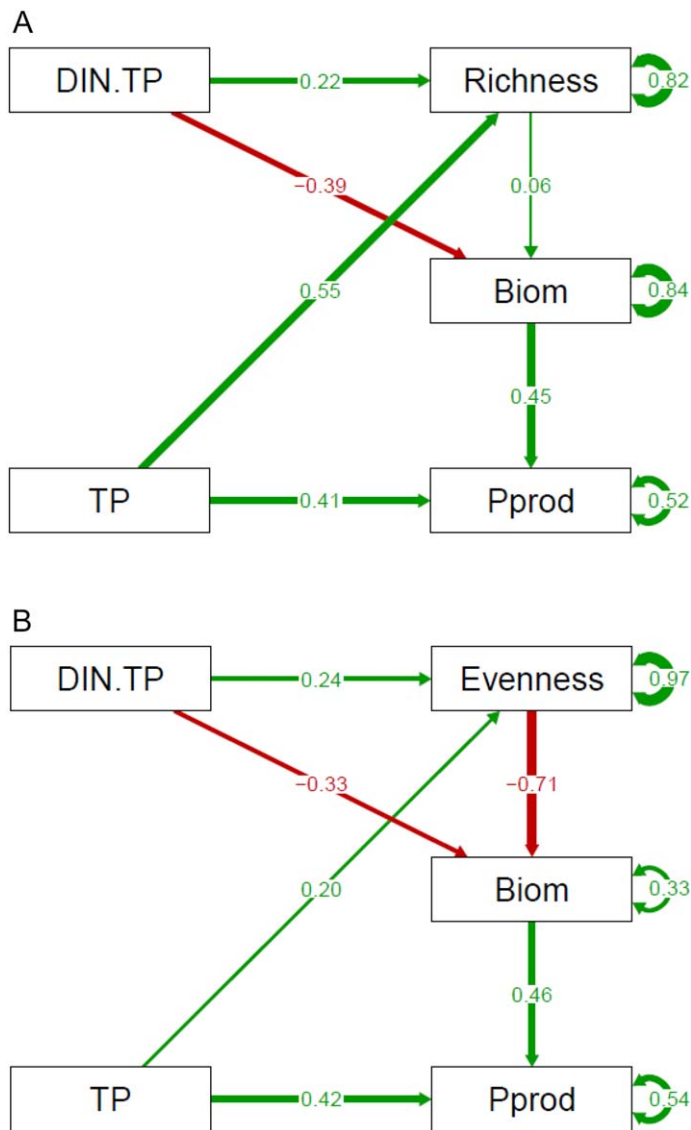


Fig. 4. Path diagrams for structural equation models relating exogenous variables representing resource availability (TP) and resource ratios (DIN : TP) on the left side of diagram to endogenous variables phytoplankton biomass (Biom), primary productivity (Pprod), and either richness (panel A) or evenness (panel B). Positive/negative path coefficients and covariances are colored green/red with line thicknesses weighted by absolute values. Path coefficients are indicated by single-headed arrows while covariances are double-headed. Covariances between exogenous variables (which are not fitted but are taken directly from the sample covariance matrix) are not shown, Path diagrams are drawn with the semPlot package for R (Epskamp 2015).

covariance structures in the sense that they had chi-square goodness-of-fit p -values = 0.528 and 0.664, respectively (both with three degrees of freedom), as well as modification indices < 1.1 and < 1.4.

The two models were very similar in the sense that all paths not involving richness/evenness showed practically the same weights (Fig. 4). The strong positive effects of

nutrient availability and biomass on production are obvious, and the relatively strong negative effects of DIN : TP on biomass indicate higher biomasses under N limitation. The main resource-related difference was the stronger link from TP to richness (“SET effect”) than to evenness. The “BEF effect” (richness to biomass) is quite negligible, while the corresponding path from evenness to biomass was very strongly negative. The latter probably reflects that the reverse path, describing decreasing evenness in bloom situations, is more plausible.

The non-metric multidimensional scaling (NMDS) positioned the communities (Fig. 5, left panel) and the typical species characterizing the communities (Fig. 5, right panel) in the NMDS ordination space based on their species-specific biomass composition. Geographic location was strongly expressed in species composition, as the communities clustered according to their geographical origin, even though samples from the Bothnian Bay and from the eastern Gulf of Finland formed two distinct clusters due to year-to-year differences in the community composition (Fig. 5, left panel).

In the eastern Gulf of Finland, samples were characterized by, e.g., the N_2 -fixing cyanobacterium *Dolichospermum*, non- N_2 -fixing cyanobacteria from order Oscillatoriales, colonial cyanobacteria from order Chroococcales, diatoms from genera *Chaetoceros*, and chlorophyte *Planctonema lauterbornii* (syn. *Binuclearia lauterbornii*) (Fig. 5, right panel). The samples from the eastern Gulf of Finland were separated into two groups based on sampling year: in year 1994, cryptophytes were more abundant and chlorophytes less abundant compared to the other study years. In the western Gulf of Finland, samples were characterized by, e.g., the N_2 -fixing cyanobacteria *Aphanizomenon flos-aquae*, dinoflagellates from genus *Dinophysis*, and diatom *Thalassiosira baltica*. Samples from the Archipelago Sea were characterized especially by the importance of the diatom *Coscinodiscus granii*. The samples from the Bay of Bothnia separated into two groups based on sampling year: most samples taken from the Bay of Bothnia were characterized by diatom *Diatoma tenuis* and chrysophytes *Uroglena* sp. and *Dinobryon divergens*. The other group of the Bay of Bothnia samples consisted of samples from year 1992, and in those samples the species composition was more similar to the species composition in the western Gulf of Finland.

To address the relative importance of conceptually different drivers for phytoplankton community structure, we used permutational multivariate analysis of variance using distance matrices (PERMANOVA) to analyze the contributions of local physical (temperature, salinity) and resource-related (total nutrients, nutrient limitation) variables, together with a regional (shoreline distance) determinant of phytoplankton community composition (Table 5). Salinity explained 14% of the variability in phytoplankton community composition, while resource-related local variables amounted to ca. 8% (TN 4.4%, DIN : TP 3.2%). Temperature and TP effects

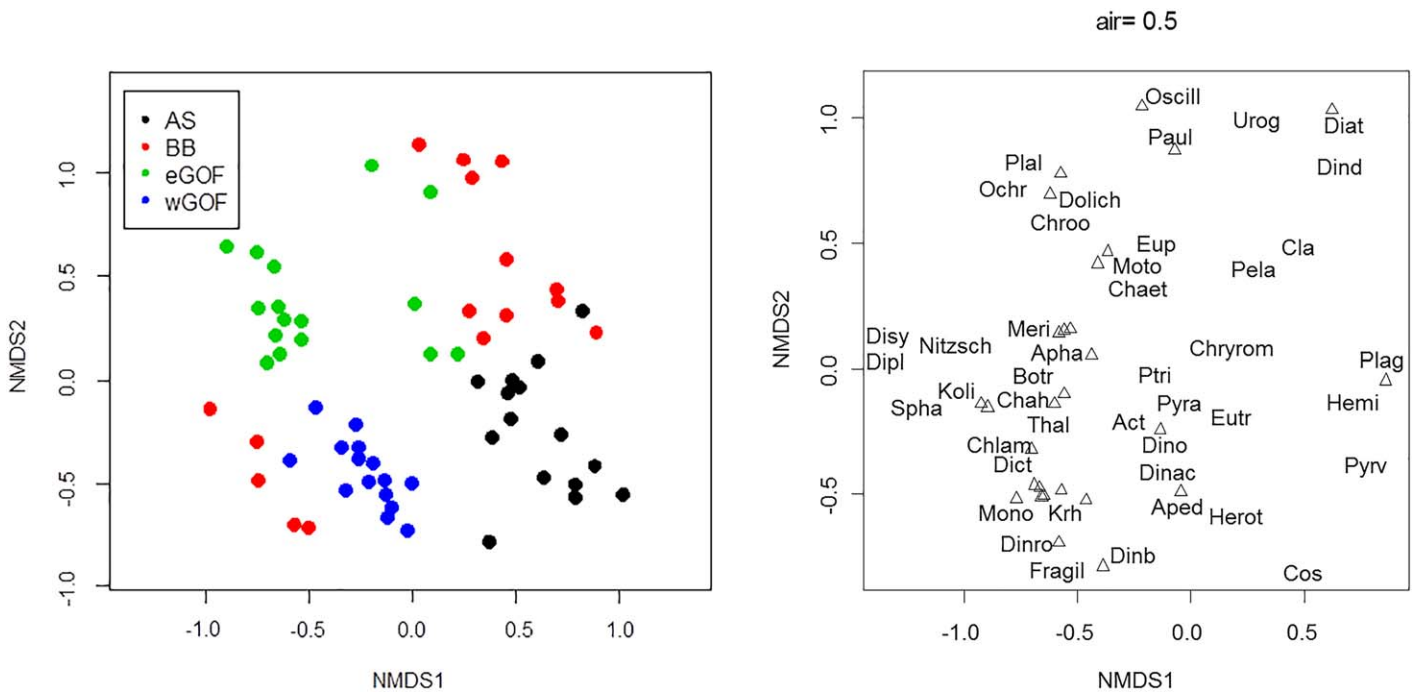


Fig. 5. The non-metric multidimensional scaling (NMDS) was run with species-level phytoplankton biomass results. Left panel shows the ordination of samples originating from four coastal regions (color code). Right panel shows the species ordination, i.e., which species characterized the samples situated in the corresponding ordination in the left panel. Abbreviations in the right panel: Act, *Actinocyclus* spp.; Aped, *Apedinella* spp.; Apha, *Aphanizomenon* spp.; Botr, *Botryococcus* spp.; Chaet, *Chaetoceros* spp.; Chah, *Chaetoceros holsaticus*; Chlam, *Chlamydomonas* spp.; Chroo, Chroococcales; Chryrom, *Chrysochromulina sensu lato*; Cla, *Closterium acutum*; Cos, *Coscinodiscus granii*; Diat, *Diatoma tenuis*; Dict, *Dictyosphaerium* spp.; Dinac, *Dinophysis acuminata*; Dinb, *Dinobryon* spp.; Dind, *Dinobryon divergens*; Dinro, *Dinophysis rotundata*; Dino, Dinophyceae; Dipl, *Diploneis* spp.; Disy, *Didymocystis* spp.; Dolich, *Dolichospermum* spp.; Eup, Eupodiscales; Eutr, *Eutreptiella* spp.; Fragil, *Fragilaria* spp.; Hemi, *Hemiselmis virescens*; Herot, *Heterocapsa rotundata*; Koli, *Koliella* spp.; Krh, *Kirchneriella* spp.; Meri, *Merismopedia warmingiana*; Mono, *Monoraphidium* spp.; Moto, *Monoraphidium contortum*; Nitzsch, *Nitzschia* spp.; Ochr, Ochromonadales; Oscill, Oscillatoriales; Paul, *Pauliella taeniata*; Pela, *Pseudopedinella elastica*; Plag, *Plagioselmis prolonga*; Plal, *Planctonema lauterbornii*; Ptri, *Pseudopedinella tricostata*; Pyra, *Pyramimonas* spp.; Pyrv, *Pyramimonas virginica*; Spha, *Sphaerocystis* spp.; Thal, *Thalassiosira baltica*; Urog, *Uroglena* spp.

Table 5. Results of a permutational multivariate analysis of variance using distance matrices (PERMANOVA) for those parameters which significantly explained species-level phytoplankton composition in samples originating from four coastal regions. Parameters which did not explain phytoplankton species-level composition significantly were total phosphorus, experimentally verified limitation indicator L14C, and temperature. Degrees of freedom was 59.

	Sums of sqs	Mean sqs	F. Model	R ²	p-value
Salinity	2.133	2.13311	13.2557	0.14444	0.001*
Distance	1.912	1.91189	10.9574	0.12946	0.001*
TN	0.654	0.65358	3.7458	0.04426	0.001*
DIN : TP	0.473	0.47339	2.7131	0.03205	0.007*
Residuals	9.5966	0.17448		0.64980	
Total	14.7686			1.00000	

TN, total nitrogen; DIN, dissolved inorganic nitrogen; TP, total phosphorus.

* Indicates statistically significant p-value ($p < 0.05$).

were not significant. Shoreline distance between the sampling stations explained 13% of the variability in the phytoplankton community composition (Table 5).

Discussion

The relationships between community properties (diversity, biomass, productivity), and their dependence on resource availability and imbalance have been studied from different perspectives, including the Species-Energy Theory (SET, Wright 1983), the Resource-Ratio Theory (RRT, Tilman 1982), and the biodiversity ecosystem functioning (BEF) paradigm (Naeem et al. 1994; Gross and Cardinale 2007; Hillebrand and Matthiessen 2009). It is conceivable that all these approaches describe necessary, but not sufficient, dimensions to explain productivity–diversity relationships in nature. Cardinale et al. (2009) thus suggested that observed diversity–productivity relationships are shaped by the interplay of four distinct, non-interchangeable variables: quantity of limiting resources, resource imbalance, total biomass, and

species richness, and tested this approach with observational, large-scale phytoplankton data. They emphasized that experimentally controlled studies, with manipulations of resource quantities, their ratios, and species richness would be desirable to examine the joint impacts of the factors on community biomass and productivity.

Our data set allows us to analyze interrelations of these non-interchangeable variables in an experimental setup, to assess their significance in natural species-rich phytoplankton communities. Most importantly, we can utilize direct carbon fixation measurements for community productivity, together with experimentally verified limitation patterns of natural communities, instead of the rather remote proxies for productivity and resource imbalance available in observational studies (e.g., Ptacnik et al. 2008; Cardinale et al. 2009). Our study did not involve manipulations of species richness, but it sampled local communities over a wide gradient of total nutrient resources (P, N), their ratios (imbalance), biomass, and community composition. Besides contributing to analysis of local interplay between resources and community properties, we therefore addressed also the role of regional connectivity for local community composition.

Resource availability and imbalance in shaping community properties

Our results showed that biomass and productivity were the highest when more resources (especially TP) were available (Fig. 2; Table 2). The weaker correlation with TN is likely to reflect the large and spatially variable share of refractory dissolved organic N in the total N pool in the Baltic Sea (Hoikkala et al. 2012). Resource availability had an overall effect on species richness (Figs. 2, 4A), along the lines of the Species Energy Theory (SET; Wright 1983), although regionally clustered variation was also evident (Table 2). Evenness was not explained by resource availability. Experimental studies using artificial communities have indicated evenness to decrease with increasing nutrient supply (Gamfeldt and Hillebrand 2011; Hillebrand and Lehmpfuhl 2011), but our results suggest that evenness of natural species-rich communities is not regulated by total nutrient availability in the temporally and spatially highly dynamic environment of the phytoplankton. This conclusion is further supported by evenness not varying much between the coastal regions, in contrast to species richness, biomass, and productivity (Table 1), despite the consistent regional differences in species-level community composition (Fig. 5).

The Resource Ratio Theory (RRT, Tilman 1982) prediction that species richness would be the highest when more than one resource is limiting, was not supported. N-limited and P-limited communities differed in their species richness, but balanced nutrient supply (combined P and N limitation) did not support higher species richness or evenness (Table 4). The imbalance index, which does not take into account the currency of limitation, was not related to

species richness or evenness. Gamfeldt and Hillebrand (2011) found the lowest evenness in artificially assembled communities when nutrient supply was the most imbalanced. This discrepancy further indicates that diversity dynamics of natural, species-rich communities are more complex than experimentation with communities that are artificially assembled from the material available in culture collections. Still, nutrient imbalance explained both biomass and production (Table 4), as did resource availability (Figs. 2, 4; Table 2). Biomass and total daily ^{14}C primary productivity very clearly peaked under N limitation, and were lowest under P limitation, whereas the productivity proxies (RUE_{chl} , RUE_{PP}) showed weak or no relationships with nutrient imbalance (Table 4).

Path analyses (SEM, Fig. 4) basically supported the strong negative effect of biomass on evenness, but also a smaller positive effect of productivity. The biomass effect is probably linked to bloom monopolization and dominance. The positive effects of both TP and DIN : TP indicates that richness increases with both amount of P and degree of P-limitation. The BEF-type linkage from richness to biomass was very weak and insignificant, while the corresponding path from evenness to biomass had negative sign indicating reverse causality.

Nutrient imbalance (in the form of N limitation) explained only weakly the biomass of N_2 -fixing cyanobacteria, but the association was weaker than between nutrient imbalance and total biomass, and consequently the share of N_2 -fixing cyanobacteria of total phytoplankton biomass did not correlate at all with nutrient imbalance (Table 4). This contrasts the assumption that competitive advantage of diazotrophs is realized in natural aquatic systems as a simple function of N limitation, and suggests that filamentous N_2 -fixing cyanobacteria are primarily regulated by other factors than ambient nutrient imbalance. Common explanations include resistance to grazing, which allows these relatively slow-growing species (Lehtimäki et al. 1997) to reach bloom densities, as well as tolerance to low light conditions (Scheffer et al. 1997). The slow build-up of bloom biomass levels obviously depends on longer-term nutrient dynamics, including in situ (Tamminen 1989) vs. external (Kononen et al. 1996; Vahtera et al. 2007) phosphorus supplies, the latter driven by local hydrographical conditions like upwelling and vertical mixing in frontal areas (see Olli et al. 2015). The reason why the share of N_2 -fixing cyanobacteria did not correlate with nutrient imbalance could also be connected to N_2 -fixation supporting not only the cyanobacteria themselves, but also other species since cyanobacteria leak significant amounts of nitrogen (Ohlendieck et al. 2007; Ploug et al. 2010, 2011). Equating ambient N limitation and occurrence of N_2 -fixing cyanobacteria blooms (e.g., Schindler 1977; Tyrrell 1999) appears to be based on a juxtaposition of conclusions from incompatible spatial and temporal scales.

The inverse relations between evenness and community performance

Our results showed that evenness had stronger relationships to functional community properties (biomass, productivity) than species richness (Figs. 3, 4). While species richness did not explain biomass, primary productivity or RUE in our data set, there was a highly significant inverse relation between evenness and biomass. When evenness was the lowest, both phytoplankton biomass and RUE_{PP} were the highest. This result supports findings presented recently by Filstrup et al. (2014) and Hodapp et al. (2015), even though it appears contradictory to several earlier results: terrestrial studies showing that high diversity, measured usually with species richness, supports higher productivity (e.g., a global meta-analysis by Zhang et al. 2012); to experimental plankton community studies showing that high species richness supports higher productivity (Striebel et al. 2009; Stockenreiter et al. 2014); and to observational lake and coastal plankton studies showing that genus richness supports higher RUE (Ptacnik et al. 2008). In the latter, highest RUE values were seen at low to moderate richness, although the mean peaked at high diversity. Negative or non-linear relationships between evenness and biomass have been documented with terrestrial plant studies (Mulder et al. 2004).

The connections of evenness with functional community properties might be best understood in context of the natural regime of planktonic ecosystems. The natural communities in our study did not represent actual bloom situations, as seen in prevailing biomass or Chl *a* levels (Table 1), but the inverse patterns of evenness with biomass or productivity were as could be expected during algal bloom events, when one or few highly competitive species temporarily succeed in monopolizing available resources, leading to low evenness. Robustness of the negative evenness-productivity relation is supported by the recurring tendency for regions to cluster when relating nutrient resources to biomasses or productivity (Fig. 2; Table 2) both in regression analyses, LME models and NMDS ordination. While communities from distinct coastal regions differed clearly in their species composition (Fig. 5), the negative relationship between evenness and biomass was consistent within separate regions as well (Fig. 3; Table 3).

It appears that in planktonic, species-rich natural communities, characterized by high overall variability (the physical framework, resource availability, and species turnover), high productivity and RUE are supported by recurrently shifting, narrow time windows suitable for events of temporary resource monopolization by few opportunistic species. The inherently fluctuating, or “bloom-oriented” mode of life of natural planktonic communities might well be the key explanation for the apparent contrasts in their diversity-productivity results to those obtained both in terrestrial systems, and with artificially assembled aquatic communities in controlled, stable environments. These planktonic “event

dynamics” are smoothed out, or entirely missed, in sparsely sampled observational data sets. Richness and evenness may also reflect community responses to the environment from different time scales. Evenness particularly expresses the very recent success of few potentially successful species under current environmental conditions, while species richness may rather be an echo from the past, integrating spatial background (horizontal advection, connectivity etc.) and a longer temporal signal (remnant populations from formerly successful taxa).

Resources and other local determinants of community composition vs. metacommunities

Species turnover, refurbishing species richness of local planktonic communities after episodic phases of resource monopolization by most successful competitors, requires supply of new taxa from neighboring areas, i.e., dispersal from metacommunities (Vyverman et al. 2007; Ptacnik et al. 2010b). In the planktonic realm, and especially in the apparent continuum of marine ecosystems, species transfer appears particularly effective due to the free-floating life mode of the communities.

NMDS analysis showed that coastal phytoplankton community composition (species-level biomass composition) differed clearly between the four regions (Fig. 5). It is conceivable that the diluted predictive power of the univariate analyses, which became evident when grouping by region in the LME models, might be a reflection of stoichiometric and other trait differences between regional communities. Total nitrogen concentration and nutrient limitation pattern (DIN : TP ratio) were the only local resource-related variables affecting significantly community composition in the Adonis results (explaining 4.4% and 3.2% of the variability, respectively). Distance between the regions, indicating the role of metacommunities in the assembly of local phytoplankton communities, explained 13% of the variability, i.e., a higher fraction than that of local resources together. In addition to distance, also salinity (the largest single explanatory variable at 14%) could mainly indicate regionality, instead of representing a truly local variable acting on the physiological level of organisms, since the regions showed quite constant salinities at the local level, while salinity differs systematically among stations (Table 1).

Species richness was significantly higher in the western Gulf of Finland compared to the other regions. Since this location is most exposed to water exchange with several adjacent areas, including the main Baltic Sea basin (Gasiūnaitė et al. 2005; Vahtera et al. 2005), species turnover through increased connectivity is also potentially higher in this area compared to the other regions. Our analyses therefore support the role of planktonic metacommunities in shaping local community composition and ensuring species turnover, responsible for maintaining local diversity (Ptacnik et al. 2010c).

Species that were able to monopolize the resources, forming high biomass and generating low evenness, differed spatially between the regions, and temporally during the summer season. However, the diversity–evenness–productivity relationships of the communities appeared comparable. For the functioning of the food web, or for environmental consequences, species identities do matter. The prevailing species composition causes cascading effects within the food web, as species forming high biomass can, e.g., be harmful or harmless, or represent high or low-quality food for grazers (Olli et al. 1996; Kozłowski-Suzuki et al. 2003; Uronen et al. 2007; Sopanen et al. 2009). Thus, species-level information of the community would be essential for also for applied functional assessments (e.g., Marine Strategy Framework Directive of the European Union (MSFD; 2008/56/EC); Rogers et al. 2010), since it cannot be replaced by only summative measures like total biomass, productivity proxies, or distributional indices of the community. However, species-level inventories should be augmented with species-specific trait information.

Diversity and function—species as carriers of traits

Diversity has been shown also to increase the stability of plankton communities (Ptacnik et al. 2008). We tested if a more diverse community was able to use experimentally added nutrients more efficiently, as a resource-related disturbance. Species richness or evenness did not explain the short-term productivity responses, in contrast to expectations. Maybe more surprisingly, neither did prevailing RUE predict the productivity increases due to additional resources. It appears that species-level traits (nutrient affinities, maximum growth rates) that are masked by community-level properties or proxies, are essential for in situ responses to sudden changes in resource availability.

Our results showing the consistent inverse relationship between evenness and phytoplankton biomass or RUE, despite the varying species composition, suggest that these relationships are driven by community dominance patterns, as opposed to being driven by any particular taxa. This finding complements the assumption that identities and characteristics of dominating species have an impact on BEF relationships (Hillebrand et al. 2008), and studies showing that a few dominant species may be behind the inverse relationship between evenness and RUE (Filstrup et al. 2014; Hodapp et al. 2015).

Phytoplankton species identity information alone has limited explanatory power for the prediction of winner species in planktonic windows of opportunity, and for the consequences of events of resource monopolization, in the absence of quantitative information on their ecological traits. These traits include description of competitive strength within a set of environmental conditions (nutrient affinities, maximum growth rates, light harvesting machinery, resistance to grazing, allelopathic potential etc.), but also the “consumer angle,” i.e., stoichiometric composition

and other nutritional value for grazers, toxicity, palatability etc. (Litchman and Klausmeier 2008).

Quantitative modelling of functional connections between resources, species richness, evenness, and production, as well as food web transfer properties like edibility and toxicity, would therefore require descriptions of species as carriers of sets of traits. In planktonic ecosystems, accumulation of such information is still in a rudimentary phase, but during the ongoing global and local changes in all aquatic environments, progress in trait-based community ecology would be essential to deepen our understanding of the multifaceted relationships between productivity and diversity.

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Conflict of Interest

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