

# Functional trait variation and nitrogen use efficiency in temperate coastal phytoplankton

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**ABSTRACT:** This study explored the relevance of the use of a trait-based approach to explain seasonal phytoplankton succession and the underlying mechanisms that structure phytoplankton assemblages and promote nitrogen use efficiency (NUE) in the temperate coastal waters of the eastern English Channel (EEC). A 3 yr time series of phytoplankton abundance assessed by flow cytometry was combined with a selection of traits related to resource utilization (light and inorganic nutrients), grazing resistance, and maximum growth, mainly sourced from the literature. Our results showed that the identified functional groups (FGs) ranged deterministically along a temporal gradient of light and nutrient availability, according to several fundamental trade-offs. These trade-offs, which reflected different ecological strategies, were distributed along 1 axis of niche differentiation. This favoured different FGs through time, and consequently allowed them to partition those available resources. The observed functional divergence of the phytoplankton community deviated from that of the expected, random one. It showed that as light increased, the phytoplankton community assembly was mainly driven by competition and grazing pressure, which eventually favoured those FGs that were fastest growing and less vulnerable to predation. Structural equation modeling evidenced that a phytoplankton community, functionally dominated by FGs that were relatively large, fast-growing, and well-adapted to higher light, promoted NUE. Together, these results confirmed the potential use of laboratory-measured functional traits to explore environment–trait linkages in phytoplankton. They also showed the possibility of further extending this approach to identify the mechanisms driving phytoplankton community assembly, structure, and related ecosystem processes.

**KEY WORDS:** Phytoplankton community · Flow cytometry · Functional traits · Nitrogen use efficiency

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## INTRODUCTION

The structure of marine phytoplankton communities plays a critical role in major ecosystem processes such as the regulation of biogeochemical cycles and climate (Falkowski et al. 2004, Arrigo 2005). However, our understanding of the drivers behind phytoplankton community structure, and consequently how this influences ecosystem processes, is still limited. Because functional traits are measurable characteristics of an organism reflecting what it is doing and how it interacts with other organisms and its

environment (Violle et al. 2007), they have become central to filling these knowledge gaps (McGill et al. 2006, Litchman & Klausmeier 2008, Hillebrand & Matthiessen 2009). Indeed, unlike traditional measures of taxonomic diversity, the metrics derived from functional traits (which have the general term of 'functional diversity'; Hooper et al. 2005, Mouchet et al. 2010) offer a potential mechanistic link between diversity and the environment, or ecological processes (McGill et al. 2006, Cadotte et al. 2011). Because functional diversity takes into account the ecological difference between species, it frequently

does not follow the taxonomic diversity (Mayfield et al. 2010, Villéger et al. 2010), and is increasingly used as a tool for understanding ecosystem functioning (Cadotte et al. 2011). In a general way, trait-based approaches provide an opportunity to make ecological generalities and predictions across ecosystems composed of different species (McGill et al. 2006).

Three non-random processes are considered to play a major role in ecological community organization, and consequently, their functional structure: trade-offs among performance-related traits; environmental filtering; and biotic interactions. Trait trade-offs reflect the diversity of ecological strategies and generally explain the coexistence, as well as the variation, in community structure across environmental gradients (Kneitel & Chase 2004). Environmental filtering is generally expected to favour species with the best-suited trait values for survival, and only under certain environmental conditions (Weiher et al. 1998, Grime 2006). This process leads to trait convergence, and as a consequence, to both a decrease in functional diversity and a shift in the functional dominance of a community along environmental gradients (Cornwell & Ackerly 2009). Conversely, competition is generally expected to increase functional diversity by leading to niche differentiation (e.g. Cornwell & Ackerly 2009), according to the principle of limiting similarity (MacArthur & Levins 1967). However, competitive interactions may also lead to convergence in traits if they are related to high competitive ability (Mayfield & Levine 2010). Likewise, high grazing pressure may lead to both trait convergence or divergence, depending on whether the grazers are generalists or specialists (Cavender-Bares et al. 2009). Accordingly, generalist consumers are expected to cluster prey communities toward species having defense traits, while specialist ones are expected to increase the functional diversity of the prey community by exerting strong pressure on their preferred species (Cavender-Bares et al. 2009).

Despite their great importance in structuring phytoplankton communities (Litchman & Klausmeier 2008, Litchman et al. 2010, 2012), these deterministic processes have rarely been explored under natural conditions (Pomati et al. 2013). The explanation for this lies in the lack of information about key functional traits for most phytoplankton species (Litchman & Klausmeier 2008). Yet characterizing how functional traits change over time in phytoplankton communities is important in understanding how these communities function. Two recent studies, however, have shown the possibility of using experimentally-measured trait values at a coarse taxonomical level to

make certain predictions. The first dealt with the functional response to environmental variations of phytoplankton functional groups in US lakes (Schwaderer et al. 2011). The second concerned ecological processes such as gross primary production, under a large range of environmental conditions (Zwart et al. 2015). In fact, although these laboratory-measured physiological traits display large variations within most phytoplankton functional groups (FGs), the majority of them differ significantly between these groups, highlighting different ecological strategies (Litchman et al. 2007, Litchman & Klausmeier 2008, Schwaderer et al. 2011, Edwards et al. 2015). Because of the existence of greater variation between groups than within, applying traits at the 'level' of FGs appears reasonable. Phytoplankton resource use efficiency (RUE) is a functional trait of phytoplankton communities that directly influences ecosystem processes. Several studies have documented a relationship between RUE and taxonomic richness (e.g. Ptacnik et al. 2008, Olli et al. 2014), suggesting that niche complementarities may play an important role in these systems. Hodapp et al. (2015) recently reported a negative relationship between RUE and taxonomic evenness, suggesting, however, that in some marine systems, some potential keystone species, rather than a set of species, may promote RUE by contributing a greater proportion to RUE than other species.

The eastern English Channel (EEC) is a productive meso-eutrophic marine ecosystem, characterized by repeating patterns in microphytoplankton succession. Based on microscopic observations, it has been established that virtually every spring an intense bloom of the fast-growing prymnesiophyte *Phaeocystis globosa* is preceded and followed by communities of fast-growing diatoms (e.g. Breton et al. 2000, Grattepanche et al. 2011a,b, Hernández-Fariñas et al. 2014). Additional cytometric surveys have shown that *Synechococcus* and picoeukaryotes were abundant during both winter and summer, and that cryptophytes and coccolithophores developed blooms mainly before the wax of the successive diatom and *Phaeocystis* blooms (Bonato et al. 2016). The *Phaeocystis* proliferation seems to depend on an excess of nitrates and a silicate limitation (Egge & Asknes 1992, Breton et al. 2006), and sufficient underwater light intensity to achieve high growth rates (Jahnke 1989, Peperzak et al. 1998). Moreover, in contrast to solitary cells, large *Phaeocystis* colonies are resistant to grazing and viral infections (Schoemann et al. 2005).

In the present study, we explored whether functional traits can explain the seasonal phytoplankton

succession observed in the EEC, and how the functional structure affects nitrogen use efficiency (NUE). We hypothesized that both an increase in light and nutrient limitation was related to the diatom and *Phaeocystis* blooms, enhancing the functional dominance of these groups and correspondingly decreasing the functional diversity of the phytoplankton community. Consequently, greater NUE should be promoted during blooms of these 2 FGs.

We defined and enumerated the phytoplankton FGs using flow cytometry, and the abundances were converted into biomass. Then we combined biomass data with trait phytoplankton values from the literature. We assumed that trait variations within FGs were far lower than between and did not change over time, and that the trait values we compiled from the literature appropriately represented the physiology of the FGs occurring in the studied area. In order to investigate the drivers (environmental variables, phytoplankton biomass, functional diversity, and functional dominance) of NUE, structural equation modelling (SEM) was performed.

## MATERIALS AND METHODS

### Sampling and flow cytometry analysis

Subsurface seawater (−2 m) samples were collected using an 8 l Niskin bottle, onboard the ‘Sepia II’ Station Research Vessel (CNRS INSU) on 47 occasions (from May 2011 to June 2014), at Coastal Station R1 (50° 48′ N, 1° 34′ E) in the EEC. Analyses were performed within 2 h of sampling, with a pulse-shape recording flow cytometer (FCM) (CytoSense; Cyto-Buoy<sup>®</sup>) specifically designed to detect cells from 1 to 800  $\mu\text{m}$  in width (Dubelaar & Jonker 2000). Seven different phytoplankton populations presenting distinct cytometric signatures were identified and enumerated: *Synechococcus* spp., picoeukaryotes, cryptophytes, coccolithophorids, diatoms, and the diploid and haploid morphotypes of the prymnesiophyte *Phaeocystis globosa*—the FGs of this study (for further details, see Bonato et al. 2015, 2016). As a note, in addition to diatom species (see Table S1 in Supplement at [www.int-res.com/articles/suppl/m563p035\\_supp.pdf](http://www.int-res.com/articles/suppl/m563p035_supp.pdf) for a list of the diatoms species identified by inverted microscopic counts, in parallel to flow cytometry analysis), *P. globosa* blooms have been well documented in the area via microscopic and molecular surveys (e.g. Breton et al. 2000, Grattepanche et al. 2011a, Christaki et al. 2014). For smaller phytoplankton, which cannot be accurately

identified by microscopy, several molecular surveys in the area (e.g. Genitsaris et al. 2015, 2016) have previously found that picoeukaryotes were dominated by Prasinophyceae (*Micromonas* spp., *Bathycoccus* spp.), among cryptophytes by *Teleaulax* spp., and coccolithophorids by *Emiliana huxleyii*. Note that *Micromonas pusilla* was also found to dominate among Prasinophyceae in coastal waters of the western English Channel (Not et al. 2004).

The biomass of each FG ( $\mu\text{g C l}^{-1}$ ) was estimated from the conversion of flow cytometry counts and/or biovolume of each of FG using appropriate carbon conversion factors. For coccolithophorids, cryptophytes, and diatoms, the equations of Menden-Deuer & Lessard (2000) were used. For the diploid and haploid morphotypes of *P. globosa*, carbon conversion factors of 14 and 8  $\text{pg C } \mu\text{m}^{-3}$ , respectively, were used (Schoemann et al. 2005). Finally, for *Synechococcus* spp. and picoeukaryotes, carbon conversion factors of 154 and 1319  $\text{fg cell}^{-1}$ , respectively, were used (Buitenhuis et al. 2012).

### Phytoplankton traits

The values and functional meaning of the 10 morphological and physiological traits used in this study are presented in Table 1. They include (1) biovolume ( $V$ ;  $\mu\text{m}^3$ ) as the ‘master trait’ for resource acquisition, reproduction, and predator avoidance (Litchman & Klausmeier 2008); (2) growth dependence on both temperature (maximum growth rate at 12.5°C [ $\mu_{\text{max}(T)12.5^\circ\text{C}}$ ;  $\text{d}^{-1}$ ]; Edwards et al. 2013) and light (maximum growth rate under saturating irradiance [ $\mu_{\text{maxLight}}$ ];  $\text{d}^{-1}$ ]; Schwaderer et al. 2011, Edwards et al. 2015); (3) traits related to the cellular requirement for nitrogen and phosphorus (C/N and C/P cell content ratios [C/N and C/P ratios; mol:mol]), which are also proxies of the palatability as food for predators (Sterner & Hessen 1994); and (4) the capacity of shade tolerance (light-saturation intensity for growth [ $E_{ki}$ ;  $\mu\text{E m}^{-2} \text{s}^{-1}$ ]). These traits relate to competitive ability under light and/or nutrient limitation as the surface-area-to-cell volume ratio (S/V ratio), and growth efficiency under low irradiance, i.e. the initial slope of the growth–irradiance curve ( $\alpha_{\mu}$ ;  $\mu\text{E}^{-1} \text{m}^2 \text{s}^{-1}$ ]; Schwaderer et al. 2011, Edwards et al. 2015). The capacity to harvest low nitrogen concentrations i.e. the nitrate scaled affinity ( $N_{\text{saff}}$ ;  $1 \mu\text{mol}^{-1} \text{d}^{-1}$ ; Edwards et al. 2012, 2013), and the presence of flagella as a trait related to predator avoidance were also considered.

Trait values, such as light and nutrient use, can differ between different species (Litchman et al. 2007,

Table 1. Trait values used in this study (see footnotes for their functional meanings). *V*: biovolume (a master trait in regards to resource acquisition, reproduction, and predation; Litchman & Klausmeier 2008); *S:V*: surface to cell volume ratio; mobility: presence/absence of flagella; C:N and C:P ratios: cellular carbon to nitrogen and phosphorous ratios;  $\mu_{\max}(T)$ : maximum growth rate at 12.5°C;  $\mu_{\max}(\text{Light})$ : maximum growth rate under saturating irradiance;  $\alpha_0$ : the initial slope of the growth-irradiance curve;  $E_k$ : saturating irradiance for growth;  $N_{\text{safr}}$ : nitrate scaled affinity. Values are averages  $\pm$  SD, except for *V* and *S:V*, which are medians. n.u.: no units

Phytoplankton group	<i>V</i> ( $\mu\text{m}^3$ )	<i>S:V</i> ratio <sup>a</sup> ( $\mu\text{m}^{-1}$ )	Mobility <sup>a,c</sup> (n.u.)	C:N ratio <sup>b,f</sup> (mol:mol)	C:P ratio <sup>b,f</sup> (mol:mol)	$\mu_{\max}(T)$ <sup>c</sup> ( $\text{d}^{-1}$ )	$\mu_{\max}(\text{Light})$ <sup>c</sup> ( $\text{d}^{-1}$ )	$\alpha_0$ <sup>a</sup> ( $\mu\text{E}^{-1} \text{m}^{-2} \text{s d}^{-1}$ )	$E_k^d$ ( $\mu\text{E}^{-1} \text{m}^{-2} \text{s}^{-1}$ )	$N_{\text{safr}}^a$ (Log l $\mu\text{mol}^{-1} \text{d}^{-1}$ )
Coccolithophores	22	2.52	1	8.3 $\pm$ 0.7	124 $\pm$ 46	1.11	1.33 $\pm$ 0.58	0.05 $\pm$ 0.03	35 $\pm$ 22	2.05
Cryptophytes	68	1.79	1	5.7 $\pm$ 0.6	155 $\pm$ 125	no data	1.13 $\pm$ 0.47	0.06 $\pm$ 0.04	33 $\pm$ 34	1.75
Diatoms	10613	1.04	0	7.1 $\pm$ 1.6	73 $\pm$ 25	1.78 (1.58–1.99)	2.06 $\pm$ 0.82	0.04 $\pm$ 0.03	79 $\pm$ 65	0.36
<i>Phaeocystis</i> diploid	997922	0.09	0	7.1 $\pm$ 0.4	80	1.2 $\pm$ 0.1	2.26 $\pm$ 0.29	0.04 $\pm$ 0.004	53 $\pm$ 10	–0.88
<i>Phaeocystis</i> haploid	63	1.8	1	7.6	157 $\pm$ 44	1.2 $\pm$ 0.1	2.55	0.03	109	1.77
Picoeukaryotes	3	4.1	0.5 <sup>g</sup>	5.7 $\pm$ 1.3	32	0.55 $\pm$ 0.23	1.04 $\pm$ 0.32	0.05 $\pm$ 0.01	23 $\pm$ 4	2.62
<i>Synechococcus</i> spp.	0.36	6.74	0	5.7 $\pm$ 0.5	125 $\pm$ 65	0.35 $\pm$ 0.20	1.6	0.07	33	3.18

<sup>a</sup>Competitive ability under low light and/or nutrient levels; <sup>b</sup>Cellular requirements in nitrogen and phosphorous; <sup>c</sup>Growth dependence on temperature or light;

<sup>d</sup>Photoadaptation capacity; <sup>e</sup>Predator avoidance; <sup>f</sup>Palatability as food for predators; <sup>g</sup>Includes both non-motile and motile species

Litchman & Klausmeier 2008) and between ecotypes of the same species, addressing differences in their light and nutrient utilization (e.g. Ahlgren & Rocap 2012). Consequently, we selected from the literature those species/strains characterizing temperate and coastal systems that were expecting to show values as close as possible to the phytoplankton of the EEC. Also, the *Phaeocystis* free-living cells, identified by flow cytometry as diploid cells, are known to have a colonial origin (Rousseau et al. 2007). Therefore, the choice of trait values for *Phaeocystis* diploid cells corresponded to those defined for *Phaeocystis* colonies. Whenever several sources of laboratory data were available for the same species, they were averaged to obtain the mean of each trait. Subsequently, all data per trait belonging to a single phytoplankton FG were averaged. Accordingly, 75 publications and reports including a total of 44 diatom, 2 haptophyte, 6 coccolithophore, 8 cryptophyte, and 11 picoeukaryote species were examined (see Supplement 2 detailing methodology for traits and references). Given the absence of  $\mu_{\max}(T)$  for marine cryptophytes in the literature, this value was lacking in our traits dataset. Finally, it is important to note that for the traits selected in this study, the standard deviations calculated for each FG were mostly lower than the mean (see Table 1).

## Environmental variables

Sea surface temperature (*T*; °C) and salinity (*S*) were measured at 2 m depth with a CTD Seabird profiler (SBE 19) equipped with a photosynthetically active radiation (PAR) sensor (QSP 2300; Biospherical Instruments). The diffuse attenuation coefficient for downwelling irradiance (*k*,  $\text{m}^{-1}$ ) was assessed from instantaneous vertical CTD profiles. The average subsurface daily light level (PAR,  $\text{E m}^{-2} \text{d}^{-1}$ ) experienced by phytoplankton for a 6 d period before the cruises was estimated using the formula of Riley (1957). The daily incident light was estimated from global solar radiation (GSR;  $\text{W m}^{-2}$ ) measured continuously with a time step of 5 min with a solar radiation sensor (Vantage Pro; Davis) mounted on the roof of our laboratory bordering the seashore. This sensor is located about 5 km from the sampling station. GSR was converted into PAR by assuming PAR to be 50 % of GSR and by considering  $1 \text{ W m}^{-2} = 0.36 \text{ E m}^{-2} \text{d}^{-1}$  (Morel & Smith 1974). Wind speed ( $\text{m s}^{-1}$ ) was obtained from the French Institute 'Météo France', based on hourly records at the coastal meteorological station of Boulogne-sur-mer, northern France, and

was normalized for a 6 d period before the cruises. Water-column stability (Stab; no units), was calculated according to Li (2002). In addition, seawater for nutrient analysis (silicate [DSi], phosphate [DIP], and nitrate + nitrite + ammonium [DIN];  $\mu\text{mol l}^{-1}$ ) were collected and assessed according to the methodologies outlined in Aminot & K erouel (2004), using the Integral Futura autoanalyser system (Alliance Instruments).

### Nitrogen use efficiency

NUE (= biomass per unit of nitrogen available  $\mu\text{g C}^{-1} \mu\text{M}^{-1}$ ; Ptacnik et al. 2008) was calculated as:

$$\text{NUE} = \text{biomass} / (\text{DIN} + \text{PON}) \quad (1)$$

where PON corresponds to particulate organic nitrogen, which was assessed on a NA2100 Frisons CHN analyzer according to Aminot & K erouel (2004), and DIN and PON are in  $\mu\text{M}$ . The possibility of organic nitrogen uptake by phytoplankton was not considered here, due to lack of available measuring equipment. However, it is worth noting that phytoplankton in the English Channel and the southern North Sea has been found to primarily use dissolved inorganic nitrogen (L'Helguen et al. 1993, Moneta et al. 2014).

### Statistical analyses

Trait data were standardized, and biomass and environmental data were log-transformed, except for nutrients and the stability index, which were quarter-root transformed to approach normal distribution (Legendre & Legendre 1998). A scheme of the 9 steps involved in the statistical methodology used in this study is detailed in Fig. 1. To identify the traits responding to the environment (and consequently being functional), as well as the inter-group trade-offs among those functional traits, the 3-table ordination RLQ method (R-mode linked to Q-mode) was performed (Dol edec et al. 1996) with the 'ade-4' package (Dray & Dufour 2007) in R (R Development Core Team 2015). The traits  $V$  and  $\mu_{\text{max}(T)}$  were discarded because  $V$  was used initially to calculate  $N_{\text{saff}}$  (see Supplement 3);  $\mu_{\text{max}(T)}$  was not used because of the missing value of cryptophytes (RLQ does not allow missing values). Thus, in the following sections, ' $\mu_{\text{max}}$ ' will refer to  $\mu_{\text{max}}$  under saturating light level, i.e.  $\mu_{\text{max}(\text{Light})}$ .

The significance of the R and Q relationship was tested with a 1000 permutation Monte Carlo randomization test. The functional community components at different sampling dates (Step 7 in Fig. 1) were estimated with the 'FD' (functional diversity) package (Lalibert e & Legendre 2010). Functional domi-

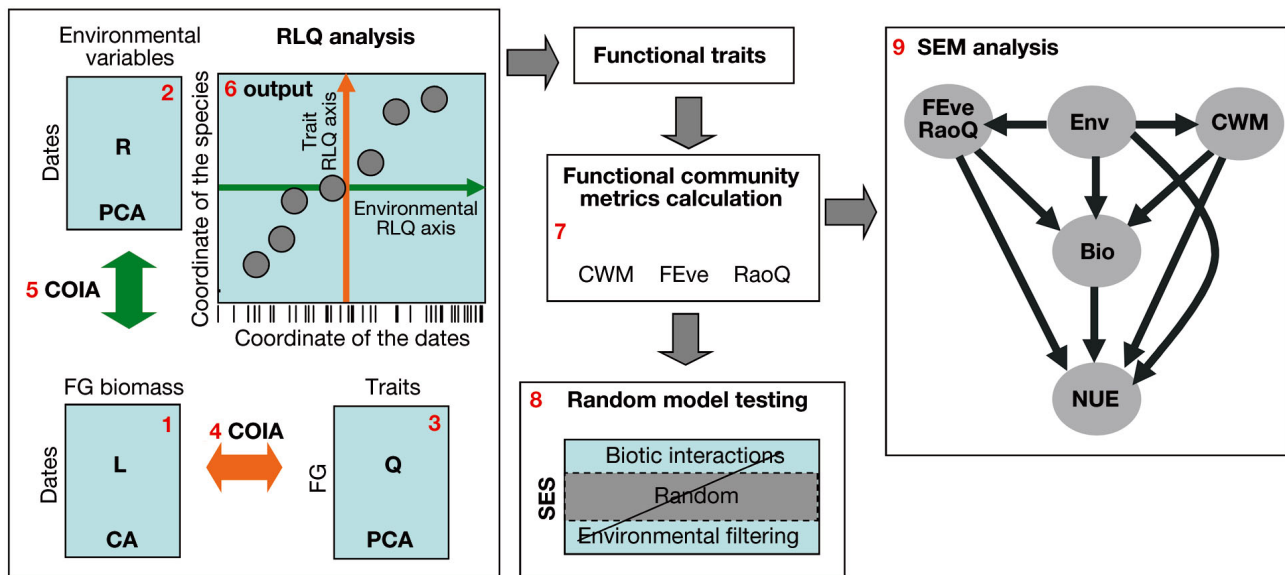


Fig. 1. The 9 steps in our statistical methodology. Steps 1 to 6: R-mode linked to Q-model (RLQ) analysis, identification of the functional traits and inter-groups trade-offs; Step 7: characterization of the community trait distributions; Step 8: testing to infer mechanisms of phytoplankton community assembly (diagonal line illustrates hypothetical gradual change in SES value); Step 9: structural equation model (SEM) analysis to identify the drivers of nitrogen use efficiency (NUE). L and R matrices (Steps 1 and 2) show abundance of functional groups (FGs) and environmental variables as columns, respectively, and sampling dates as rows. Q matrix (Step 3) shows FG labels as rows and trait values as columns. PCA: principal component analysis; CA: correspondence analysis; COIA: co-inertia analysis; SES: standardized effect size; CWM: community-weighted mean; RaoQ: functional divergence; FEve: functional evenness

nance was estimated as the community-weighted mean of trait values (CWM; Garnier et al. 2004), functional evenness (FEve) was estimated according to Villéger et al. (2008), and functional divergence (RaoQ; Rao quadratic entropy) according to de Bello et al. (2010). Although RaoQ is a combined measure of functional richness and functional divergence (Mouchet et al. 2010), in our study, it represents only a measure of functional divergence, since all FGs detected by flow cytometry were present throughout the study. RaoQ was preferred over the functional divergence measure of Villéger et al. (2008), because it has a greater power to detect community assembly rules (Mouchet et al. 2010, Mason et al. 2013).

The CWM corresponds to the mean trait value amongst each community, weighted by the relative carbon biomass of each FG (Garnier et al. 2004). FEve corresponds to the regularity with which FG biomass in a community is distributed along the minimum spanning tree which links all FGs in the multidimensional trait space, whereas RaoQ corresponds to the sum of pairwise distances between FGs weighted by the relative biomass within the multidimensional trait space (de Bello et al. 2010). For calculation of the multidimensional trait space, a preliminary principal coordinate analysis (PCoA) was performed with the traits on a matrix of Gower distances. All the axes of the PCoA were retained. The inference of environmental filtering and biotic interactions were estimated from the deviation (standardized effect size [SES]; Gotelli & McCabe 2002) of the observed functional diversity of one phytoplankton community from that expected from a random community by randomizing trait data 999 times for each date but maintaining richness and abundances (Step 8 in Fig. 1). Randomization was performed with the function 'permatfull' in the 'Vegan' package (Oksanen et al. 2011). SES was calculated according to the equation:

$$SES = \frac{x_{\text{obs}} - \bar{x}_{\text{ran}}}{\sigma_{\text{ran}}} \quad (2)$$

where  $x_{\text{obs}}$  represents the observed value of functional diversity at one date, and  $\bar{x}_{\text{ran}}$  and  $\sigma_{\text{ran}}$ , the mean and standard deviation of the functional diversity of the randomly assembled communities. Assuming a normal distribution of the random communities, the traits derived from the sampling dates falling into the 95<sup>th</sup> (or higher) percentile of the random distribution were considered 'over dispersed' (i.e. niche differentiation being the major process driving community assembly), whereas those falling into the 5<sup>th</sup> (or lower) percentile were considered 'under dispersed' (i.e. environmental filtering as the major process)

(e.g. Coyle et al. 2014). In order to investigate the biotic drivers (phytoplankton biomass, functional diversity, and functional dominance) of NUE, an SEM was performed with the 'lavaan' package (Rosseel 2012). The hypothesized structural equation model followed that of Hodapp et al. (2015) (Fig. 1). Goodness-of-fit of SEM was evaluated on the basis of the chi-square test (good model:  $p > 0.05$ ), chi-square degrees of freedom (CMIN / df < 2), comparative fit index (CFI > 0.9) and the standardized root mean residual (SRMR < 0.08) (Hu & Bentler 1999, Kline 2011). In order to prevent spurious correlations between phytoplankton biomass and NUE, the first was calculated differently from that for NUE, i.e. using independent chlorophyll *a* (chl *a*) measurements carried out in parallel with flow cytometry, according to the analytical method of Holm Hansen et al. (1965).

## RESULTS

Diatom biomass was important throughout the year, except during *Phaeocystis* blooms in April and May. From September to mid-February, *Synechococcus* spp., picoeukaryotes, and cryptophytes also contributed to an important part of the phytoplankton biomass (Fig. 2). The RLQ analysis showed a strong association ( $p < 0.001$ ) between the environmental variables and phytoplankton traits through the responses (i.e. biomass) of the FGs (Fig. 3). Since the first RLQ axis accounted for 91.3% of the total variance of the matrix crossing the environmental structure and traits, only this first RLQ axis was considered thereafter. This axis represented a typical resource gradient such that sampling dates were ordered according to nutrient concentration (DIN, DIP, DSi, located on the right side of the RLQ axis), irradiance (PAR) and salinity (S) located on the left side (Fig. 3A,B). Note that nutrients and light intensity varied greatly along this axis, by 2 and 1 orders of magnitude, respectively (Table 2). In the present study, neither seawater temperature, wind speed, nor water column stability played a significant role in defining phytoplankton temporal succession (Fig. 3B). Consequently, 2 distinct periods were shown: the first period, from July to February when light and salinity decreased and nutrients increased, and the second, from March to June, when those variables showed the opposite trend. Along this resource gradient, FGs were ranked according to the timing of their peak biomass (Fig. 3A) and for 5 out of the 8 pre-selected traits (Fig. 3C). These 5 traits were those characterizing competitive ability for low re-

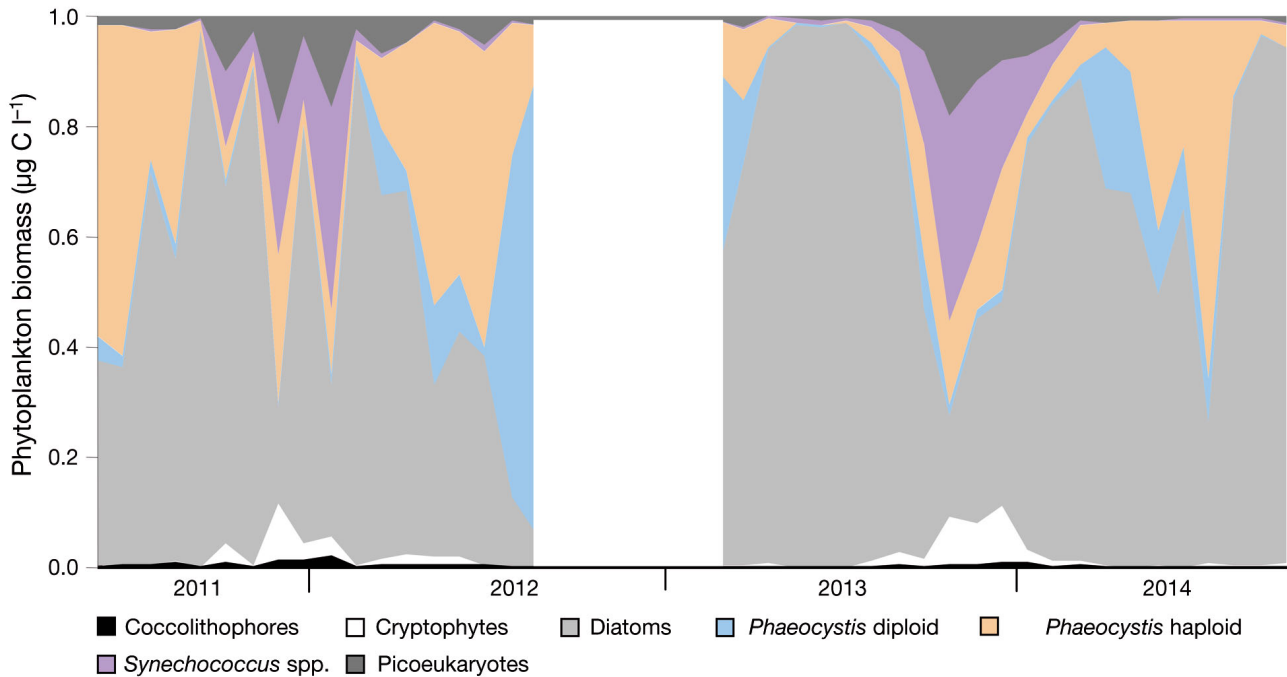


Fig. 2. Temporal variation of the relative biomass of functional phytoplankton groups identified and quantified by flow cytometry in coastal waters of the eastern English Channel over the period 2011 to 2014

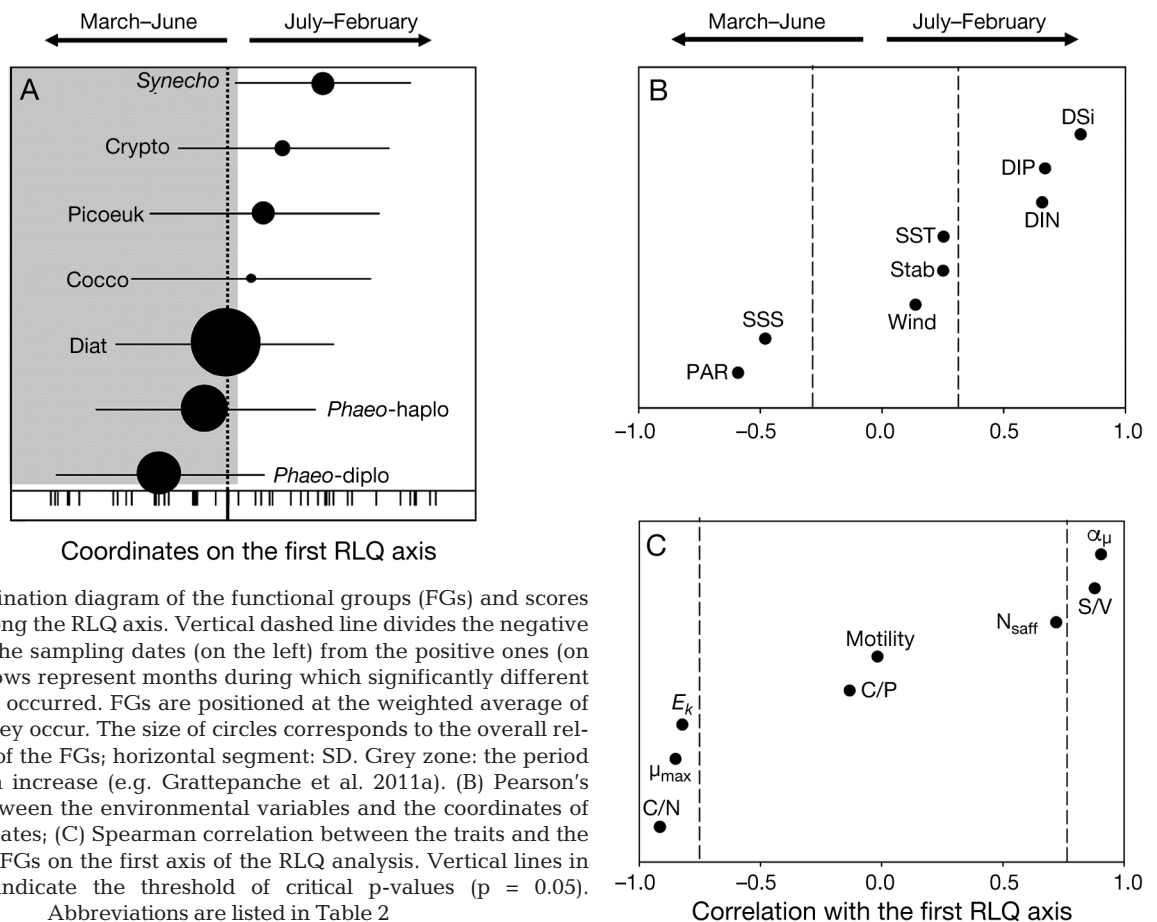


Fig. 3. (A) Ordination diagram of the functional groups (FGs) and scores of the dates along the RLQ axis. Vertical dashed line divides the negative coordinate of the sampling dates (on the left) from the positive ones (on the right). Arrows represent months during which significantly different FG dominance occurred. FGs are positioned at the weighted average of dates where they occur. The size of circles corresponds to the overall relative biomass of the FGs; horizontal segment: SD. Grey zone: the period of zooplankton increase (e.g. Grattepanche et al. 2011a). (B) Pearson's correlation between the environmental variables and the coordinates of the sampling dates; (C) Spearman correlation between the traits and the coordinates of FGs on the first axis of the RLQ analysis. Vertical lines in (B) and (C) indicate the threshold of critical p-values ( $p = 0.05$ ). Abbreviations are listed in Table 2

Table 2. Range values of environmental variables measured during this study and biomass of the functional groups defined by flow cytometry in coastal waters of the eastern English Channel over the period 2011 to 2014. n.u.: no units

Variable	Abbreviation	Unit	Range
<b>Environment</b>			
Wind speed	Wind	m s <sup>-1</sup>	3–9
Subsurface incident light	PAR	E m <sup>-2</sup> d <sup>-1</sup>	4.5–55
Sea surface temperature	SST	°C	5–18
Sea surface salinity	SSS	n.u.	33.3–34.7
Stability	Stab	n.u.	$2 \times 10^{-5}$ – $3 \times 10^{-2}$
Dissolved inorganic nitrogen	DIN	µM	0.05–31.84
Dissolved inorganic phosphorus	DIP	µM	0.01–1.29
Dissolved silicates	DSi	µM	0.1–12.9
<b>Phytoplankton</b>			
Coccolithophores	Cocco	µg C l <sup>-1</sup>	0.02–1
Cryptophytes	Crypto	µg C l <sup>-1</sup>	0.09–6
Diatoms	Diat	µg C l <sup>-1</sup>	5.03–288
<i>Phaeocystis</i> diploid	<i>Phaeo</i> -diplo	µg C l <sup>-1</sup>	0.27–212
<i>Phaeocystis</i> haploid	<i>Phaeo</i> -haplo	µg C l <sup>-1</sup>	0.39–92
Picoeukaryotes	Picoeuk	µg C l <sup>-1</sup>	0.22–13
<i>Synechococcus</i> spp.	<i>Synecho</i>	µg C l <sup>-1</sup>	0.06–21

sources (S/V ratio,  $\alpha_{\mu}$ ) displaying high values on the right side, whereas light-saturation intensity for growth ( $E_k$ ), maximum growth rate ( $\mu_{\max}$ ), and the cellular requirement for nitrogen (C/N ratio) displayed high values on the left side (Fig. 3C).

The normed row scores of the environmental matrix on the RLQ axis were used to relate variations in CWM, FEve, and RaoQ to the resource gradient, in an attempt to reveal different mechanisms of phytoplankton community assembly. The CWM of the S/V ratio and  $\alpha_{\mu}$ , which are 2 traits related to competitive capability, and that of  $\mu_{\max}$ ,  $E_k$ , and C/N ratio, showed each one had a significant relationship with the resource gradient (Fig. 4). Accordingly, the CWM of the S/V ratio and  $\alpha_{\mu}$  decreased as light and salinity increased, but nutrients decreased (Fig. 4A,B); whereas the CWM of  $\mu_{\max}$ ,  $E_k$ , and C/N ratio showed the opposite trend (Fig. 4C–E). Moreover, non-parametric Mann-Whitney tests showed that phytoplankton communities from March to June had significantly lower S/V ratio and  $\alpha_{\mu}$ , but higher  $\mu_{\max}$ ,  $E_k$ , and C/N ratio than those from July to February (Fig. 5). It is noteworthy that the relationship between CWM and S/V for each diatom species value (see Table S1 in Supplement 1) and the one considering the overall median value (Table 1) were significantly related ( $r^2 = 0.68$ ,  $p < 0.0001$ ; Supplement 4).

Unlike the standardized effect size of functional evenness (SES-FEve), functional divergence (SES-RaoQ) showed a significant difference between the 2 periods (Fig. 5). Accordingly, SES-RaoQ was signifi-

cantly lower from March to June than from July to February (Fig. 5). Furthermore, although overall it was not significantly different from the ones expected by random community analysis (i.e. 5th < SES < 95th percentile of the random distribution; Fig. 6A,B, see Supplement 5 for more details), SES-RaoQ displayed a significant quadratic relationship with the resource gradient. Accordingly, SES-RaoQ decreased as light increased but nutrients decreased (Fig. 6B).

The results from SEM showed that the data used in this study fit well with the hypothesized model ( $\chi^2 = 2.263$ ,  $df = 10$ ,  $p = 0.132$ ,  $CMIN / df = 0.013$ ,  $CFI = 0.983$ ,  $SRMR = 0.05$ ). According to SEM, the environmental variables and the multiple-traits CWM (represented through the first axis of PCoA analysis) were the only significant drivers of NUE (Table 3, Fig. 7). This relationship ( $p < 0.0001$ ) explained 53% of NUE. Hence, relatively high values of light intensity, salinity,  $\mu_{\max}$ ,  $E_k$ , and C/N ratio, and low values of nutrients,  $\alpha_{\mu}$  and S/V ratio, favoured NUE.

## DISCUSSION

A major challenge in applying functional trait-based approaches is that their values can vary tremendously according to environmental variables, within taxonomic groups, and even the physiological state of the cell. However, this approach, which consists of using laboratory-measured traits at a coarse taxonomic level, has been successfully used in several studies, and has provided consistent results (e.g. Litchman et al. 2007, Litchman & Klausmeier 2008, Schwaderer et al. 2011, Zwart et al. 2015). Here, we tested the potential of such a traits-based approach to explore an environment–trait linkage in phytoplankton in the coastal waters of the EEC. Despite the coarse taxonomic level, our results showed some coherent general patterns in the mechanisms driving the coexistence of the different FGs considered in this study (derived from flow cytometry analysis), the functional structure of the phytoplankton communities, and NUE.

FGs, determined by pulse shape-recording flow cytometry, ranged deterministically along a temporal gradient of light and nutrient availability according



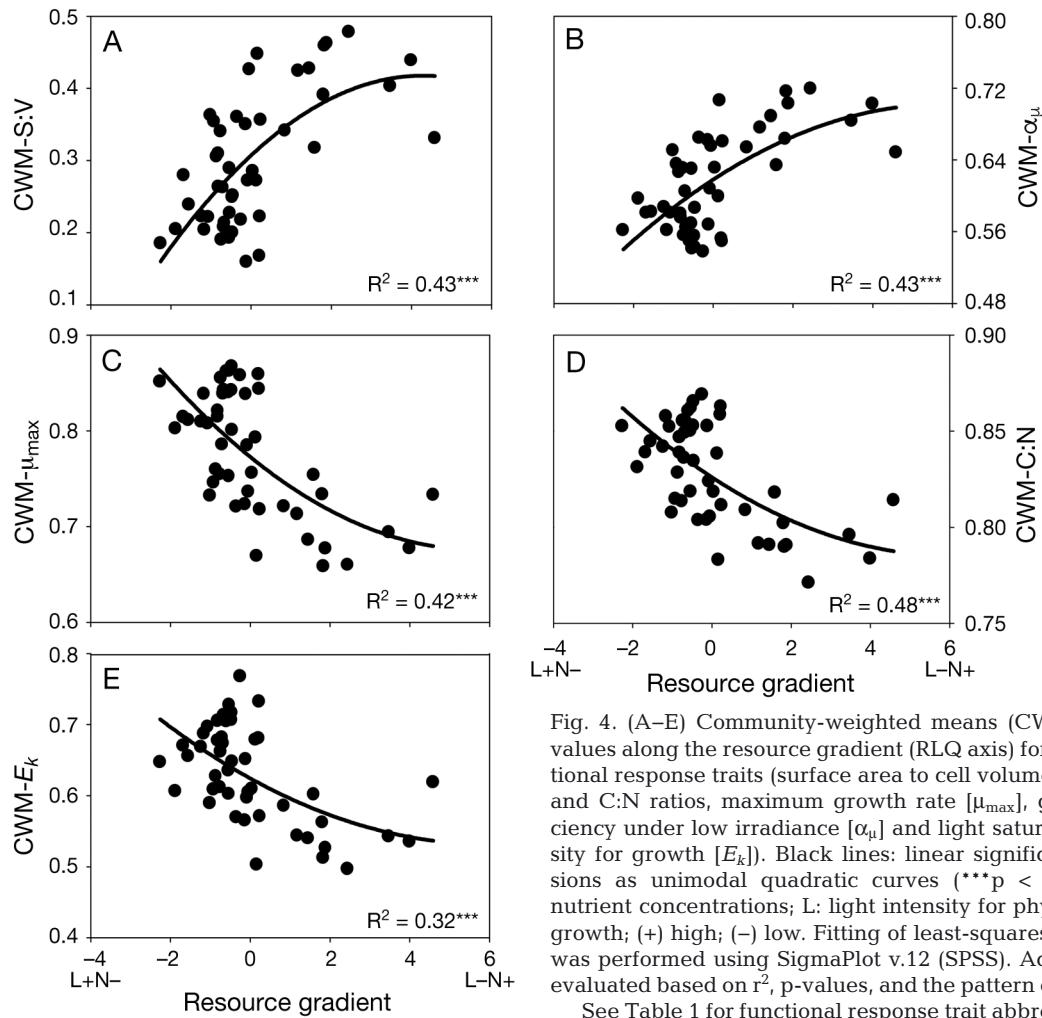


Fig. 4. (A–E) Community-weighted means (CWM) of trait values along the resource gradient (RLQ axis) for the 5 functional response traits (surface area to cell volume ratio [S:V] and C:N ratios, maximum growth rate [ $\mu_{\max}$ ], growth efficiency under low irradiance [ $\alpha_{\mu}$ ] and light saturation intensity for growth [ $E_k$ ]). Black lines: linear significant regressions as unimodal quadratic curves (\*\*\* $p < 0.0001$ ). N: nutrient concentrations; L: light intensity for phytoplankton growth; (+) high; (–) low. Fitting of least-squares regression was performed using SigmaPlot v.12 (SPSS). Accuracy was evaluated based on  $r^2$ , p-values, and the pattern of residuals. See Table 1 for functional response trait abbreviations

to several functional trait trade-offs (Fig. 3A–C). Thus, different FGs were favoured through time, allowing them to partition the resources, and therefore, their non-equilibrium coexistence throughout the year. Such coexistence mechanisms are considered to be particularly important in highly variable environments (Kneitel & Chase 2004), which is typically the case in the EEC (e.g. Lefebvre et al. 2011, Grattepanche et al. 2011a, Genitsaris et al. 2016). Three fundamental functional trait trade-offs can be depicted in the present study: (1) a gleaner-opportunistic trade-off (Grover 1990), which represents a trade-off between the  $K$  (e.g. *Synechococcus* and picoeukaryotes; Stawiarski 2014) and  $r$  (diatoms and *Phaeocystis* diploid; Reynolds 2006, Rousseau et al. 2007) strategies (Reznick et al. 2002); (2) a trade-off between growth efficiency under low irradiance and an increase in nitrogen demand (low C/N cell ratio; Edwards et al. 2015); and (3) a competitive-grazing resistance/tolerance trade-off (e.g. Kneitel & Chase

2004). The last trade-off was deduced from (1) the high relative maximal growth rate (i.e.  $\mu_{\max}$ ) positioned towards higher light but lower nutrient levels, which may allow a partial counteraction of grazing pressure; and (2) the expected position of biovolume (i.e.  $V$ ) along the resource gradient, which is generally considered as a good proxy of grazing resistance (Irigoiien et al. 2004). Given that  $V$  is inversely related to  $S/V$ , it would be positioned opposite to  $S/V$  along the resource gradient, i.e. towards higher light but lower nutrient levels. In addition, the increase in the C/N cell content ratio in the same direction as  $V$  and  $\mu_{\max}$  may confer some degree of unpalatability of the dominant FGs at this time of the year (Sterner & Hessen 1994). These multiple trade-offs were distributed along a single main axis of functional differentiation, indicating that strong competitors for nutrients and light (i.e. high  $S/V$  and  $\alpha_{\mu}$ ) are also shade-tolerant (low  $E_k$ ; Fig. 3C). Moreover, they have a lower  $\mu_{\max}$ , a higher vulnerability to grazing due to their smaller

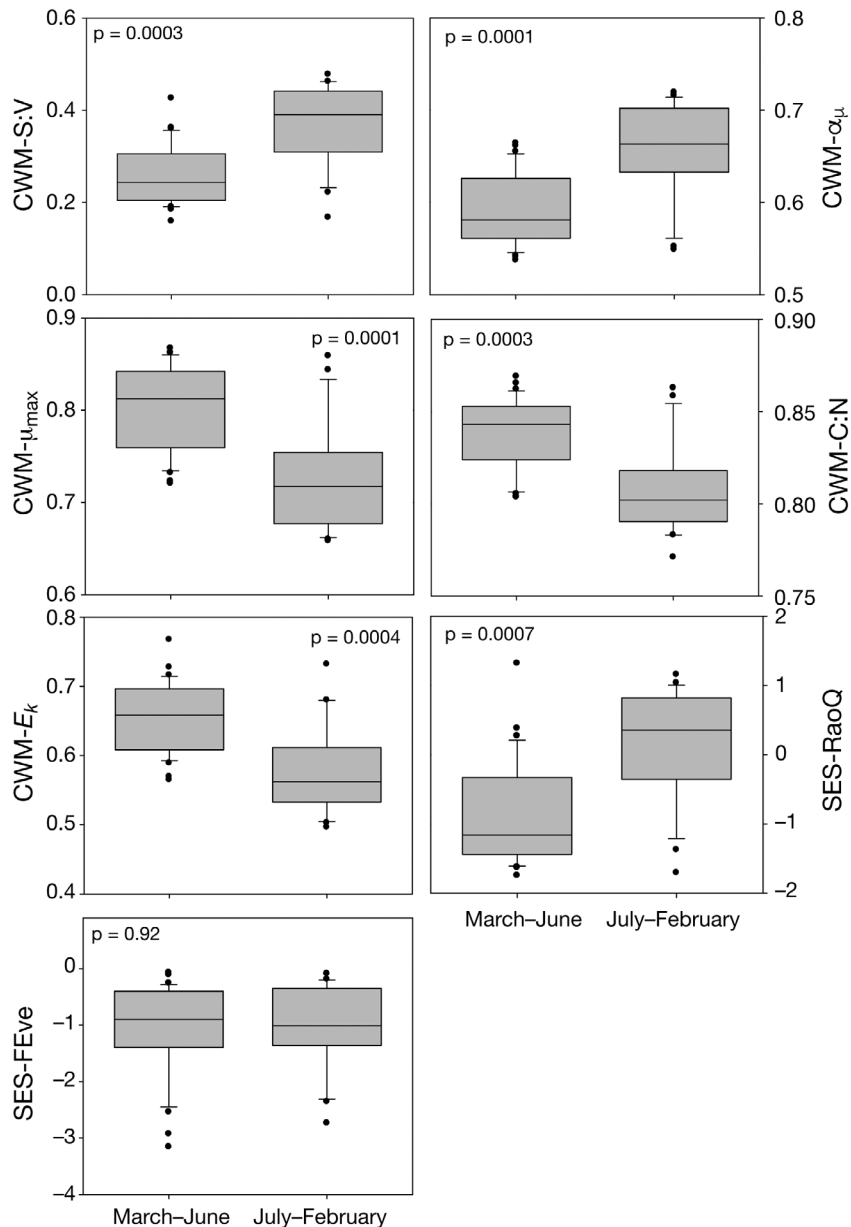


Fig. 5. Box-plot and Mann-Whitney test of community-weighted mean trait values (CWM-S:V; CWM- $\alpha_{\mu}$ , CWM- $\mu_{\max}$ , CWM- $E_k$ , and CWM-C:N—see Fig. 4 for abbreviations), and functional diversity (standardized effect size of functional divergence and of functional evenness, SES-RaoQ and SES-FEve, respectively) between the periods March–June and July–February. Horizontal line inside box: median; box: 0.75th to 0.25th quartiles; whiskers: 1.5 times the interquartile range (IQR); dots: outliers (>1.5 times IQR). See Table 1 for functional response trait abbreviations

size, and higher nitrogen content (Fig. 3C). Carbon-to-nutrient ratio shifts in the response of phytoplankton to light and grazing changes have already been recorded in experimental studies (Urabe & Sterner 1996, Hessen et al. 2002). Moreover, carbon-to-nutrient shifts have been invoked in models addressing the trigger of the spring diatom bloom in the coastal

waters of the western English Channel (Polimene et al. 2015). The gleaner-opportunist and competitive-grazing resistance trade-offs have been also indirectly observed in the field (Litchman & Klausmeier 2008 and references therein) or directly in manipulated field experiments (Hillebrand et al. 2000). In these studies, however, opportunists (i.e. fast-growing species) were more vulnerable to grazing, and their dominance occurred when nutrient levels were high. Despite relatively low nutrient levels, large diatoms species (e.g. *Guinardia flaccida* and *Rhizosolenia imbricata*) often dominate in the EEC during and after the *Phaeocystis* bloom (Hernández-Fariñas et al. 2014). It is possible that their relatively high nutrient storage capacity due to their large size gives them additional competitive advantages under low nutrient availability (Raven 1997, Schoemann et al. 2005). Furthermore, Litchman et al. (2009) demonstrated in a modelling study that nitrogen limitation and pulsed supply of this nutrient may select large diatom cell sizes in marine waters. Moreover, when DIN and DIP are depleted, *Phaeocystis* has the capacity to use dissolved organic nitrogen and phosphorus (van Boekel & Veldhuis 1990, Sanderson et al. 2008) provided by biotic processes (e.g. cell lysis, zooplankton excretion, etc.).

The gradual changes in CWM of phytoplankton traits across the resource gradient support the idea that abiotic filtering plays a role in shaping phytoplankton community structure (e.g. Cornwell & Ackerly 2009) as previously evidenced from RLQ analysis. However, the gradual changes in the SES-RaoQ, with the tendency towards traits convergence as phytoplankton (and presumably, zooplankton) biomass increased, indicates that biotic interactions would become the dominant phytoplankton assembly process as light increased. Indeed, an increase in abiotic stress as phytoplankton biomass increases is unlikely, and both competitive interactions and pressure by generalist consumers may lead

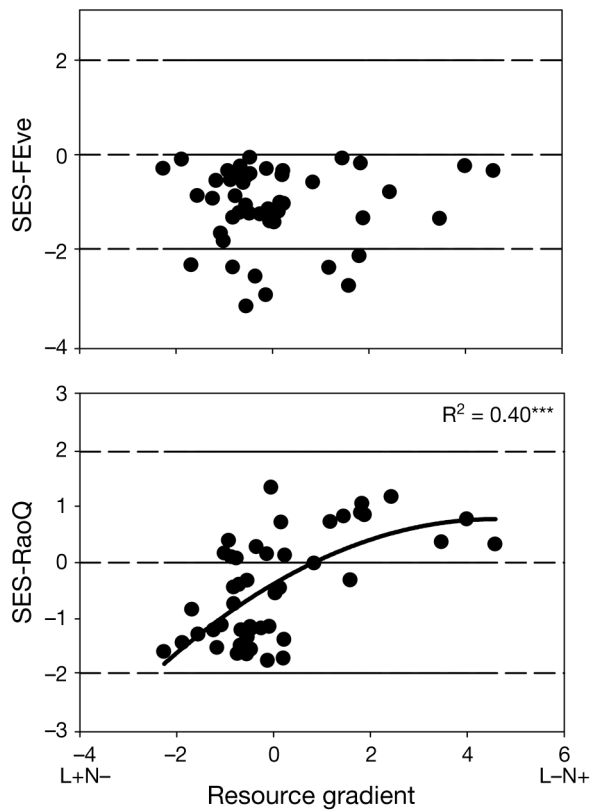


Fig. 6. Relationships between the standardized effect size of (A) functional evenness (SES-FEve) and (B) functional divergence (SES-RaoQ) of the phytoplankton community, and the resource gradient (RLQ axis). Black lines: linear significant regressions as unimodal quadratic curves (\*\* $p < 0.0001$ ). See Fig. 4 for abbreviations, fitting of least-squares regression, and evaluation of their accuracy

Table 3. Statistical results: path-coefficient and its standard error, Z-value, p-value, and standardized path coefficients (Std estimate) of the structure equation model for nitrogen use efficiency (NUE). FEve: functional evenness; CWM: community-weight mean based on the first component of principal coordinate analysis of functional groups mean trait values for functional traits (S:V,  $\mu$ ,  $\mu_{\max}$ ,  $E_k$ , and C:N ratio; see Table 1 for trait abbreviation definitions); Env: RLQ axis

Regressions	Path-coefficient	Std error	Z-value	p-value	Std estimate
NUE ~	-0.152	0.325	-0.468	0.640	-0.075
Biomass + FEve + CWM + Env	-0.688	0.632	-1.088	0.277	-0.131
	-2.402	0.864	-2.780	0.005	-0.435
	-0.175	0.084	-2.084	0.037	-0.366
Biomass ~	-0.276	0.317	-0.871	0.384	-0.106
FEve + CWM + Env	-0.647	0.424	-1.526	0.127	-0.236
	-0.117	0.038	-3.089	0.002	-0.493
FEve ~ Env	0.029	0.014	2.066	0.039	0.322
CWM ~ Env	0.058	0.011	5.501	0.000	0.671

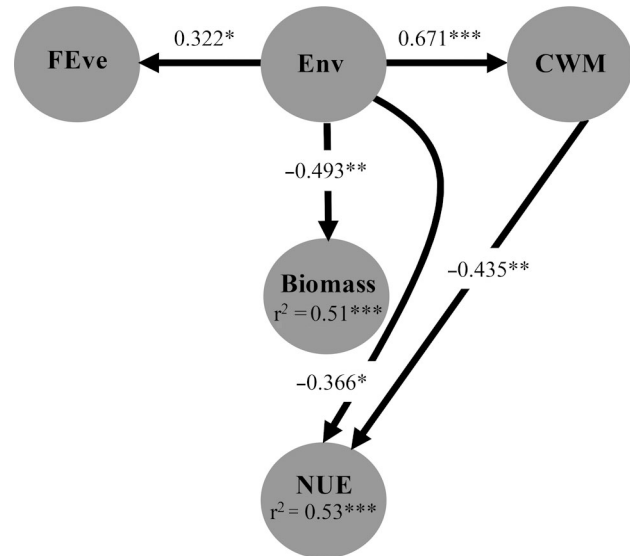


Fig. 7. Final structural equation model ( $\chi^2 = 2.263$ ,  $df = 10$ ,  $p = 0.132$ , chi-square degrees of freedom [CMIN / df] = 0.013, comparative fit index [CFI] = 0.983, standardized root mean residual [SRMR] = 0.05). Only significant relationships are shown. Values in arrows are standardized path-coefficients. \* $p < 0.05$ ; \*\* $p < 0.005$ ; \*\*\* $p < 0.0001$

to convergence in traits related to competitive dominance and defense, respectively (Cavender-Bares et al. 2009, Mayfield & Levine 2010). Like the S/V ratio and  $\alpha_{\mu}$ ,  $\mu_{\max}$  is also related to competitive capability, since faster-growing species may out-compete those with lower growth rates, and better tolerate grazing pressure. Grazing resistance can be achieved with either defensive strategies such as large size, and/or unpalatable physical structures like silica frustule for diatoms and mucopolysaccharidic colonies for *Phaeocystis* (Gasparini et al. 2000, Jakobsen & Tang 2002). Moreover, phytoplankton cells with high C relative to N are often less palatable to herbivores (Urabe & Sterner 1996, Hessen et al. 2002). During the productive season (from March to mid-July), microzooplankton are always abundant in the EEC (e.g. Grattepanche et al. 2011a, Christaki et al. 2014, Genitsaris et al. 2015), and exert high grazing pressure on smaller phytoplankton ( $< 5 \mu\text{m}$ ; Grattepanche et al. 2011b). Moreover, a field study in the coastal waters of the North Sea has shown that *Synechococcus* and picoeukaryotes are strongly controlled by microzooplankton grazing pressure (Baudoux et al.

2008). This strong grazing pressure may have counteracted the effect of competition by preventing the increase of these highly competitive FGs under low nutrient levels. Unlike picophytoplankton, *Phaeocystis* haploid cells, which are preferentially grazed by microzooplankton (Grattepanche et al. 2011b), have a higher  $\mu_{\max}$ .

Overall, our results indicated that the CWM of trait values and environmental variables were the most important drivers of NUE. By contrast, functional diversity (functional evenness and/or functional divergence) showed no significant influence on NUE. These results also indicated that increase in light and compositional shifts of the phytoplankton community towards the dominance of faster growing and particularly productive FGs (i.e. *Phaeocystis* and diatoms) well adapted to relatively high light, allow a better use of the nitrogen available in the EEC. From this point of view, the results of the present study support the 'mass-ratio hypothesis' (Grime 2006) rather than the 'niche-complementary hypothesis' (Tilman et al. 1997) as playing an important role in nitrogen storage capacity in the EEC. Our results are in accordance with those of Filstrup et al. (2014) from the Baltic Sea, and Hodapp et al. (2015), in the Wadden Sea. These both showed that the dominance of few species explained most of RUE, in contrast to Ptacnik et al. (2008), who found a positive relationship between RUE and phytoplankton genus richness in Scandinavian lakes.

## CONCLUSIONS

The significant response of the flow cytometry-defined FGs to a resource gradient found in this study indicates the potential use of laboratory-measured functional traits to explore an environment-trait linkage in phytoplankton, and further to identify the mechanisms driving phytoplankton community organization. Accordingly, fundamental intergroup trade-offs, an increase in biotic interactions, together with an increase in light levels were identified as important coexistence mechanisms shaping phytoplankton community structure in the EEC. The trend towards traits convergence, along with the observed decrease in nutrients, indicate the combined effect of selective-grazing-mediated facilitation of opportunists and their capacity to grow faster than strong competitors for nutrients, once light becomes sufficient.

As light increased, the resulting functional dominance of particularly fast-growing and productive

FGs (i.e. *Phaeocystis* and diatoms) in phytoplankton communities allowed a better use of the nitrogen available in the EEC. It should be made clear that the present work does not suggest that natural assemblages can easily be characterized by placing them into coarse FGs and assigning them traits. However, given the current paucity of data on trait dynamics of specific planktonic taxa, applying traits at the level of FGs — although it has limitations — has allowed us to work with this problem. It would be of great interest to confirm and extend our observations to other coastal and/or open sea phytoplankton communities under different environmental forcings, taking into consideration turbulence conditions and compare the values of the laboratory-measured traits with the flow cytometry and physiological traits of the FGs measured in the field.

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