



Seasonal phytoplankton nutrient limitation patterns as revealed by bioassays over Baltic Sea gradients of salinity and eutrophication

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ABSTRACT: The *in situ* P versus N limitation of northern Baltic Sea phytoplankton was studied over 3 seasonal cycles at 6 locations, encompassing the large-scale salinity and eutrophy gradients of the Baltic Sea. Limitation patterns were inferred from 170 time-series (3 d) experiments with a replicated factorial experimental design, analyzed using a novel model selection-based classification. Seven limitation classes describe exclusive and primary limitation by N, P, combined N and P, or none. Response parameters were chlorophyll *a* (chl *a*) and primary productivity. Although chl *a* responses reported more N-limited, and ¹⁴C responses more P-limited cases, the responses of both parameters were highly similar. Distinct seasonal patterns of *in situ* P versus N limitation were evident, reproducible, and dissimilar even in closely related environments. The most pristine low-saline areas were dominantly P-limited, while more eutrophied areas showed predominant N limitation even at comparable salinities. The typical Baltic Sea surface salinity regions (5 to 6 PSU) were clearly N-limited either for summer months (e.g. the mildly eutrophied Bothnian Sea), or throughout the growth season (e.g. the eutrophied Gulf of Finland), although terrestrial loading ratios for the Baltic Sea exceed the Redfield ratio several-fold. The commonly vigorous N₂-fixing cyanobacterial blooms in the southern basins do not alleviate the basic N-limitation pattern, and do not appear in the summertime N-limited Bothnian Sea. Management strategies should include toning down the N-limited spring bloom in eutrophied areas, which fuels summertime P release from sediments, in turn favoring cyanobacteria. Terrestrial nutrient loading does not reach offshore areas without significant modification and loss, emphasizing the role of coastal zones in global nutrient cycles and stoichiometry.

KEY WORDS: Nutrient limitation · Coastal eutrophication · Bioassays · Phytoplankton · Nitrogen · Phosphorus · Baltic Sea

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INTRODUCTION

Nutrient limitation is one of the perennial topics in the limnological research tradition, and among those with the most obvious connection to the environmental management of water bodies. Without knowledge of the limiting factors of aquatic primary production, interventions to eutrophication caused by anthropogenic nutrient discharges are at best haphazard and ineffective, and at worst counter-effective. The paradigm of P limitation in freshwater ecosystems, based especially on the cornerstone works of Vollenweider

(1968, 1976) and Schindler (1974, 1977), has been repeatedly supported directly by experiments and indirectly by relative nutrient availability analyses. During the last decades, the picture has somewhat diversified and N limitation appears to be more common in freshwater systems than was previously considered (Morris & Lewis 1988, Elser et al. 1990).

Marine research on nutrients has, contrary to lake studies, largely concentrated on N (Hecky & Kilham 1988, Howarth 1988, National Research Council 2000) since the early work by Ryther & Dunstan (1971); with some notable exceptions such as P limitation in the

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Mediterranean Sea (Thingstad et al. 2005). Eutrophication problems in coastal systems have developed far later than in lakes, and consequently the nutrient limitation data available from saline environments are, although accumulating, still sparse compared to the limnological data (Cloern 2001). In addition to its importance in coastal eutrophication management, marine phytoplankton nutrient limitation has recently become a central issue in studies of global biogeochemical nutrient cycles and ecological stoichiometry of the oceans (Arrigo 2005).

Nutrient limitation relates to the immediate time scales of planktonic primary production in the euphotic surface layer. However, the underlying driving forces behind the *in situ* conditions span larger biogeochemical temporal and spatial scales, involving not only whole water bodies but also processes extending far up into terrestrial catchments. Despite the obvious and challenging multidimensionality of drivers, including both anthropogenic and natural nutrient sources and losses, it is fair to conclude that an essential component of any analysis of nutrient limitation of a particular aquatic system should be the verification of nutrient limitation at the focal point of action, *in situ* planktonic primary production.

Methods for verifying the limitation pattern of this domain all carry inherent constraints, which also relate to the temporal and spatial scales of the processes involved (e.g. Beardall et al. 2001). Limitation by a particular nutrient and its availability should be closely knit aspects of nutrient cycles in any system. It is therefore conceivable that sufficiently imbalanced availability of main nutrients would provide enough information on the nutrient limitation patterns to allow us to infer these from hierarchically distant properties of the system, such as large-scale nutrient budgets, nutrient loading ratios, or pool sizes of inorganic nutrients. In several cases, however, this nutrient limitation information is either not readily available, or it appears inconclusive due to the apparently low availability of more than one potentially limiting resource. It is in this situation that methods for directly assessing *in situ* nutrient limitation of phytoplankton should be evaluated for their strengths and constraints.

It is not immediately apparent how salinity as such produces the dichotomy between the key nutrients in freshwater and marine environments. Terrestrial nutrient runoff to the coastal zone is characterized by a surplus of nitrogen both globally (Seitzinger et al. 2002, Harrison et al. 2005), and often the more so in coastal areas under strong anthropogenic pressure, like the Baltic Sea (terrestrial loading ratio being ca. triple the Redfield ratio; Stålnacke et al. 1999, HELCOM 2004). This is affected by riverine inputs flowing from P-limited freshwater domains or point sources with efficient

P removal established. Despite the N surplus in nutrient loading, productive surface layers of the coastal recipients tend to converge towards the Redfield ratio benchmarking average phytoplankton nutrient demands, and often even below that ratio (e.g. Fisher et al. 1992, 1999, Pitkänen & Tamminen 1995). Modification of nutrient pools in the brackish transition zone must therefore be substantial.

A common rationale for different roles of P and N in different salinity regimes has been the general absence of marine phytoplankton species capable of fixing atmospheric N₂ (with the notable offshore exception of *Trichodesmium* spp.), while there are a considerable number of freshwater cyanobacteria sharing this N flow shortcut (Howarth 1988, Vitousek & Howarth 1991). Another specific N pathway working towards the same outcome is loss through denitrification, which is suggested to be more efficient in shallow coastal regions compared to freshwater habitats (Nixon 1981, Seitzinger 1988). However, salinity also directly affects the relative availabilities of inorganic P and N through bottom water and sediment chemistry, as a major component of marine salt, sulfate, plays a key role in sequestering iron and thus weakening the permanent removal of inorganic P from the water column by iron precipitation, which is a central mechanism maintaining P limitation in freshwater (Caraco et al. 1989, 1990, Blomqvist et al. 2004). Several biogeochemical processes thus jointly affect the N:P availability transition along the salinity gradient, amounting to more effective removal of N from, and/or more effective retention of P in the productive surface layer of the system.

Each of these processes operates on a characteristic time scale, and the appropriate time scale to use for estimating nutrient limitation along the gradient is not always obvious, especially with environmental management interests in mind. The essentially instantaneous assessment of phytoplankton nutrient limitation, based on physiological indices like cellular composition or those obtained by fast repetition rate fluorometry (see Beardall et al. 2001), describes preconditions for primary production. However, linkages between the physiological state of cells, or short-term photochemical responses, and long-term biomass accumulation are often far from obvious. Physiological nutrient limitation of instantaneous phytoplankton growth is also, from the eutrophication control point of view, less central than limitation manifested in biomass changes, as most of the negative eutrophication effects are connected to the fate of the increased phytoplankton biomass.

Changes of autotroph biomass in bioassays of various kinds, all sharing the traditional Liebigian concept of treatments with alternative nutrient combinations in order to increase the carrying capacity and thus iden-

tify which nutrient is lacking, allow for the estimation of the limiting nutrient it seems, without dispute. Growth and biomass accumulation are, however, outcomes of processes operating on several scales, the more so if the test target is a natural planktonic community where the batch culture analogy has obvious limitations. Nutrient addition may stimulate autotroph growth (thus indicating limitation by that particular nutrient) without any concomitant biomass increase, either because the increased production is efficiently harvested by secondary producers (Kivi et al. 1993), or because stimulation of one species may suppress another species not limited by the same nutrient.

Leaving *in situ* food web interactions aside would clearly diminish the naturalness and thus the applicability of the results. Short-term bioassays with the natural planktonic community thus reflect the nutrient limitation of the ambient phytoplankton, but the signal is potentially modified by prevailing trophic interactions within the community, as would happen in the open system under study within time scales compatible with algal growth response.

We studied the *in situ* nutrient limitation patterns of one of the largest estuarine areas of the world, the Baltic Sea. This northern European non-tidal environment hosts exceptionally extensive and relatively stable gradients of low-end salinities, where the coastal shifts in P to N limitation predicted by current thinking should take place. The northern basins (Gulf of Bothnia and Gulf of Finland) have distinct salinity gradients but also exhibit large differences in the levels of eutrophication due to terrestrial nutrient sources, to the extent that the Gulf of Finland is one of the most eutrophied major estuarine areas, whereas the Gulf of Bothnia remains relatively pristine (Larsson et al. 1985, Stålnacke et al. 1999, HELCOM 2002). Growth season concentrations of both inorganic P and N are generally low, often close to or even below the detection limits of standard analyses, so that assessment of the limiting nutrient on the basis of monitoring nutrient data is uncertain. The Baltic Sea is also one of the few major estuaries in the world where N₂-fixing cyanobacteria appear in significant quantities during the late growth season (Kahru et al. 1994, Larsson et al. 2001). The Baltic coastal gradients therefore provide an exceptionally suitable testing ground for experimental *in situ* nutrient limitation assessment.

We chose to study the *in situ* limitation patterns by running 3 d time series bioassays with natural planktonic communities, with daily sampling under controlled laboratory conditions simulating the *in situ* annual development of temperature and light–dark cycles. Six coastal locations distributed along 1000 km of the Finnish coastline were selected to represent the large-scale coastal gradients, and the bioassays

were repeated ca. 10 times annually for 3 growth seasons at each location, producing 170 bioassays in total. In this paper we describe the phytoplankton responses as measured by chl *a* and primary productivity. The amount of data (>8000 chl *a* measurements and >5000 primary productivity measurements) from exactly comparable experiments, with both measurement- and treatment-level replication, both necessitated and allowed for the development of strict quality control routines, which were developed after the analysis of the statistical properties of the data (Appendix 1; available at www.int-res.com/articles/suppl/m340p121_app.pdf). Statistical modeling of nutrient enrichment responses was based on a novel model selection-based classification described in full detail in Andersen et al. (2007). This method allowed quantitative assessment of temporal and spatial *in situ* limitation patterns.

The implications of *in situ* limitation patterns for environmental management is discussed in the context of seasonal biogeochemical cycles of the coastal environment. Detailed analysis of the relations between the observed *in situ* limitation and other environmental data, like basin-scale nutrient stoichiometry and long-term trends, will remain the topics of separate studies in preparation.

MATERIALS AND METHODS

Sampling program and organization. The data originate from a 3 yr study carried out during the seasonal growth periods of years 1992 to 1994 at 6 sampling stations (Fig. 1, see coordinates in Table 1) distributed along the entire Finnish coastline of the Baltic Sea (ca. 1000 km). Stations were selected to be representative of the major sub-basins of the northern Baltic Sea, and for their location in the vicinity of a laboratory of the network of the Finnish environmental administration (the laboratories of the Water and Environment Districts of Oulu, Kokkola, Vaasa, Turku, Helsinki and Kymi). During each study year, stations were visited 7 to 12 times, yielding 24 to 30 experiments per location during the whole study period, and a total of 170 experiments (Table 1). A combined 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 pooled sample was taken to the laboratory in two 30 l polyethylene carboys and stored overnight in the dark in a thermostated water bath adjusted to the *in situ* surface temperature. No prescreening was applied, and the experiment was started the following morning according to the experimental design presented below. Incubations and sample preparations were done at the site, and sampling took place accord-

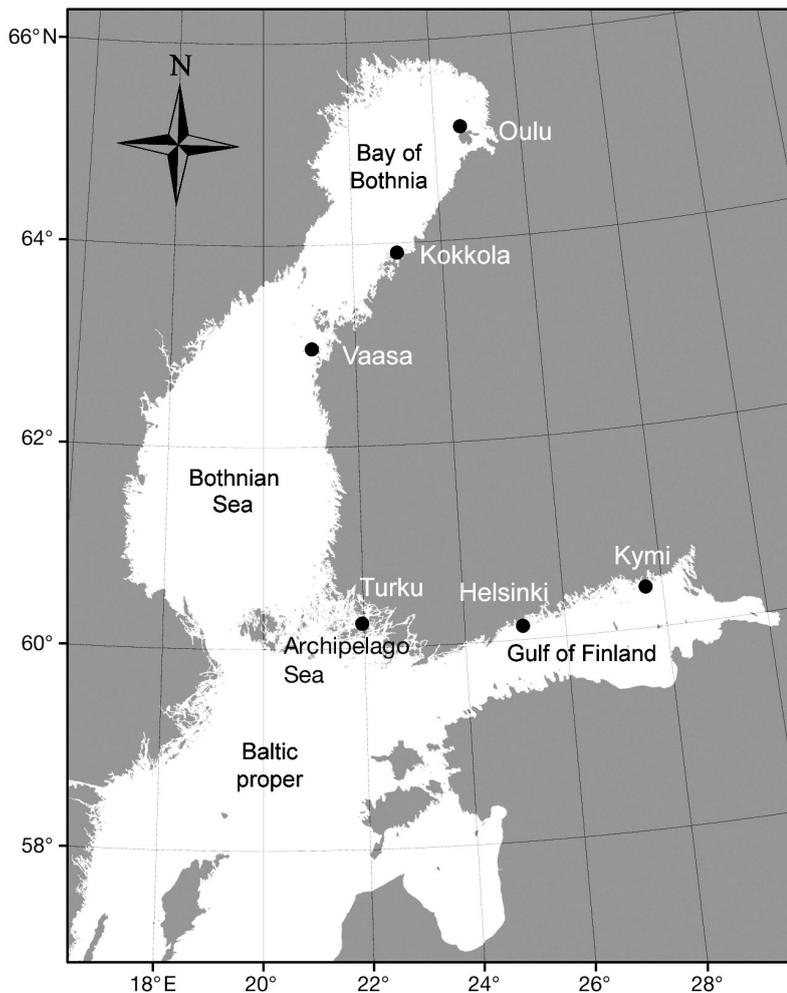


Fig. 1. Study area, showing the location of the 6 sampling stations along the Finnish coastline of the Baltic Sea. For the precise coordinates of the sampling stations, see Table 1

ing to a fixed weekly program, with little variability (within ca. 1 h) in the timing of daily sampling (09:00 h) and the subsequent incubations and sample preparations. Preserved samples were shipped to the laboratory of the Tvärminne Zoological Station (University of Helsinki) for measurement.

Experiment design and measurements.

Each experiment consisted of 8 experimental units (1 unit = 6 l water in an 8 l polycarbonate bottle) that were filled from the carefully stirred water sample and manipulated with phosphate ($20 \mu\text{g P l}^{-1}$) and/or ammonium ($80 \mu\text{g N l}^{-1}$) additions according to a 2^2 factorial design with replicates (see Fig. 2). All experiments were carried out at *in situ* temperature (thermostated water bath) under the locally prevailing light-dark cycles of the experimental week, 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. The bottles were gently shaken twice a day during the experiment, otherwise no mixing was performed. Samples for time-zero measurement of chl *a* and ^{14}C -uptake were taken only from unmanipulated water, while all experimental units were sampled for chl *a* on Days 1 to 3 and ^{14}C -uptake on Days 1 and 2. Parameters were measured in duplicate, giving a total of 50 chl *a* samples and 34 ^{14}C samples from each experiment. A total of 170 experiments were performed over the 3 yr period, of which 2 were lost entirely due to mistakes in the nutrient additions at the start. A reasonably small number of analytical errors at some stage of the procedure reduced the data pool slightly, and after final quality control routines (see description in Appendix 1), ca. 95% of the data for both parameters remained for further analysis. As the lacking or discarded data for these parameters were non-overlapping, a total of 154 experiments (91%) produced a full set of both parameters.

Chl *a* was measured fluorometrically (Shimadzu RF-5000) with pure chl *a* (Sigma) as standards. Samples were prepared in duplicate by filtering 100 ml subsamples onto Whatman GF/F glass-fiber filters, which

Table 1. Sampling stations and no. of experiments made annually. Station names used here correspond to the location of the laboratory of the respective Finnish Water and Environment District. Stations are characterized by the average values of salinity, total N, total P and chl *a* of the initial sample for the experiments of the study period ($n = 24$ to 30)

Station	Depth (m)	Latitude ($^{\circ}\text{N}$)	Longitude ($^{\circ}\text{E}$)	Salinity (PSU)	Total N ($\mu\text{g N l}^{-1}$)	Total P ($\mu\text{g P l}^{-1}$)	chl <i>a</i> ($\mu\text{g l}^{-1}$)	Experiments (n)		
								1992	1993	1994
Oulu	24	65.1333	24.6000	2.5	269	8	3.0	7	9	8
Kokkola	19	63.9186	22.9840	3.3	323	12	7.0	7	11	12
Vaasa	18	62.9848	21.0368	5.1	269	13	3.3	7	12	10
Turku	51	60.2555	21.9566	6.0	359	18	4.7	9	11	10
Helsinki	33	60.1495	25.1442	5.1	420	27	10.3	9	11	10
Kymi	48	60.4201	27.6520	3.6	389	23	10.3	7	10	10

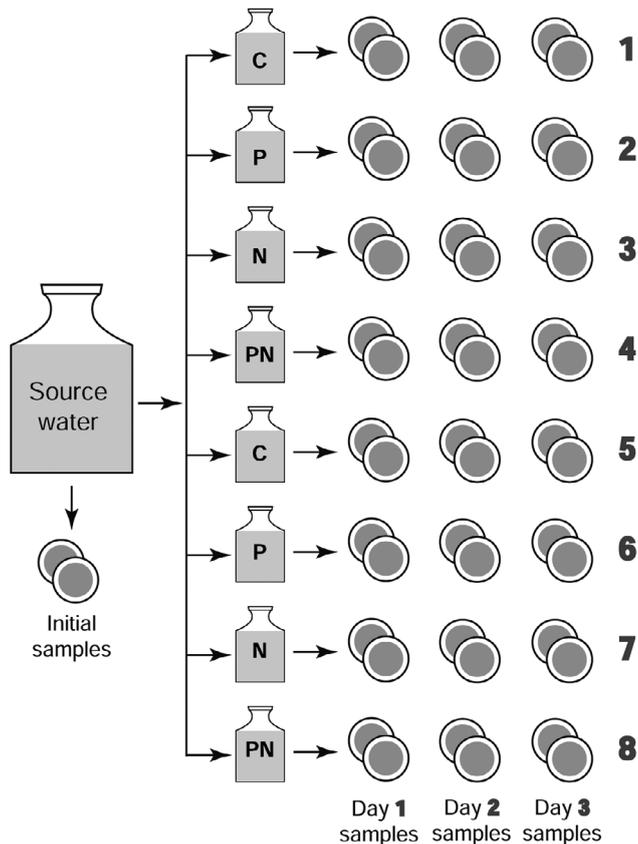


Fig. 2. Basic experimental design (replicated 2^2 factorial designs) and sampling scheme for 3 repeated observations of chl *a* response. Treatment codes: C = control (no nutrient addition), P = $20 \mu\text{M P l}^{-1}$, N = $80 \mu\text{g N l}^{-1}$, PN = combined P+N addition

were deep-frozen in scintillation vials until analysis. Samples were extracted in 94 % ethanol for 24 h in the dark at room temperature prior to measurement. Primary productivity was measured using the ^{14}C technique, by preparing dark and duplicate light subsamples for each sample. Incubation took place in the thermostated water bath at the same irradiance as the experimental units, in 20 ml acid-washed and precombusted (450°C) glass scintillation vials after adding $100 \mu\text{l}$ of $20 \mu\text{Ci ml}^{-1}$ bicarbonate (Amersham). Incubations were terminated after 2 to 4 h by adding $200 \mu\text{l}$ of 38 % formaldehyde. After transportation to the Tvärminne laboratory, a 4 ml subsample was acidified in a ventilation cupboard by adding $100 \mu\text{l}$ of 1 N HCl. After 24 h, 7 ml of scintillation cocktail (Hi-Safe 3) was added and the radioactivity was measured with a Wallac Rack-Beta scintillation counter with the external standard channel ratio method.

Statistical modeling of nutrient addition responses.

The statistical properties of the data, preconditions for the modeling of responses, and quality control procedures are summarized in Appendix 1 (available

at www.int-res.com/articles/suppl/m340p121_app.pdf). The statistical treatment is based on a novel model selection procedure for unsupervised classification of nutrient limitation bioassays (see Andersen et al. 2006 for a detailed description). Briefly, the method defines a set of *a priori* treatment contrast structures, or nutrient limitation classes, which can be given direct biological interpretations. Combining treatment contrast structures and time effects (represented by orthogonal polynomials) results in a set of linear models, from which a 'best' model can be selected through confrontation with data. The model selection is based on minimizing the Akaike Information Criterion (AIC) which depends on both the goodness of fit and the complexity of a model (the principle of parsimony). The treatment contrast structure of the selected 'best' model (the one with the lowest AIC) for a particular set of experimental data will thus implicitly yield its nutrient limitation class. The following 7 nutrient limitation classes were used (with their abbreviations):

(1) No response (00): No effect upon nutrient addition: all 4 treatments behave identically, even though their common time course can be highly non-linear (up to 3rd order polynomial). Typically observed when both inorganic nutrients are abundant during off-season or phytoplankton growth is constrained by low temperatures or extreme turbidity, or strongly controlled by grazers.

(2) Exclusive P limitation (XP): The supply of N is so much in excess of demand that adding it has no effect. The N alone treatment behaves identically to the control, and the P alone treatment is indistinguishable from the PN treatment.

(3) Primary P limitation (P1): The supply of N is sufficient for the N alone and control treatments to behave identically, but addition of P alone will soon lead to N deficiency. Thus the P alone treatment will not behave identically to the PN treatment.

(4) Exclusive combined limitation (XC): The supplies of P and N are closely balanced to the demands, so that neither the P alone nor the N alone treatments will behave differently from the control. Only the combined addition of P and N will produce a response, which will be different from the 3 other treatments.

(5) Primary combined limitation (C1): All addition treatments behave differently from each other and from the control. Combined P and N additions have effects on the phytoplankton community, but in contrast to the XC class, P and N have effects also as single additions.

(6) Primary N limitation (N1): The supply of P is sufficient for the P alone and control treatments to behave identically, but addition of N alone will soon lead to P deficiency. Thus the N alone treatment will not behave identically to the PN treatment.

(7) Exclusive N limitation (XN): The supply of P is so much in excess of demand that adding it has no effect. The P alone treatment behaves identically to the control, and the N alone treatment is indistinguishable from the PN treatment.

The primary combined (C1) pattern can occur if different components of the planktonic community (e.g. different phytoplankton species, or phytoplankton versus bacterioplankton) are limited by different nutrients. The C1 category would also include apparent nonsense results that have a completely unrelated set of responses to manipulations, such as single nutrient responses exceeding the combined PN response, or the control exceeding nutrient additions. However, the majority of responses falling into this category in the present data showed single P and N responses enveloped by the PN addition and control. For the sake of keeping the classification scheme as simple as possible, no further classes were introduced.

The experimental design includes an extra level of replication since duplicate samples were taken from each experimental unit. This replication is valuable for measurement of quality control, as well as for safeguarding against accidentally lost measurements and obvious outliers. In order not to inflate the degrees of freedom, the model fitting was based on the means of the unit-level replicates. We also did bootstrap analyses in which we generated input data to the classification procedure through random selection among the unit-level replicates. Since there are 25 duplicated chlorophyll measurements in a single experiment, there are $2^{25} = 33\,554\,432$ possible ways to do this. If this is repeated many times (1000 in our case), the resulting frequency distribution of limitation classes will give an indication of classification uncertainty due to measurement noise.

It should be noted that this bootstrapping procedure does not include all sources of error, and will give an optimistic estimate of the ambiguity of the classification. On the other hand, Table 2 shows that between-unit measurement noise is actually of the same order as the within-unit measurement noise in the present data set, so our bootstrap analysis should yield some important insights into the robustness of the experimental classification.

RESULTS

Seasonal succession of initial conditions

The geographical differences in the initial conditions for the experiments are summarized in Fig. 3 for inorganic P and N, together with chl *a*. The northernmost basin (Bay of Bothnia; stations Oulu and Kokkola) displayed a pattern for inorganic N that differed from the

Table 2. Coefficients of variation between replicate samples (ω_R) and between replicate treatments in different laboratories (ω_G) for ^{14}C -uptake and chl *a* samples

Laboratory	^{14}C		Chl <i>a</i>	
	Sample ω_R (%)	Treatment ω_G (%)	Sample ω_R (%)	Treatment ω_G (%)
1	4.7	6.5	4.9	5.0
2	4.2	5.4	5.3	5.8
3	6.2	6.5	6.0	4.8
4	5.6	5.9	5.2	6.0
5	5.5	7.1	5.9	7.0
6	6.6	9.4	7.7	8.6

other basins, with late and only partial depletion of the wintertime dissolved inorganic nitrogen (DIN) storage during the late, and modest, spring bloom. Although both northernmost stations showed some summertime samplings with almost entirely depleted DIN, reaccumulation had already started during late summer. Dissolved inorganic phosphorus (DIP) remained low throughout the season, often below the detection limit of standard monitoring programs ($<2\ \mu\text{gP l}^{-1}$). Dissolved silicate (DSi) was abundant through the years in the northern basin (data not shown).

At the other locations, experiments started from late spring bloom stages, with inorganic nutrients already depleted, and decreasing chl *a* values (Fig 3). Summertime concentrations of both inorganic P and N were generally low, close to or below standard detection limits, making it difficult to infer phytoplankton limitation with any certainty from the concentration data only (note in Fig. 3 the scaling of DIN and DIP panels in Redfield weight ratio N:P = 7.2). Silicate might potentially limit diatom growth during the late spring bloom in the southern basins, as occasional values below $100\ \mu\text{g l}^{-1}$ (ca. $3\ \mu\text{mol l}^{-1}$ DSi) were recorded (data not shown). However, the phytoplankton communities responded to experimental N and P additions, indicating that lack of silicate did not play a major role in their growth limitation.

Temporal development of responses at different locations

The contrast-based classification method can be used to compute the relative distribution of responses to nutrient addition among different stations along the coastal gradient from the northernmost Bay of Bothnia to the easternmost Gulf of Finland. Such distributions can be based on either of the 2 response parameters and any particular day of the experiment (Days 1 and 2 for ^{14}C -uptake and Days 1 to 3 for chl *a*). Fig. 4 summarizes location-wise (geographical gradient N to S, then

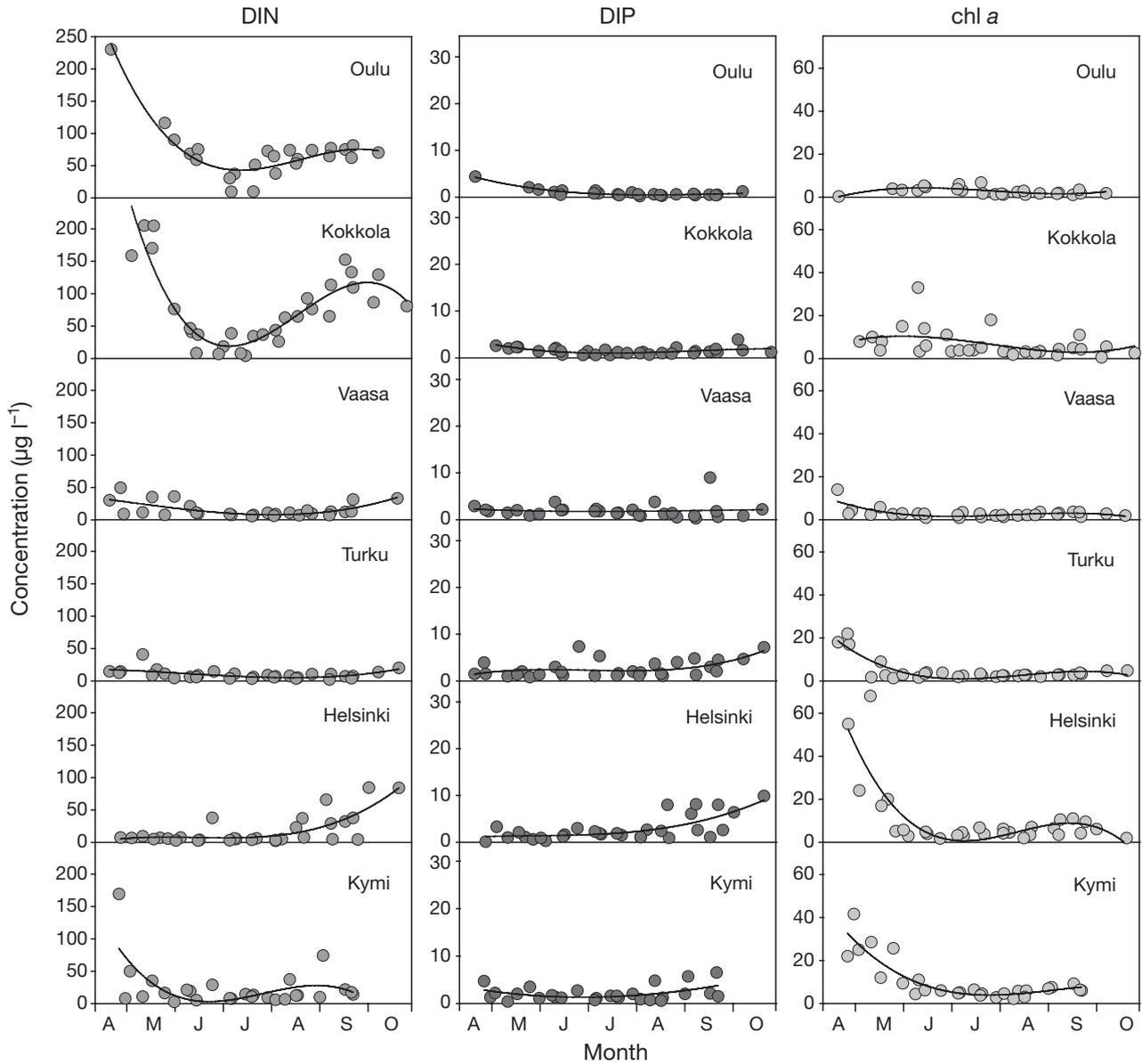


Fig. 3. Seasonal succession of the initial conditions (dissolved inorganic nitrogen [DIN] = inorganic N as the sum of NO_3^- and NH_4^+ ; Dissolved inorganic phosphorus [DIP] = PO_4^{3-}) for the 170 experiments conducted at the 6 stations (see Fig. 1 for their locations). All concentrations in weight units ($\mu\text{g l}^{-1}$). The 3 years of the study are pooled on a joint seasonal axis. Note: DIN and DIP panels are scaled to the Redfield weight ratio N:P = 7.2. Thin black line = nonlinear regression (polynomic; cubic) with dynamic fitting

to E running in each panel from left to right, see also Fig. 1) the temporal development of both response parameters, revealing several interesting patterns with respect to location, parameter, and duration of the experiment.

The most obvious pattern in Fig. 4 is the shift in P limitation dominance in the northernmost stations (Bay of Bothnia locations Oulu and Kokkola) to N limitation dominance in the south (Gulf of Finland locations Helsinki and Kymi), with the intermediate locations (Bothnian Sea and Archipelago Sea locations

Vaasa and Turku) strongly resembling the latter. It also appears that there are minor, but consistent differences in classifications based on different parameters, in the sense that ^{14}C -uptake based classifications tend to give more cases of P limitation, while chl a based classifications tend to produce more cases of N limitation.

The primary productivity responses (Fig. 4, first row panels) developed faster than chl a responses (Fig. 4, second row panels) in the sense that the share of no-response experiments (the difference between 1 and

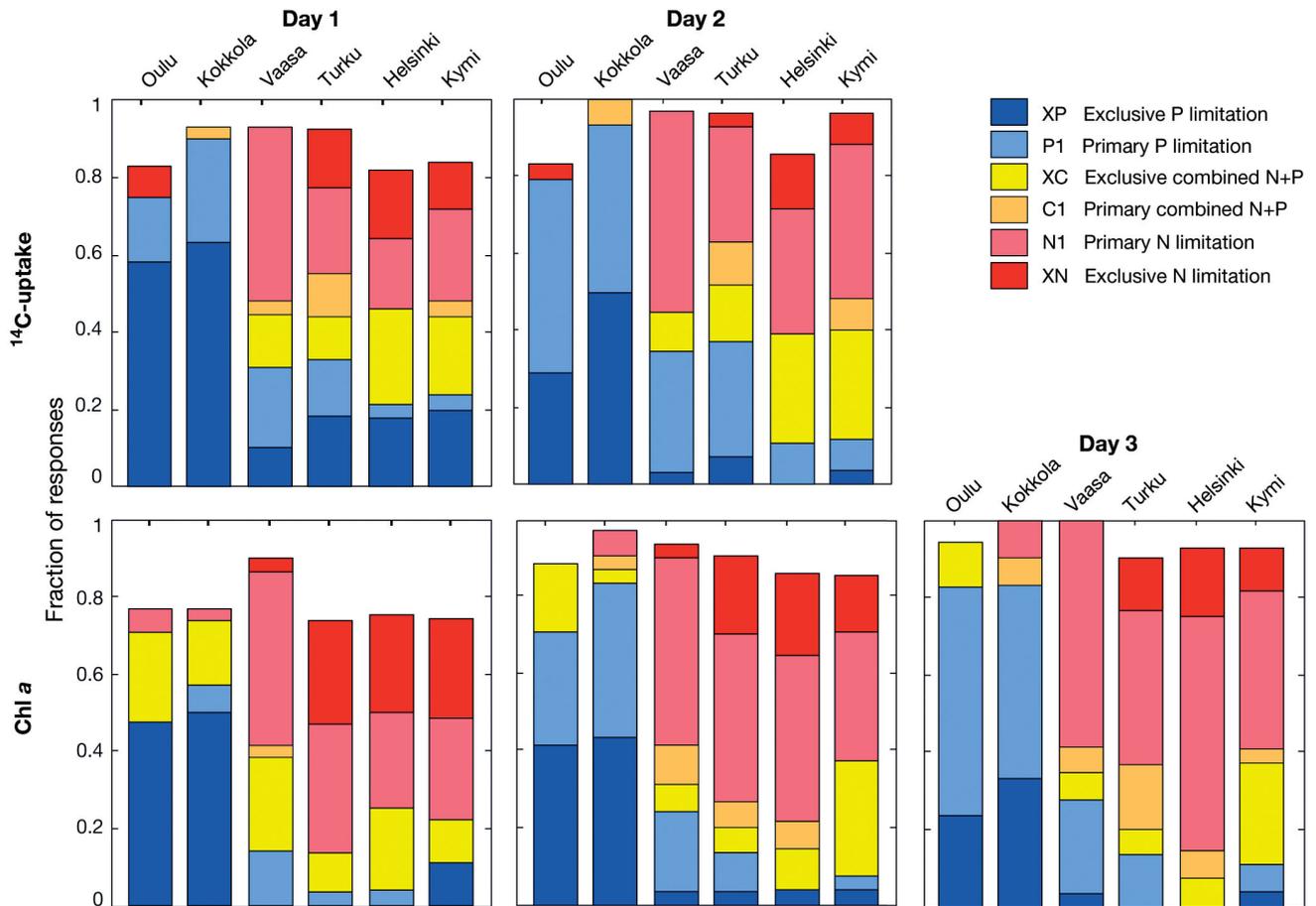


Fig. 4. Relative frequencies of limitation classes by location, response parameter, and incubation time. First row: ^{14}C -uptake responses; second row: chl *a* responses. First column classifications are based on Day 1 responses only, second column on Days 1 and 2, and third column on all measurements on Days 1 to 3 (chl *a* response only). The frequency of experiments with no significant response to nutrient addition (class 00) is represented by the difference between 1 and height of bar

height of bar in Fig. 4 panels) was smaller and decreased faster with time. Exclusive P limitation (XP) of the ^{14}C -response was apparent in ca. 20% of the cases on Day 1 (Fig. 4, upper left panel) even at southern locations, where chl *a* responses were strongly dominated by N limitation (Fig. 4, lower left panel). This role of P limitation in ^{14}C -uptake responses was attenuated during Day 2 for the 4 southern stations but remained the dominant response in the north, although generally shifting from exclusive (XP) to primary P (P1) limitation during the course of the experiment (Fig. 4, first row panels).

Chl *a* responses initially contained a relatively large share of combined limitation responses (XC, C1), even at the northern stations, but with the exception of the easternmost Kymi station, they became marginal during the experiment (Fig. 4, second row panels). Also the relatively large share of exclusive limitation responses on Day 1 (XP at the 2 northernmost stations and XN at

the others) gave way to corresponding primary limitation categories (P1, N1) during Days 2 and 3, when combined limitation responses also developed somewhat from exclusive (XC) to primary (C1) limitation. In general, chl *a* responses indicated a central role of N limitation in the southern basins, representing up to 80% of cases in Helsinki toward the end of the pooled 3 d experiment, while agreeing with primary productivity responses on the overwhelming P limitation (>80% of cases) of the northernmost, Bay of Bothnia basin.

Although the responses in primary productivity and chl *a* varied systematically between the locations, the overall development indicated some level of temporal uncoupling between the 2 parameters. The primary productivity responses developed faster and typically resembled the chl *a* responses of the subsequent day. All other pair-wise comparisons between the 2 parameters were less related (Fig. 4; based both on the percentage of identical classifications and the contingency

analysis likelihood ratio Chi-square test, $p < 0.001$). Evidently the increased photosynthetic activity, in direct response to increased nutrient availability, manifested as a matching biomass increase with a time lag. Although increased chl *a* per unit biomass cannot be ruled out, it is reasonable to expect that accumulated algal growth is the major contribution to the chl *a* responses.

When the responses of the 2 parameters were cross-tabulated experiment-wise (Table 3, ¹⁴C responses on Day 2 versus chl *a* responses on Day 3) 56% (86 out of 154) of the experiments showed an identical classification by both parameters (diagonal cells in Table 3). It is apparent that there was a consistent tendency for ¹⁴C uptake responses to produce classification leaning more towards P limitation, while chl *a* response classifications were biased towards N limitation. If offsets of one category along the gradient of classifications are pooled with identical classifications, the parameters agree in 74% of the cases (114 out of 154). No-response categories (00) were scarce in both parameters (7 or 8 cases out of 154, or 5%).

The most notable dissimilarity between the 2 classifications (27 out of 154, or 18%) represented cases where chlorophyll responses reported classifications 2 categories or more off the ¹⁴C response towards N limitation. Chl *a* response classifications were biased correspondingly towards P limitation in only 2 cases out of 154 (Table 3). In general, the association between the 2 response parameter classifications was high (likelihood ratio $\chi^2 = 189.59$, $p < 0.001$). The marginal distributions, however, summarize the tendency of chl *a* responses to report more N-limitation cases and ¹⁴C responses more leaning towards P-limitation (Fig. 5).

Seasonal patterns of the responses

For an overview of the limitation patterns over the seasonal succession at each location, the limitation classes were pooled into P limitation (classes XP and P1), combined P and N limitation (classes XC and C1) and N limitation categories (classes XN and N1), expressed as bootstrap probabilities based on the relative frequencies of limitation classes after 1000 random selections among the unit-level replicates. The 3 successive experimental years are jointly displayed for ¹⁴C uptake and chl *a* responses (Figs. 6 & 7, respectively).

Table 3. Cross-tabulation of nutrient limitation classes based on ¹⁴C-uptake response of Day 2 (columns) versus chl *a* response of Day 3 (rows). Limitation class codes: 00 = No response; XP = Exclusive P limitation; P1 = Primary P limitation; XC = Exclusive combined limitation; C1 = Primary combined limitation; N1 = Primary N limitation; XN = Exclusive N limitation (see 'Materials and methods' for details)

Chl <i>a</i> classification	¹⁴ C-uptake classification							Σ
	00	XP	P1	XC	C1	N1	XN	
00	2	2	0	0	1	1	1	7
XP	0	14	1	1	0	0	0	16
P1	1	5	28	3	1	0	0	38
XC	1	1	6	6	0	0	0	14
C1	1	1	4	0	1	5	0	12
N1	0	0	8	9	3	33	4	57
XN	3	0	0	3	1	1	2	10
Σ	8	23	47	22	7	40	7	154

The 2 northernmost stations (Oulu and Kokkola, in the Bay of Bothnia) showed very consistent and strong P limitation indicated by both response parameters throughout the growth season, with only occasional combined or N limitation incidents around midsummer, mainly manifested in chl *a* responses at the Kokkola station (Fig. 7). The intermediate Bothnian Sea and Archipelago Sea stations (Vaasa, Turku), however, demonstrated a very clear seasonal development in terms of P and N limitation.

At the Bothnian Sea station (Vaasa), there was a clear-cut shift from P limitation in spring to N limitation in summer (June to August), and back to P limitation in autumn, especially according to primary productivity responses (Fig. 6). The chl *a* responses also clearly

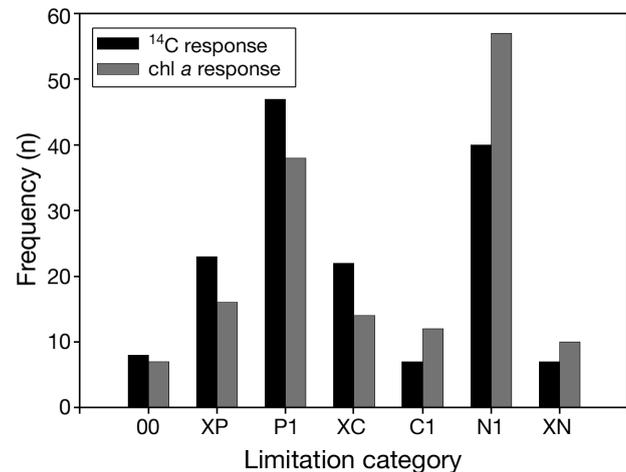


Fig. 5. Nutrient limitation classes based on ¹⁴C-uptake response on Day 2 versus chl *a* response on Day 3 (the marginal distributions of Table 3). Limitation class codes: 00 = No response; XP = Exclusive P limitation; P1 = Primary P limitation; XC = Exclusive combined limitation; C1 = Primary combined limitation; N1 = Primary N limitation; XN = Exclusive N limitation

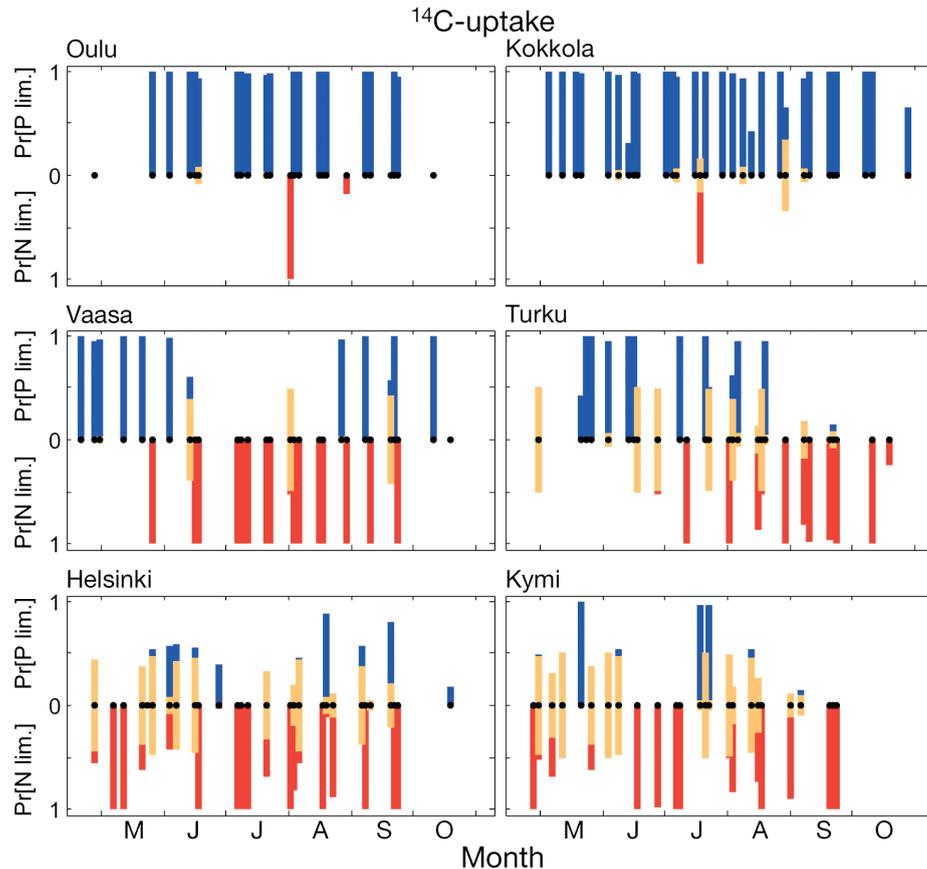


Fig. 6. Bootstrap probabilities (Pr) of P and N limitation inferred from ^{14}C -uptake (Day 1–2 responses), by location and season. Each experiment is classified 1000 times based on random selection within each duplicate measurement at the lowest replication level, and probabilities estimated by relative frequencies of different limitation classes. Accumulated probabilities for the 2 P limitation classes (XP and P1; blue bars) shown positive upward, the 2 N limitation classes (XN and N1; red bars) positive downward, and the combined P and N limitation classes (XC and C1; yellow bars) divided evenly between the two. Experiments with no significant response to nutrient addition (class 00) are marked only by black dots. Observations from the same week in different years are offset by ± 2 d to avoid overlap

showed more evidence of N, or combined N and P limitation during the spring and autumn periods (Fig. 7), but both parameters agreed on complete N limitation during the summer months.

At the Archipelago Sea station (Turku), the shift from P limitation of primary productivity prevailing in early summer to N limitation occurred later in the season (late August), with N limitation dominating throughout the autumn (Fig. 6). Chl *a* responses during the spring bloom (late April, early May) were clearly N-limited (Fig. 7); unfortunately spring bloom primary productivity samples from this location were available only for one sampling over the study years (Fig. 6). The share of combined N and P limitation in both parameter responses was high at this station throughout almost the entire growth season, but especially during summer months, and the successional pattern was in general much more variable than in the northern Bothnian

Sea (Vaasa), as successive years could also produce contrasting limitation results for equal time of season during summer months.

The 2 southernmost locations (Helsinki and Kymi, Gulf of Finland) showed generally consistent N limitation, although periods with significant P responses occurred, especially reflected in primary productivity responses of the post-spring bloom season (late May and early June), and autumn (Fig. 6). The share of combined N and P limitation was relatively high for primary productivity throughout the growth season in the Gulf of Finland, but at the Helsinki station the chl *a* responses were unequivocally N-limited (Fig. 7). Despite the N limitation dominance in chl *a* responses at the easternmost Kymi station as well, this station showed clearly more overall heterogeneity over the seasonal cycle, resembling the Archipelago Sea station (Turku) in this respect.

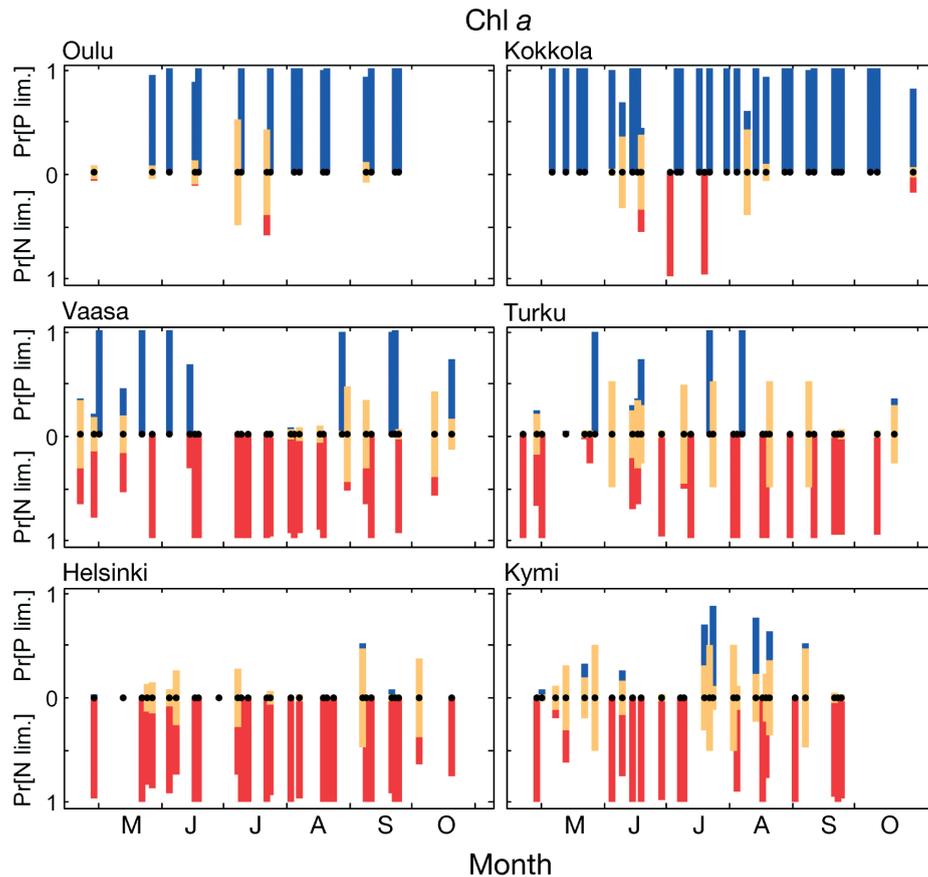


Fig. 7. Bootstrap probabilities (Pr) of P and N limitation inferred from chl *a* (Day 1–3 responses), by location and season. See legend to Fig. 6 for detailed explanation

DISCUSSION

Bioassay responses and *in situ* phytoplankton limitation

Both the loading ratios to the northern Baltic Sea (Stålnacke et al. 1999), and total N:P ratios at all stations (Table 1), suggest P as the dominant limiting nutrient throughout the northern Baltic Sea. The initial inorganic nutrients, however, would imply predominant P limitation only at the 2 northernmost stations (situated in the Bay of Bothnia), while in the remaining areas the low DIN and DIP levels provide a very uncertain basis for limitation assessment, and no seasonal succession is evident (Fig. 3). In contrast, experimental assessment of *in situ* limitation produced consistent patterns of limitation both between the areas, and over the growth season during the 3 yr study period (Figs. 4, 6 & 7). It thus appears that active experimentation, with short-term responses, is able to reveal reproducible *in situ* limitation patterns in situations, where more static or hierarchically distant ecosystem properties appear indecisive.

The consistent tendency of primary productivity to be more P-limited and chl *a* increase more N-limited (Figs. 4 & 5, Table 3) evidently relates to the different roles of P and N in phytoplankton cellular metabolism. P is mainly required for basic cellular regulatory and energy needs (nucleic acids of which ribosomal RNA constitutes the major pool, but also free nucleotides and phospholipids), while N is mainly used in proteins but is also needed for nucleic acids and key components of the photosynthetic machinery such as chlorophyll (Falkowski 2000, Geider & La Roche 2002, and references therein). The unequal association of P and N limitation patterns with the response parameters makes general sense if we assume that relatively more P is needed for the cellular energy and regulatory processes more closely related to the ^{14}C uptake response, while more N is needed for the biomass increase estimated by the chl *a* response.

The temporal development of responses in primary productivity and chl *a* during the 3 experimental days was gradual but distinct (Fig. 4). The share of indistinguishable responses became marginal in ^{14}C uptake after Day 1, while it took one day more for chl *a*

responses. Statistically, all dates for both parameters showed high resemblance ($p < 0.001$ for all pairwise likelihood ratio Chi-square tests), but a response succession pattern was evident for both parameters. Successive days produced identical chl *a* limitation classes in 52% (Day 1 to Day 2) and 68% (Day 2 to Day 3) of the experiments, but only 35% of chl *a* response classifications were identical on Days 1 and 3. This gradual development was mainly due to the exclusive limitation classes (N, P, and combined) diluting to corresponding primary limitation classes.

The exclusive limitation categories as defined here (see 'Materials and methods' and Andersen et al. 2006) are very strict in the sense that for the single limitation cases (XN or XP), any departure of the non-limiting nutrient from control, or the limiting nutrient from the combined (P + N) treatment, will shift the classification into one of the other limitation classes. The generally observed difference between the exclusive and primary single nutrient limitation classes in the data was that the single limiting nutrient treatment started to lag behind the combined nutrient addition at some phase of the experiment, i.e. the effect of the non-limiting nutrient was only visible through its effect in combination with the limiting one (see 'Materials and methods'). The exclusive limitation outcomes thus describe planktonic communities with abundant supply of the non-limiting nutrient over the whole duration of the experiment. The interpretation on both the cellular and community metabolism level is straightforward: only one nutrient is lacking.

The dominant XP limitation in the northernmost Bay of Bothnia basin (Fig. 4, stations Oulu and Kokkola) represents the most clear-cut example of a 'pure and simple' limitation pattern. However, even at these stations, only ca. 60% of the responses produced the exclusive P (XP) limitation class in Day 1 ^{14}C -uptake responses, while almost 50% of all experiments were classified as primary P limitation (P1) on Day 2 (Fig. 4). A similar replacement of XN by N1 classes was even more pronounced in chl *a* responses from Day 1 to 3. At the predominantly N-limited southern sites, the share of primary N limitation (N1) was higher to start with, and the dominance of this class increased among chl *a* responses during the successive days (Fig. 4).

It appears that planktonic communities growing under relative abundance of the non-limiting nutrient have adapted their metabolism by stocking their intracellular reserves with the non-limiting nutrient. The initial boosting of production and consequent biomass synthesis may thus be based on utilization of the experimentally increased supply of the limiting nutrient alone for a limited period of time, corresponding to a few cell divisions (1 to 2 d, Fig. 4). After this, exclusive limitation is replaced by primary limitation by the same nutrient, as the initially non-limiting one is exhausted from cellular reserves.

When the responses are used to interpret nutrient limitation prevailing in the initial community, the exclusive and primary limitation categories would thus seem to be successive temporal stages of a main limitation pattern, and for most applied purposes the limitation classes could be pooled into 3 basic categories: P, N and combined limitation (XP+P1, XN+N1, XC+C1).

Although the response patterns of ^{14}C uptake and chl *a* suggested different roles of P and N in phytoplankton limitation, both responses were overall significantly alike. The degree of uncoupling of primary production and biomass synthesis appears thus to be minor and mainly temporal. Such decoupling would imply an adaptively valuable buffering plasticity of phytoplankton metabolism with respect to the fluctuating resource availability of the planktonic habitat.

Food web modifications and experimental artifacts in responses

The goal of running bioassays is to produce information on the planktonic assemblage at the time of sampling. The inherent problem of any bioassay setup is therefore a tradeoff between 2 concerns. On one hand, the duration of the experiment should be sufficient to produce reliably measurable, as well as unambiguously interpretable responses to nutrient manipulations. On the other hand, accumulating experimental artifacts ('bottle effects') will also increase with time, possibly confounding the extrapolation of observed responses back to the initial situation.

These artifacts could arise from several sources. The confined experimental conditions can select for or against different algal or grazer species present in the natural community; the prevailing balance between nutrient assimilation and regeneration within the planktonic food web can be disrupted by the exclusion or uneven distribution of larger and less abundant components of the food web; and the potentially important spatial flows of nutrients affecting their *in situ* availability in the euphotic layer of the natural system (like sedimentation and advection) are cut off completely.

Phytoplankton community succession is a potentially confounding effect in bioassays (e.g. Pollinger et al. 1988), but constraining the length of the assay should safeguard against excessive community changes. Mimicking the natural conditions in terms of temperature and light quality is obviously important for minimizing artificial effects in assays. In order to attain reliable limitation patterns for nutrients, light levels should match saturating conditions and not impose additional limiting factors in the setup, invalidating the analysis (Andersen et al. 2006).

Perhaps the most difficult experimental artifact to avoid is the confinement effect creating an artificial turbulence regime. In our experiments, no attempt was made to mimic natural turbulence conditions, so it is possible that non-motile species were not performing naturally in spite of mixing the experimental bottles twice daily, and sinking out of saturating light conditions is not possible in confinement. In the absence of microscopy data, no estimate of this type of artificial succession is available. However, the regular mixing of experimental bottles during the short-term experiments should also rule out excessive pH changes and related CO₂ shortage, which according to our culture experience takes place at phytoplankton cell densities far above the natural community levels encountered in the experiments (for initial chl *a* levels, see Fig. 3).

The time series approach of our assays allowed us to distinguish between linear and higher-order (quadratic, cubic) time effects of the treatments (see Andersen et al. 2006), of which the latter were the most common in the data (percentages of linear, quadratic and cubic time effects in chl *a* responses were 14%, 32% and 55%, respectively), and they typically reflected the transition from exclusive to primary limitation of a single nutrient. Significant 3rd-order time effects were often accompanied by decreasing phytoplankton biomass at the end of the experiment, very probably reflecting a delayed response of grazers to increased prey abundance. As the natural planktonic communities were not subject to any pre-screening treatment, the microbial food web was present at natural abundances, including crustacean zooplankton.

It is obvious that over the seasonal succession, smaller grazers (rotifers, ciliates, nanoflagellates) will also be important components of the food web in the Baltic Sea as elsewhere (Kivi 1986, Kuosa & Kivi 1989), with ciliate and nanoflagellate growth rates matching those of their algal prey (Kuuppo-Leinikki et al. 1994). Tight grazing control would dampen down the phytoplankton biomass responses to nutrient enrichment. Kivi et al. (1993) demonstrated type of cascading enrichment effect with a comparable experimental setup, as ciliate biomass showed clear responses to nutrient additions in the time scale of corresponding but minor phytoplankton responses.

An interesting indication of non-intuitive food web effects was a response pattern that was reproduced several times (ca. 5% of the experiments) over the experimental campaign, and which appears at first sight nonsensical: the combined N+P addition produced a strong chl *a* response accompanied by a weaker but distinct N response, as well as by a significant negative P response (see examples in Andersen et al. 2006). The classification method reports the out-

come as primary combined limitation (C1), as it does not take into account the signs of responses.

This might seem a handicap of the classification scheme, as combined limitation categories with positive responses are easily conceived as describing a phytoplankton community with compartments limited by different nutrients, but inhibition by moderate P addition is not really explainable. However, bacterioplankton are naturally present in the bioassays, and if they have access to an organic N source unavailable to N-limited phytoplankton, their competition for P can lead to decrease in algal biomass, as demonstrated by Thingstad et al. (2005). Although both algae and bacteria have been shown to utilize organic N and P, this is more widespread in heterotrophic nutrition (e.g. Berman & Bronk 2003). All below-control responses in our experiments were for single P treatment, never for single N, suggesting more intense competition between algae and bacteria for DIP than for DIN.

In summary, time series based bioassays with appropriate setup and analysis of the responses can yield important insights on the *in situ* limitation, as well as on the production dynamics and functioning of the planktonic food web. This includes both basic phytoplankton responses, and their interactions with other food web compartments. However, reliable interpretation of different types of cascading food web effects, though indicated by phytoplankton responses, would naturally require additional information on the main heterotrophic organism groups.

Seasonality in nutrient limitation patterns

The high frequency of combined or non-exclusive limitation outcomes at all locations indicates close couplings between P and N cycles in the planktonic ecosystem. This is further emphasized by the seasonal patterns of nutrient limitation (Figs. 6 & 7). Even in the cases of relatively clear-cut P limitation in the Bay of Bothnia and N limitation of the Gulf of Finland, the severity of the dominant limitation pattern changes during the season. The post-spring bloom and/or summer period showed instances of combined limitation, or even occasional limitation by the non-predominant nutrient. The Bothnian Sea station (Vaasa) showed a very consistent pattern of seasonal development of the limiting nutrient, as spring and autumn were clearly P-limited, but summertime showed unequivocal N limitation during all study years (Figs. 6 & 7). Seasonality in limitation patterns was apparent at the Archipelago Sea (Turku) and Gulf of Finland (Helsinki, Kymi) stations as well, although variability during all seasons was clearly higher in the southern basins, especially at the Turku and Kymi stations.

Seasonality in nutrient limitation of the planktonic community has been previously observed in several environments, mainly in lakes (Pollinger et al. 1988, Vanni & Temte 1990, Elser et al. 1990, Sterner 1994) but also in a variety of coastal environments (D'Elia et al. 1986, Paasche & Erga 1988, Fisher et al. 1999). Our results demonstrate that the seasonal patterns can be highly reproducible, and they can vary distinctly even between closely related environments (as in the northern basins Bay of Bothnia and Bothnian Sea; Figs. 6 & 7).

In systems exhibiting seasonal patterns in nutrient limitation, an analysis of the basin-wide biogeochemical cycles behind the limitation patterns is obviously a major challenge for targeting eutrophication control actions effectively. Basic load-response models without explicit seasonal dynamics appear insufficient for these environments. Understanding the causes of seasonal limitation shifts requires combining experimental limitation assessment and monitoring data in order to analyze the biogeochemical nutrient cycles, and especially their interactions.

Hydrographically mediated, seasonal vertical nutrient fluxes between the surface and bottom layers are potentially important features of environments where seasonal patterns in nutrient limitation are encountered. Mechanisms for these shifts could include selective (non-Redfield) removal of nutrients from the euphotic zone via sedimentation (Heiskanen et al. 1996, Thomas et al. 1999), unbalanced losses of N and P at the sediment-water interface via denitrification and, according to oxygen conditions, iron precipitation or release of PO_4 (Seitzinger 1988, Blomqvist et al. 2004). Seasonal and episodic mixing events introduce the modified (in terms of N:P) near-bottom waters back to the productive layers. Seasonal limitation shifts would thus appear most likely in relatively shallow temperate waters.

Cyanobacteria and N-limitation

In the case of the Baltic Sea, the picture is further complicated by the occurrence of N_2 -fixing filamentous cyanobacterial blooms, common in lakes but generally absent from oceanic coastal regions. These blooms (with dominant species *Nodularia spumigena* and *Aphanizomenon* sp.) have regularly occurred in the southern basins including the Baltic Proper, but not in the Gulf of Bothnia (Kahru et al. 1994, Larsson et al. 2001). Sedimentary evidence suggests that they have been a component of the Baltic Sea ecosystem throughout its present brackish state (Bianchi et al. 2000). Cyanobacterial blooms should primarily be nutritionally dependent on dissolved P due to their access to atmospheric N_2 .

The southern basins around Finland (Archipelago Sea, Gulf of Finland) were predominantly N-limited throughout the whole growth season, as was the Bothnian Sea for the summer months (station Vaasa, Figs. 6 & 7). Yet cyanobacterial blooms appear for limited summertime periods, and in the southern basins only (Kahru et al. 1994). It is therefore evident that causes for the cyanobacterial blooms in the Baltic Sea are more complicated than just a matter of P surplus and sufficiently high temperature, which has so far been the standard interpretation (see Larsson et al. 2001). Recent evidence on the variability in species-specific responses of filamentous and colonial cyanobacteria to N and P enrichment supports this conclusion (Kangro et al. 2007).

The spring bloom is by far the most productive period of the growth season in the northern Baltic Sea (Lignell et al. 1993), supplying the largest sedimentation flux to bottoms (Heiskanen 1998). This season is unequivocally N-limited in the areas where cyanobacterial blooms occur (Figs. 4, 6 & 7). Monitoring data since the experimental period of this study suggest that N limitation of the spring bloom has even intensified, as inorganic P levels remaining after the bloom period have clearly increased in the Gulf of Finland since mid-1990s (Finnish Environment Institute unpubl. data).

The interactions and feedbacks between N and P cycles appear clearly on the system level in the Baltic Sea. The consequence of increasing N availability at the commencement of the N-limited spring bloom is higher biomass production and sedimentation, leading to enhanced P release from sediments in summer due to enhanced oxygen consumption. Aggravated P release from the sediments has indeed been recently demonstrated in the Gulf of Finland, in connection to increased anoxic coastal bottom areas over the permanent halocline (Pitkänen et al. 2001).

High springtime N levels would therefore tend to maintain high summertime P levels, favoring the cyanobacterial blooms, importing more N to the system—a vicious eutrophication circle which is obviously impossible to tone down without significantly decreasing both the N and P loads to the system. Nitrogen fixation does not appear to compensate for the recently escalated relative shortage of N on the system level, as the increasing P levels in the southern basins have not been followed by Redfield-proportional N increases over a decade—to the contrary, N concentrations have shown a simultaneous decrease (Finnish Environment Institute, Swedish Meteorological and Hydrological Institute unpubl. data). This indicates that on time scales relevant for management perspectives, decreased N loads to dampen down the spring bloom production would very unlikely be quantitatively compensated by enhanced N_2 -fixation.

The northern Baltic Sea basins: lakes or coastal zones?

Our results confirm and extend previous evidence of prevailing N limitation of the southern Baltic Sea basins (Gráneli et al. 1990, Elmgren & Larsson 1997), Gulf of Finland (Kivi et al. 1993, Pitkänen & Tamminen 1995) and Gulf of Riga (Seppälä et al. 1999). The only predominantly P-limited coastal areas (Figs. 4, 6 & 7) were situated in the Bay of Bothnia (Fig. 1; Oulu, Kokkola), where average salinities were below 3.5 PSU (Table 1). The transition between P and N limitation patterns occurs over a surprisingly narrow salinity range. The eastern Gulf of Finland (Kymi), with an average salinity of 3.6 PSU, showed predominant N limitation, and the northern part of Bothnian Sea (Vaasa), which is situated only some 150 km south of the P-limited Kokkola station, was clearly N-limited throughout the summer months.

The persistent difference in the average salinities of the stations of the 2 northernmost basins (Bay of Bothnia and Bothnian Sea; < 3.5 and 5.1 PSU, respectively; Table 1) is a reflection of the anti-clockwise circulation patterns of the basins, which force the voluminous freshwater inflow into the Bay of Bothnia (from northern Finnish and Swedish rivers) southward along the western, Swedish coastline. The eastern, Finnish side of the Bothnian Sea thus receives more saline waters originating from the south, Archipelago Sea and Baltic Proper (Mälkki & Tamsalu 1985). It is therefore possible that the P-limited regime extends further south to the northern Bothnian Sea on the western, Swedish coast. However, the Quark strait that divides the Gulf of Bothnia into 2 major basins (Bay of Bothnia and Bothnian Sea) seems to be a geographical threshold for transition from predominant P to N limitation.

Besides the salinity regimes, the study areas differ significantly in the degree of anthropogenic eutrophication as reflected in the average total nutrient and chl *a* concentrations (Table 1, Fig. 3). The 2 Gulf of Finland stations (Helsinki and Kymi) stand out in terms of eutrophication, with the Archipelago Sea in an intermediate position. These large-scale differences are due to intensive agriculture and large municipal and industrial point sources around the southern basins. The Gulf of Finland catchment area includes the largest river discharging into the Baltic Sea (the Neva) flowing through the largest coastal city, St. Petersburg, located at the easternmost end of the Gulf. In contrast, the Gulf of Bothnia catchment is very sparsely populated with modest point sources of nutrients and land use dominated by forestry (Stålnacke et al. 1999, HELCOM 2004).

Increasing eutrophication appears to shift the P limitation of Baltic pristine low-salinity areas towards N lim-

itation, as demonstrated by the contrast between Kokkola and Kymi stations with closely corresponding salinities. The persistent N limitation of the eutrophic Helsinki station differs clearly from the seasonally shifting P and N limitation of the Vaasa station, although their average salinities are similar (Table 1, Figs. 4, 6 & 7). Previous studies from the most eutrophied eastern Gulf of Finland (Pitkänen & Tamminen 1995) and the eutrophic Gulf of Riga (Seppälä et al. 1999) have demonstrated that N limitation occurs in these environments at salinities comparable to the P-limited Bay of Bothnia.

The mechanisms for the tendency of eutrophication to drive the system towards N limitation are most likely connected to intensified pelagic-benthic coupling in the eutrophied Baltic areas. This causes less permanent removal of P through sedimentation, as well as increasing removal of N through denitrification, under conditions of reduced oxygen levels in bottom layers. Despite high N:P ratios prevailing in terrestrial loading (for the Baltic Sea as a whole, triple the Redfield ratio; Stålnacke et al. 1999, HELCOM 2004), the surface layer inorganic N:P ratio tends to decrease rapidly, even clearly below the Redfield ratio, under eutrophic conditions (Pitkänen & Tamminen 1995, Tamminen & Seppälä 1999), a phenomenon observed also in other coastal systems (Fisher et al. 1999).

The highest variability in nutrient limitation patterns both seasonally (Figs. 6 & 7) and in general (Fig. 4) was found at the Turku (Archipelago Sea) and Kymi (eastern Gulf of Finland) stations. The most likely explanation for this heterogeneity is their closest proximity to the anthropogenic point sources of nutrients. Local fish farming constitutes a large fraction of the nutrient load within the mosaic of the Archipelago Sea (Hänninen et al. 2000), whereas the largest nutrient load to the Gulf of Finland is discharged into the easternmost estuary from the Neva river and city of St. Petersburg (HELCOM 2004, Kuuppo et al. 2006), only ~150 km from the Kymi station. Continuous nutrient discharges, conveyed by weather-driven variable surface currents, obviously diversify the annual development of limitation patterns in these areas.

Our results indicate that nutrient limitation patterns in coastal low-salinity regimes are modified towards N limitation when exposed to eutrophication pressure. Prediction of nutrient limitation on the basis of salinity or loading ratios is not reliable. The high fraction of non-exclusive limitation categories in our experiments demonstrates that phytoplankton can grow close to balanced limitation for extended periods of the season, which creates considerable methodological challenges for limitation assessment, and necessitates dedicated experimentation.

P vs. N limitation

The demonstration of closely coupled P and N cycles and their seasonal interplay in nutrient limitation of the coastal Baltic Sea carries obvious implications for environmental management in the catchment area, but also points out some important aspects of the role of coastal zones in general. The dynamic processes transforming the terrestrial nutrient loading within the coastal system make nutrient limitation patterns inferred from loading ratios very uncertain. Seasonality in nutrient limitation has been demonstrated in widely differing coastal areas like the Baltic Sea (Kivi et al. 1993, this study), the Gulf of Mexico (Lohrenz et al. 1999), the Oslo fjord (Paasche & Erga 1988), and Chesapeake Bay (D'Elia et al. 1986, Fisher et al. 1999). Seasonality further underlines the significance of coastal biogeochemical cycles in modifying the terrestrial nutrient signals at a distance within the range of human vision from coastal point sources.

A potentially interesting corollary of the dynamic role of the coastal zone relates to the discussions on the nutrient limitation of the World Ocean. Redfield's original concept of self-regulating oceans via planktonic stoichiometry (Redfield 1934, 1958) was recently formulated into a 1-dimensional model (Tyrrell 1999) built on 2 basic assumptions: terrestrial nutrient fluxes are evenly distributed over the World Ocean, and temporary N-limited phases are relieved by N₂-fixation. From these assumptions it appears that an 'ultimate P limitation' cannot be avoided.

In the northern Baltic Sea we could verify neither of these assumptions. The nutrient loading does not reach offshore areas without major modifications and losses, as N-rich terrestrial nutrient exports are transformed in near-coast environments into N-limited pelagic domains. The verified N limitation does not automatically lead to appearance of N₂-fixing plankton blooms (Bothnian Sea), and even in areas where the whole growth season is predominantly N-limited (Archipelago Sea, Gulf of Finland), the N₂-fixing blooms appear only for a fraction of the season. The main cyanobacterial blooms appear in the most offshore areas of the Baltic Proper (Kahru et al. 1994), with maximal distance from terrestrial nutrient sources—not unlike the global distribution of the major diazotroph *Trichodesmium* (Capone et al. 1997). As the seasonal cycle in the southern Baltic Sea basins recurrently starts from a strongly N-limited spring bloom, it appears that N₂-fixation during late summer cannot alleviate the basic N-limitation pattern on the basin scale.

If this geographical and temporal uncoupling of terrestrial nutrient loads and offshore N₂-fixation appears in the relatively small scale of the Baltic Sea, where the

nitrogen-fixing planktonic species portray globally exceptional visibility, it is not immediately apparent how the 1-dimensional concept of the homeostatic World Ocean (Tyrrell 1999) could describe such a vast system realistically. It is obvious that the complexity of the nutrient cycles within marine microbial communities, including coastal zone dynamics as well as non-Redfield behavior and multiple resource co-limitations (Arrigo 2005), still remains a domain of aquatic sciences where immediate management interests (Cloern 2001) overlap with challenges for basic plankton physiology, stoichiometric system analyses, and understanding of biogeochemical cycles from local to global scales.

CONCLUSIONS

Nutrient limitation of natural phytoplankton communities cannot be reliably inferred from large-scale nutrient budgets, pool sizes, or loading ratios, in cases where several potential limiting nutrients show apparently low availability, or where these ecosystem qualities produce apparently contrasting conclusions. The Baltic Sea is an example where coastal processes strongly modify the nutrient loading signals, to the effect that widespread N₂-fixing cyanobacterial blooms occur despite high excess of N in nutrient inputs to the system. We have demonstrated that active experimentation, comprising of short-term time series bioassays, coupled to a novel model selection procedure for unsupervised classification of responses, is able to reveal reproducible *in situ* nutrient limitation patterns that cannot be inferred from nutrient data alone. We advocate the use of *a priori* classification of bioassay limitation responses instead of site-specific protocols, to facilitate meaningful comparative studies of different aquatic systems.

Our limitation patterns highlighted the seasonal and vertical interplay of the cycles of N, P, and O in the coastal Baltic Sea, and the results help to explain the apparent discrepancy between nutrient loading and planktonic responses. The management conclusion is to delimit both N and P loading to alleviate eutrophication symptoms, because of both direct and cascading biogeochemical effects within the system. It is evident that a combination of experimental assessment of *in situ* limitation, monitoring data on the biogeochemical cycles of main nutrients, and seasonal hydrographical forcing of the system, is able to provide insights into the coastal modification of nutrient pools that are important both for coastal management, and for assessing the role of the coastal zone in large-scale nutrient and production dynamics of the marine environment.

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