Long-term phytoplankton–nutrient interactions in a shallow coastal sea: Algal community structure, nutrient budgets, and denitrification potential

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Abstract

In the eutrophic Marsdiep, the westernmost tidal inlet of the Wadden Sea, phytoplankton biomass, and production almost doubled at the end of the 1970s and remained high ever since. Principal component analysis of 21-yr (1974–1994) high-resolution time series of the 32 most numerous marine algal species revealed that the phytoplankton community changed drastically both between 1976 and 1978 and again between 1987 and 1988, and that it was relatively stable in-between (1974–1976, 1978–1987) and thereafter (1988–1994). These major changes in phytoplankton biomass and species composition coincided with changes in absolute and relative (TN : TP) nutrient concentrations. During the summer of 1977, the Marsdiep shifted from a rich, but phosphorus-controlled system to an even more eutrophic but nitrogen-controlled environment. The system reshifted towards P-control between 1987 and 1988. The coincidence of the shifts in relative nutrient concentrations and phytoplankton species composition implies a strong causal relationship between TN : TP ratios and phytoplankton community structure. Among diatoms, the observed increase in phytoplankton biomass under eutrophic N-controlled conditions was particularly due to an increase of the abundance of larger algae. Our results indicate that the N budget of the area is correlated with the community structure, suggesting enhanced loss of nitrogen to the sediment through increased deposition of larger algal cells.

In shallow coastal marine waters, the total primary production and biomass of phytoplankton is generally assumed to be hyperbolically related to nutrient loadings from land and subsequent availability of these nutrients in the water column (e.g., Borum and Sand-Jensen 1996). However, understanding the effect of nutrient enrichment on living resources requires detailed knowledge of how nutrients enter and leave these waters. Dissolved and particulate materials as well as living organisms are exchanged between the coastal waters and the open sea, and net material fluxes appear to depend on physical and biological responses within these systems to changes in nutrient loadings from land (e.g., Dame and Allen 1996).

Phytoplankton species obviously respond in different ways to nutrient enrichment, most probably depending on their specific life-history characteristics such as growth curves and storage capacities (Grover 1997; Roelke et al. 1997). Current theories on plankton ecology predict that an eutrophic and nitrogen-controlled environment gives rise to a dominance of large phytoplankton species (Stolte and Riegman 1995) because due to their larger storage capacity they are better competitors under high and fluctuating nutrient regimes (Sommer 1984; Stolte et al. 1994; Grover 1997), while the biomass of smaller algae is controlled by microzooplankton (Thingstad and Sakshaug 1990; Riegman et al. 1993). This will subsequently result in an enhanced flux of large phytoplankton species to benthic communities via sedimentation (Thingstad and Sakshaug 1990; Riegman et al. 1993), where part of the associated nutrients is buried or mineralized. Algal cells can store nitrate in their vacuoles (Dortch 1982; Stolte et al. 1994; Stolte and Riegman 1996). Deposition of such cells may result in accumulation in the sediment (Cadée 1996) with intact nitrate pools. After disruption of the cells, these pools may form an additional source of nitrate to the denitrifying community resulting in further enhancement of benthic denitrification rates (Lomstein et al. 1990).

The above-described theories are almost exclusively based on modeling exercises and laboratory experiments. Long-term field observations on marine phytoplankton in the Marsdiep, the westernmost tidal inlet of the Wadden Sea (Fig. 1A), showed that the mean annual chlorophyll content and primary production of the phytoplankton community almost doubled between 1976 and 1978 and remained high thereafter (Cadée 1992; Cadée and Hegeman 1993; Beukema and Cadée 1997). These changes in general phytoplankton characteristics are considered to be induced by the gradual increase in freshwater nutrient supply (Beukema and Cadée 1986; Van der Veer et al. 1989; Schaub and Gieskes 1991; Cadée 1992; Cadée and Hegeman 1993). The coinciding increased blooming of the colonial flagellate *Phaeocystis* spp. was attributed to a shift from phosphorus- to nitrogen-controlled phytoplankton production (Riegman et al. 1992).

In this study, we relate long-term changes in the phytoplankton community structure with those in the sources and

Fig. 1. The study area with locations of the phytoplankton sampling station in the Marsdiep tidal inlet (Sta. N, NIOZ Jetty, 53°00′18″N, 04°47′42″E), and the nutrient sampling stations in the North Sea (Sta. a, Callantsoog, 52°51′6″N, 04°40′27″E), the western Wadden Sea (Sta. b, Marsdiep, 52°59′02″N, 04°45′02″E; Sta. c, Vliestroom, 53°18′52″N, 05°09′37″E), and near the sluices in the dam that closes off the man-made freshwater lake IJsselmeer from the Wadden Sea (Sta. d, Den Oever; Sta. e, Kornwerderzand). (A) Geographical map of the study area. (B) One-compartment representation of the western Wadden Sea. Solid arrows represent tidally-averaged advective water transport (Q_1, Q_2) and bimonthly averaged major freshwater inputs (Q_d, Q_e), the dashed arrow (K_1) represents the dispersive exchange with the North Sea (Ridderinkhof et al. 1990).

sinks of nutrients in the western Wadden Sea ecosystem. First, we examined if the shift toward eutrophic conditions was accompanied by a shift in phytoplankton species composition, in particular toward larger species with relatively large storage capacity. For this purpose, we analyzed a 21-yr time series of phytoplankton species composition in the Marsdiep tidal inlet for the existence of covariability (i.e., coexistence) between phytoplankton species with the assumption that their numbers may be related to common environmental conditions such as limiting resources. Second, we compiled bimonthly phosphorus and nitrogen budgets of the western Wadden Sea to assess the impact of changes in nutrient loadings from land on other fluxes to and from the system, i.e., exchange with the open sea, burial, and denitrification. Finally, we discuss the link between long-term changes in system-wide denitrification and phytoplankton biomass and species composition, focusing particularly on size structure and nitrate storage capacity.

Material and methods

Phytoplankton

Sampling—Bucket water samples were collected at high water from the so-called NIOZ jetty, which is located at the northern shore of the Marsdiep tidal inlet (Fig. 1), between 1974 and 1994. Sampling frequency varied from once to twice a month in winter up to twice a week during phytoplankton spring blooms.

Algal densities—Phytoplankton samples were preserved with Lugol and cells were counted with a Zeiss inverted microscope using 5-ml counting chambers. Samples were processed within one week to one month to avoid cell deterioration (Cadée 1986). Most algae were identified to the species level; the others were clustered into larger groups. The second author performed all identifications and counts of the algae during the entire study period. Identification of the phytoplankton species was based on Drebes (1974); nomenclature of the phytoplankton was updated according to Tomas and Hasle (1997). The group of flagellates *Phaeocystis* spp. probably contains only *P. globosa*. A few of the irregular colonies of *P. pouchetii* (*see* Jahnke and Baumann 1987) were with certainty observed only later in the Marsdiep, viz. in the summers of 1996 and 1997. Note that *P. globosa* is, erroneously, often referred to as *P. pouchetii* in earlier Marsdiep publications (Cadée and Hegeman 1991).

Statistical analysis of changes in the phytoplankton covered the 32 most numerous marine algal taxa, i.e., 27 diatoms and 5 flagellates (Table 1), which together contributed more than 99.9% to the total numbers of marine algae in the Marsdiep between 1974 and 1994. Freshwater algae originating from the man-made freshwater lake IJsselmeer (Fig. 1) were excluded. For each taxon, the density (cells mL^{-1}) was averaged for every month from 1974 to 1994.

Biomass—For biomass (expressed as Chl *a*) measurements, additional 0.5 to 1 liter water samples were filtered over MgCO$_3$ coated filters (Whatman GF/C); acetone extracts were measured following Lorenzen (1967). Chl *a* concentrations (mg m^{-3}) were averaged for every month from 1974 to 1994.

Species composition—In order to account for the seasonality in limiting resources, viz. light during winter and nutrients during summer, multivariate analyses of phytoplankton species composition were performed separately for six different bimonthly periods. The average interannual covariability between algal species (i.e., coexistence) was examined by means of a Principal Component Analysis (PCA) that was performed on the correlation matrices of species abundances by means of SYSTAT (Wilkinson 1988). Data on algae densities were logarithmically transformed and standardized to mean zero and variance one for each species before analysis. As the result of standardization, all algal species had a similar weight.

The results of the PCA were visualized in biplots showing the correlations between each pair of species (Gabriel 1971). The product of the cosine of the angle between the two abundance vectors and the length of the two vectors indicates the

Table 1. Taxon code as used in biplots (flagellates in italics), scientific name, the type of possible grouping of cells (based on observations by the second author), individual cell volume (μm^3), and average monthly total cell volume (cm^3 m^{-3}) of the 32 most numerous marine phytoplankton species or groups of species in the Marsdiep tidal inlet between 1974 and 1994. Individual cell sizes as listed by U. Tillmann (Research and Technology Centre Westcoast, University of Kiel, Germany).

| | | | Biovolume | |
Code	Taxon	Appearance	(μm^3 cell^{-1})	(cm^3 m^{-3})
	Flagellates			
FLAGL	large *Flagellates* spp.	single cells	612	3
FLAGS	small *Flagellates* spp.	single cells	60	9
GYROS	*Gyrodinium* spp.	single cells	5,891	1
PHAES	*Phaeocystis* spp. (mainly *P. globosa*)	single cells or colonies	65	42
RHODS	*Rhodomonas* spp.	single cells	221	1
	Diatoms			
ASTEG	*Asterionellopsis glacialis*	chains	345	2
ASTEK	*Asterionella kariana*	chains	411	<1
BROCB	*Brockmanniella brockmannii*	chains	350	2
CERAP	*Cerataulina pelagica*	chains	6,595	6
CERAPE	*Cerataulina pelagica* var. *elongata*†	chains	1,649	1
CHAEL	large *Chaetoceros* spp.	chains	2,941	3
CHAES	small *Chaetoceros* spp. (mainly *C. socialis*)	chains or colonies*	318	6
CYLIC	*Cylindrotheca closterium*	single cells	204	<1
CYMAB	*Cymatosira belgica*	single cells	300	<1
DYTIB	*Ditylum brightwellii*	single cells	11,796	2
EUCAZ	*Eucampia zodiacus*	chains	3,427	1
GAILS	*Gaillonella sulcata*	chains	1,767	3
GUIND	*Guinardia delicatula*	chains	2,050	11
LAUDA	*Lauderia annulata*	chains	7,310	2
LEPTD	*Leptocylindrus danicus*	chains	477	<1
LEPTM	*Leptocylindrus minimus*	chains	160	2
NITZL	*Nitzschia longissima*	single cells	204	<1
ODONA	*Odontella aurita*	chains	2,399	2
PSEUS	*Pseudo-nitzschia* spp. (incl. *P. seriata, delicatissima, pungens*)	single cells	160	1
PENND	Pennate diatoms	single cells	895	2
PLAGV	*Plagiogrammopsis vanheurckii*	chains	611	<1
RHIZI	*Rhizosolenia imbricata*	chains	8,416	6
RHIZS	*Rhizosolenia hebetata* & *R. setigera*	chains	9,465	8
SKELC	*Skeletonema costatum*	chains	145	3
THALL	large *Thalassiosira* spp.	chains	4,925	22
THALN	*Thalassionema nitzschioides*	chains	827	1
THALS	small *Thalassiosira* spp.	chains	423	<1

* In most cases, the main species in this group (*C. socialis*) has appeared in colonies.
† A very slender growth form of *Cerataulina pelagica* without taxonomic status (distinguished from common growth form because of its difference in biovolume).

correlation. So, long vectors which are pointing in the same direction indicate a high positive correlation between species abundances, while orthogonal vectors indicate a zero correlation. Additionally, the orthogonal projection of each bimonthly observation on an abundance vector approximates the relative value of that species in that particular year. The lengths of the vectors indicate the reliability of these results: the approximation is almost perfect when vectors approach the unit circle.

Size structure—Due to the absence of long-term time series on individual cell volumes of the phytoplankton involved, we had to assume that individual cell sizes were constant in time, i.e., that there was no seasonal or interannual variation in cell size (e.g., due to auxosporulation cycles). The total cell volume (biovolume) of the phytoplankton community was then calculated as the sum of individual cell volumes of all species present. The species-specific cell volumes were derived from lists supplied by U. Tillmann (Research and Technology Centre Westcoast, University of Kiel, Germany), that were based on taxa-specific allometric formulae derived and adjusted from Rick (1990) and Edler (1979). Additionally, we applied the data on cell volume to examine the size-specific contribution of the 27 diatom species (Table 1) to changes in the summed-cell volume of all diatoms present.

Nitrate storage capacity—Total vacuole volume is considered to be an index of the ability of diatoms to store inorganic nitrogen (Dortch et al. 1985; Stolte et al. 1994). This volume was calculated as the sum of individual vacuole volumes of all diatoms present derived from their total cell

Table 2. Main characteristics and water mass fluxes of the western Wadden Sea as based on the hydrodynamical model by Ridderinkhof et al. (1990) and data on freshwater inputs between 1975 and 1993 (114 bi-monthly averages) supplied by the Dutch Ministry of Transport and Public Works.

	Symbol	Value	Unit
Characteristics			
Volume		4.66×10^9	m^3
Surface area		1.41×10^9	m^2
Average depth		3.3	m
Tidal exchange		3.60×10^7	m^3 tide^{-1}
Tidal frequency		1.92	tides day^{-1}
Residence time		9	days
Inputs			
Freshwater discharges IJsselmeer	Q_d	294 ± 137	m^3 s^{-1}
	Q_c	194 ± 121	m^3 s^{-1}
Advective transport from North Sea	Q_2	703 ± 60	m^3 s^{-1}
Outputs			
Dispersive transport (tidal exchange)	K_1	3,556	m^3 s^{-1}
Advective transport to North Sea	Q_1	$1,192 \pm 192$	m^3 s^{-1}

volume according to Stolte and Riegman (1996). To compensate for coinciding changes in total phytoplankton biomass, we divided the total vacuole volume by ambient chlorophyll concentrations.

Since we have no actual data on the intracellular nitrate pools for the western Wadden Sea, their magnitude had to be assessed on the basis of available data from other coastal marine waters (Dortch et al. 1985; Petterson 1991), from which we established an allometric regression between internal nitrate pools (IC$_{NO_3}$, nmol L^{-1}) and chlorophyll concentrations (Chl *a*, μg L^{-1}): IC$_{NO_3}$ = 1.04·Chl *a*$^{2.11}$ ($n = 19$, $r^2 = 0.88$, $P < 0.0001$).

Nutrients

Nutrient dynamics—Time series on nutrient loadings and concentrations were taken from the water quality monitoring database (DONAR) of the Dutch Ministry of Transport and Public Works. Total phosphorus (TP) includes phosphate (PO$_4^{3-}$), dissolved organic phosphorus (DOP), and particulate compounds of phosphorus; total nitrogen (TN) is the sum of ammonium (NH$_4^+$), nitrate plus nitrite (NO$_x$), dissolved organic nitrogen (DON), and particulate compounds of nitrogen.

Nutrient budgets—To determine the nutrient fluxes to and from the western Wadden Sea, we have compiled the phosphorus and nitrogen budgets for this system for every two-month period from January–February 1975 to November–December 1993 ($n = 114$). The nutrient budgets were based on a hydrodynamical model of this well-mixed system (Table 2) containing advective water transport and tidal exchange rates as estimated by Ridderinkhof et al. (1990). Nutrient mass fluxes were calculated by multiplying the advective water transport rates (Q_1 and Q_2 in Fig. 1B) with corresponding nutrient concentrations, and the tidal exchange rate (K_1 in Fig. 1B) with the corresponding nutrient gradient (Van Raaphorst and Van der Veer 1990). The atmospheric nitrogen input was based on values estimated for the southern North Sea by Rendell et al. (1993).

Nutrient loadings were determined as the summed outputs by two sluices, Den Oever and Kornwerderzand, from the freshwater lake IJsselmeer (Sta. d and Sta. e in Fig. 1). The advective transport rates were based on nutrient concentrations at the interior (Sta. c in Fig. 1) and at the main tidal inlet (Sta. b in Fig. 1) of the western Wadden Sea. The dispersive transport rates between the North Sea and the western Wadden Sea were based on nutrient concentrations at two monitoring stations at the seaward and at the shoreward side of the main tidal inlet (Sta. a and Sta. b in Fig. 1) which are representative for the tidal exchange in this inlet (Ridderinkhof et al. 1990).

No consistent nutrient data were available for the North Sea station (Sta. a in Fig. 1) for the entire study period. Therefore, we estimated the nutrient concentrations at this station from nutrient time series of other upstream stations in the North Sea, viz. the average values of two coastal stations (52°03′12″N, 04°09′36″E; 52°15′41″N, 04°24′22″E) and of two offshore stations (52°26′06″N, 03°23′24″E; 52°35′10″N, 03°31′53″E). Salinity at Sta. a was then used as an index for mixing of the coastal and offshore waters.

Each bimonthly phosphorus budget was closed with a residual TP-flux, which included the accumulation of particulate matter originating from the open sea, a process described for the Wadden Sea (Postma 1961) and several other coastal areas (Postma 1980). Subsequently, the inward transport rates of nitrogen were calculated from coinciding phosphorus fluxes using ambient nutrient ratios (mol mol^{-1}) according to:

$$F_{TN, inward} = (N_{part} \cdot P_{part}^{-1}) \cdot F_{TP, inward}$$

Finally, the nitrogen budget was closed with a residual N-specific flux which comprises net inputs ($+$) or outputs ($-$) not accounted for in the TP budget.

Missing values

Missing values within the data sets (phytoplankton species, Chl *a*, and nutrients) were filled in under the assumption that the expected monthly averaged density of each spe-

Table 3. Number of phytoplankton species (*n*) and percentage of variance explained by the first two axes (PC1 and PC2) in biplots of most numerous marine phytoplankton species in the Marsdiep tidal inlet between 1974 and 1994 during different bimonthly periods of the year.

Period	Months	*n*	Explained variance (%)		
			PC1	PC2	Sum
I	Jan–Feb	27	20	17	37
II	Mar–Apr	31	24	15	39
III	May–Jun	32	21	16	37
IV	Jul–Aug	32	19	16	35
V	Sep–Oct	32	18	16	34
VI	Nov–Dec	29	20	15	35

cies was a product of a 'year effect' and a 'month effect.' These effects were estimated by a linear Poisson regression (McCullagh and Nelder 1989). For phytoplankton abundances and Chl *a*, we excluded two years (1977 and 1981) from further analysis due to their relatively high proportion of missing values. For the remaining 19 years, the monthly densities of each phytoplankton species were based on 218 actual averages and 10 imputed values and those of Chl *a* on 217 actual averages, and 11 imputed values. The number of missing values within the nutrient data sets of 19 years ranged from 24 to 27, i.e., the number of monthly observations ranged from 201 to 205. The method for filling in the missing data assumes that the differences among years were the same for all months, and the seasonal trends were the same for all years. These assumptions are probably unrealistic because they do not include year-to-year differences in the seasonality of phytoplankton blooms. However, the biases in the final results of this technique are thought to be small due to more or less random distribution and the low proportion of missing values (4–12%) in the data sets.

Results

Phytoplankton

Species composition—The first two principal components (PCs) of the PCA of the abundance of algal species accounted for 34% to 39% of the total variance of the log$_e$-transformed and standardized annual averages (Table 3). Covariability between species was relatively high considering that the explained variance by the first two PCs is approximately six times higher than it would have been if the time series of the 32 algal species were not correlated at all (i.e., $2/32 \cdot 100\% \approx 6\%$). Thus, more than one-third of the year-to-year variation in 32 algal species is accounted for in just two dimensions.

In the biplot of March–April (Fig. 2A), the study period shows four clusters, viz. for the successive periods 1974–1978, 1979–1987, 1988–1992, and 1993–1994. The first few years of the study period (1974–1978) were characterized by relatively low abundances of most algal species considered. The following years were characterized by (i) an increase of most algae, in particular *Guinardia delicatula* and unidentified large flagellates, and (ii) an alternation of two different groups of dominant algal species. In some years

(1979–1987 and 1993–1994), the phytoplankton community in March–April was characterized by relatively high abundances of *Brockmanniella brockmannii, Odontella aurita, Gaillonella sulcata*, and unidentified pennate diatoms. In other years (1988–1992), other species such as *Leptocylindrus minimus* and small *Thalassiosira* species were relatively more abundant.

Based on the biplot of July–August (Fig. 2B), the study period can be divided into three successive periods, i.e., 1974–1976, 1978–1987, and 1988–1994. As was observed for March–April, the first three years (1974–1976) were characterized by relatively low abundances of most algal species considered. The following period was characterized by (i) an increase in most algae, in particular *Rhodomonas* and other (small and large) flagellates, and (ii) an alternation of two different groups of dominant algal species. Between 1978 and 1987, the algae community was characterized by relatively high abundances of small *Chaetoceros* species and pennate diatoms. In the following years (1988–1994), *Leptocylindrus minimus* appeared in relatively high abundances.

The biplots for the other periods in the year did not show such clear patterns of well separated clusters for successive years (figures not shown), but some characteristics were found to be consistent with the ones observed for March–April and July–August. They all showed relatively low abundances of unidentified small and large flagellates during the first few years of the study period. A rapid increase in these flagellates occurred between 1978 and 1979 according to the biplots of January–February and March–April, and between 1976 and 1978 according to the other biplots. These results suggest that a major increase in the flagellates occurred around May–June 1977, and that densities of these algae stayed relatively high thereafter, i.e., all year round for the rest of the study period. Furthermore, the species composition of the algae in the Marsdiep changed between 1987 and 1988 and the biomass and species composition remained stable thereafter.

Biomass—Changes in species compositions as described above were accompanied by changes in Chl *a* concentrations (Fig. 3A). Annual mean values ranged from 4.6 mg m^{-3} in period A (1974–1976) to more than 9 mg m^{-3} thereafter (periods B1 and B2). During the entire study period, monthly averaged chlorophyll concentrations were more or less similarly low during the beginning of the year, viz. from January to March. However, the magnitude of the spring bloom doubled between 1974–1976 (period A) and 1978–1994 (periods B1 and B2). After 1988 (period B2), the timing of the peak of the spring bloom appeared to have shifted one month forward, viz. from May to April (Fig. 4).

Size structure—The interannual changes in phytoplankton biomass and species compositions were accompanied by changes in size distribution. Not all species contributed equally to the changes in total cell volume (Table 1). The increase after 1978 was mainly due to species with large individual cell volumes (Fig. 5), particularly when going from period A (1974–1976) to period B1 (1978–1987). The additional biomass of the algae community during the nutrient-controlled period of the year (July–August) then was
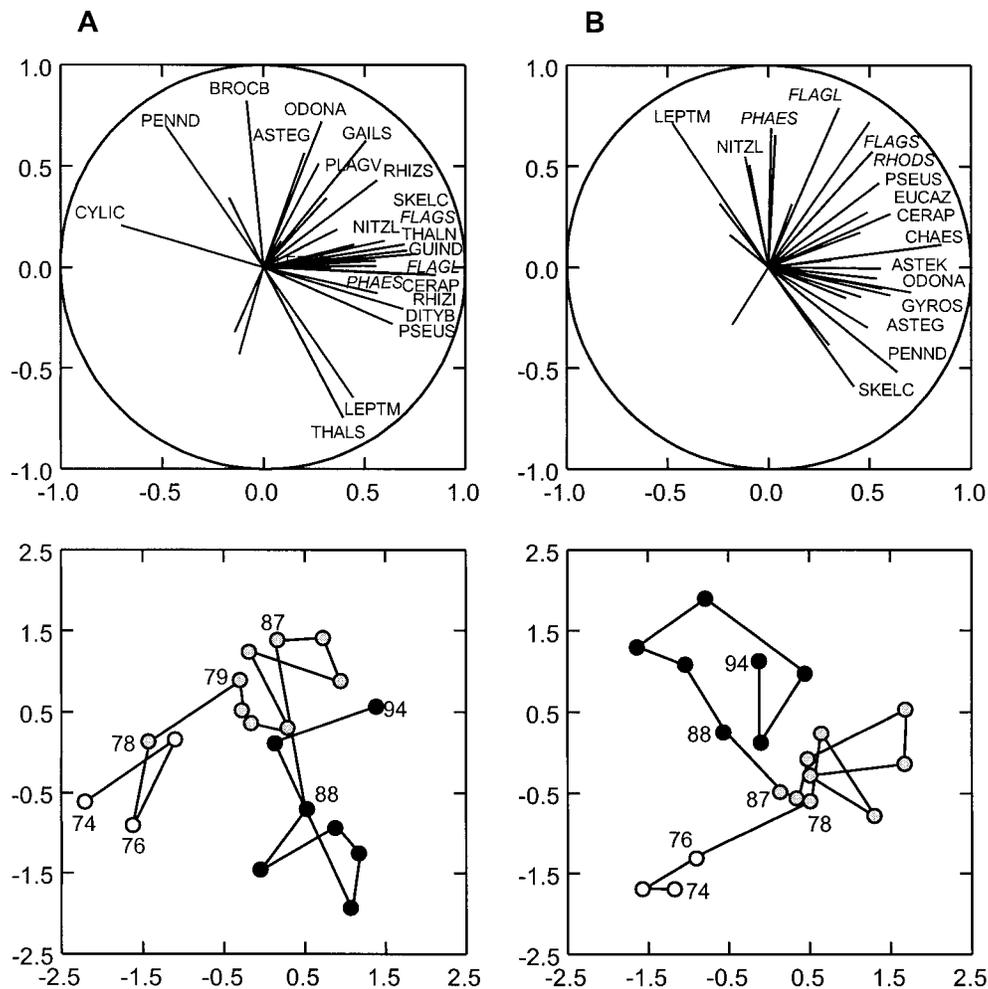
Fig. 2. Scores (upper panels) and loadings (lower panels) for the first two Principal Components of log-transformed and standardized bimonthly averaged densities of the 32 most numerous phytoplankton species or groups of species (see Table 3 for percentage of variance explained) between 1974 and 1994. Phytoplankton species (upper panels) are represented by vectors (see Table 1 for species' codes; only species with a vector length >0.5 are labeled). Years (lower panels) are represented by dots (white dots: 1974–1976; grey dots: 1978–1987; black dots: 1988–1994) chronologically connected by lines. (A) March–April. (B) July–August.

characterized by a high proportion (>65%) of diatom species with an individual cell size of more than 1,000 μm^3 (Fig. 5) such as large *Thalassiosira* sp. (8%), *Cerataulina pelagica* (8%), *Rhizosolenia imbricata* (14%), *R. hebetata*, and *R. setigera* (together 20%), and by smaller diatoms that are able to form colonies, i.e., *Chaeotoceros socialis* (30%).

Nitrate storage capacity—The annual mean of the total diatom vacuole volume increased from less than 0.4 cm³ m⁻³ during the mid-1970s (period A) to more than 1.5 cm³ m⁻³ until the mid-1980s (period B1), and decreased thereafter to less than 1.3 cm³ m⁻³ (Fig. 3B). Relative storage capacity (total diatom vacuole volume per unit of Chl *a*; Fig. 3C) increased from more than 80 cm³ mg⁻¹ to almost 170 cm³ mg⁻¹ from period A to period B1, and decreased to less than 140 cm³ mg⁻¹ during the last years of the study period (1988–1994). Relative storage capacity followed a similar trend as chlorophyll for the entire study period (Fig. 3A).

Applying the empirical relationship from other coastal marine waters to ambient chlorophyll data (Fig. 3A), the annual mean intracellular pool of nitrate in the water column was 0.2 mmol m⁻³ after 1978, corresponding to an intracellular nitrate concentration of about 200 mM. Such an accumulation of nitrate by phytoplankton is high compared to the range reported for marine diatoms between 20 and 150 mM (Dortch et al. 1985; Stolte and Riegman 1995; Villareal and Lipschultz 1995).

Nutrients

Nutrient dynamics—The increases of N and P inputs from land during the mid-1970s until the end of the 1980s were followed by distinct decreases (Fig. 6A,D). Concentrations of nitrate plus nitrite (NO_x; Fig. 6C), total phosphorus (TP; Fig. 6E), and phosphate (PO_4^{3-}; Fig. 6F) in the Marsdiep reflected these changes in the loadings. In contrast, mean total nitrogen concentrations (TN; Fig. 6B) dropped sharply
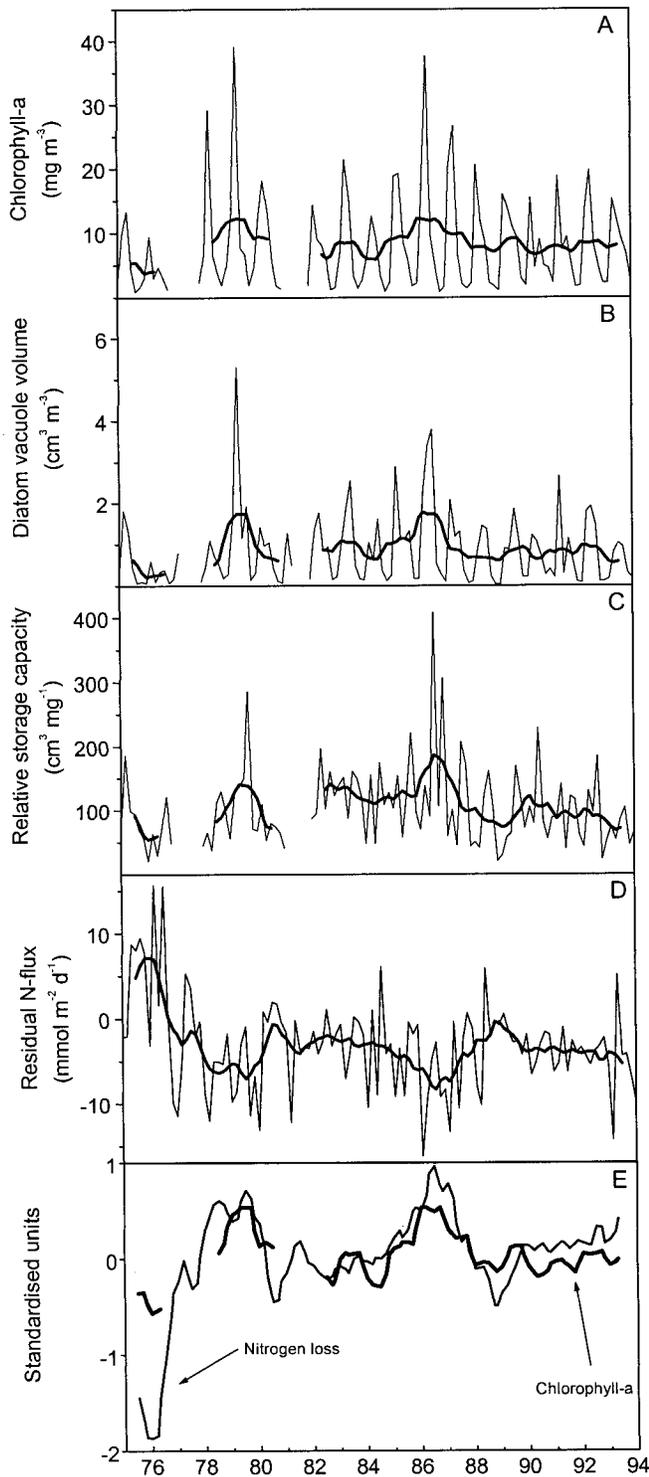
Fig. 3. Bimonthly averaged time series and 12-month running means in the western Wadden Sea from 1975 to 1993. (A) Chl *a* concentrations (mg m^{-3}). (B) Total vacuole volume of diatoms (cm^3 m^{-3}). (C) Relative storage capacity (total diatom vacuole volume per unit of Chl *a*; cm^3 mg^{-1}). (D) Residual nitrogen fluxes (F$_{TN,res}$ in Table 4; mmol m^{-2} d^{-1}) as estimated by means of nitrogen budgeting. (E) Running means of standardized (zero mean, unit variance) Chl *a* concentrations (thickest line) and nitrogen loss.
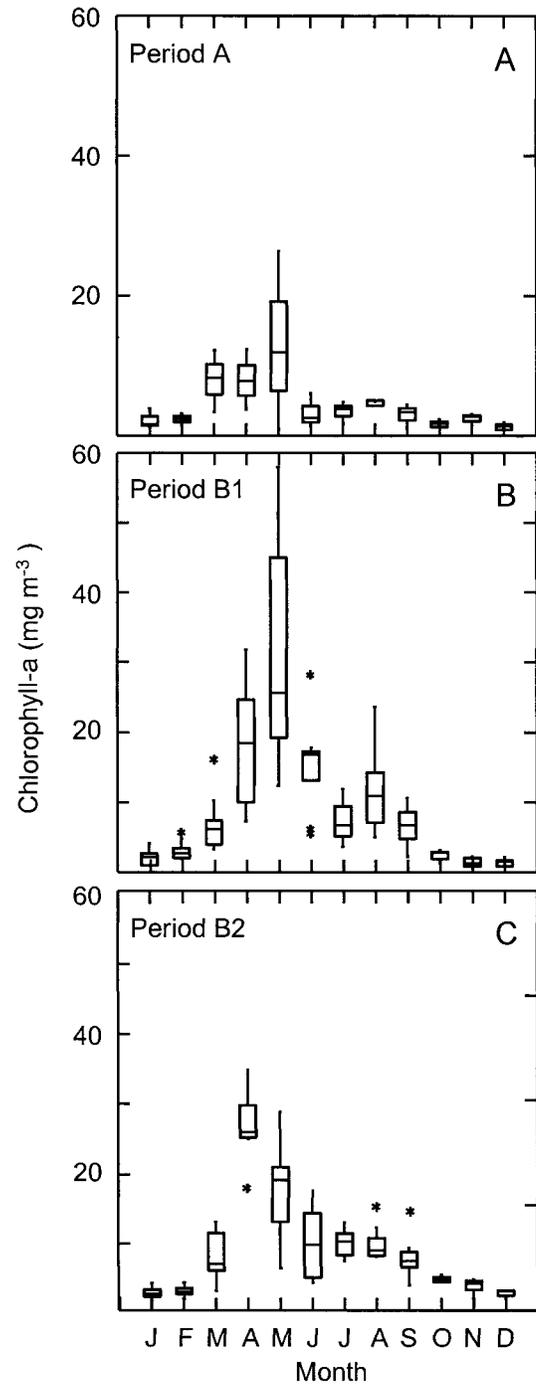
Fig. 4. Box-and-whiskers plots of seasonal (monthly averaged) time-series of Chl *a* concentrations (mg m^{-3}) of the phytoplankton community in Marsdiep waters from 1974 to 1994. (A) Period A (1974–1976). (B) Period B1 (1978–1987). (C) Period B2 (1988–1994).

from 100–150 to 50–100 mmol m^{-3} between 1977 and 1978, and remained at this lower level ever since.

Up to mid-1977, the mean TN : TP ratio during the growing season from March to August was mostly much higher (40 ± 6) than the Redfield ratio (Redfield et al. 1963) (Fig. 6G). This suggests that, on average, phosphorus rather than
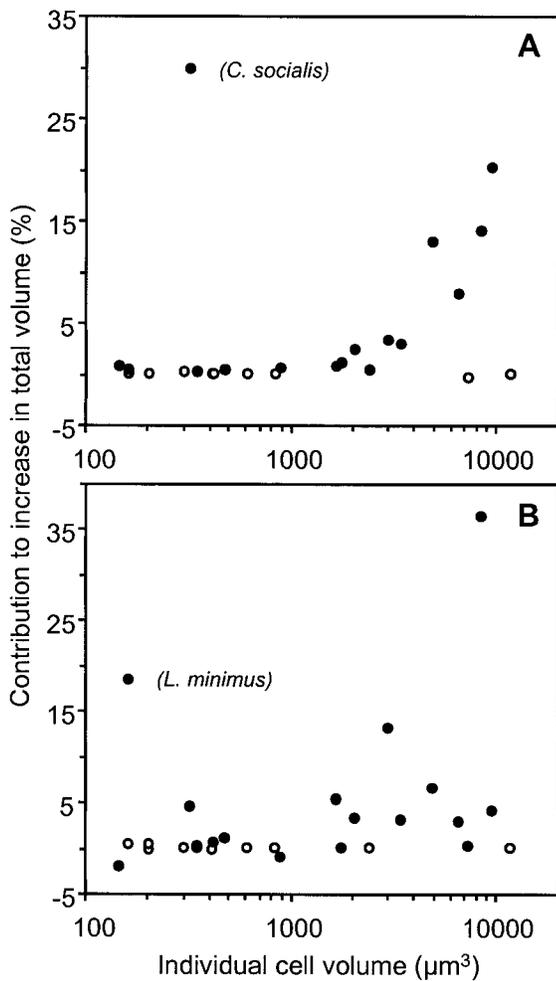
Fig. 5. Relationship between individual cell size (μm^3; see Table 1) and relative contribution to increase of total cell volume ($\mu m^3 \cdot \mu m^{-3} \cdot 100\%$) of diatom species during the nutrient-controlled periods of the growing season (July–August) from 1974 to 1994. (A) Biovolume difference between period B1 (1978–1987) and period A (1974–1976). (B) Biovolume difference between period B2 (1988–1994) and period A (1974–1976). Solid dots represent diatom species that contributed ≥ 0.01 cm^3 m^{-3} to the total biovolume of phytoplankton in the western Wadden Sea, open dots refer to the other species. Note that the diatom *Chaeotoceros socialis*, the main species contributing to the group of small *Chaeotoceros* spp. (species code CHAES in Table 1), appears in colonies, while other diatom species such as *Leptocylindrus minimus* may form chains.

nitrogen was controlling net annual community phytoplankton production. Between 1978 and 1987 with relatively low TN : TP ratios (15 ± 2), nitrogen was probably more often controlling than phosphorus. During this period, nitrate was the main nitrogen source (Riegman et al. 1992) with NO_x concentrations significantly higher than 1 mmol m^{-3} even in

$\rightarrow$
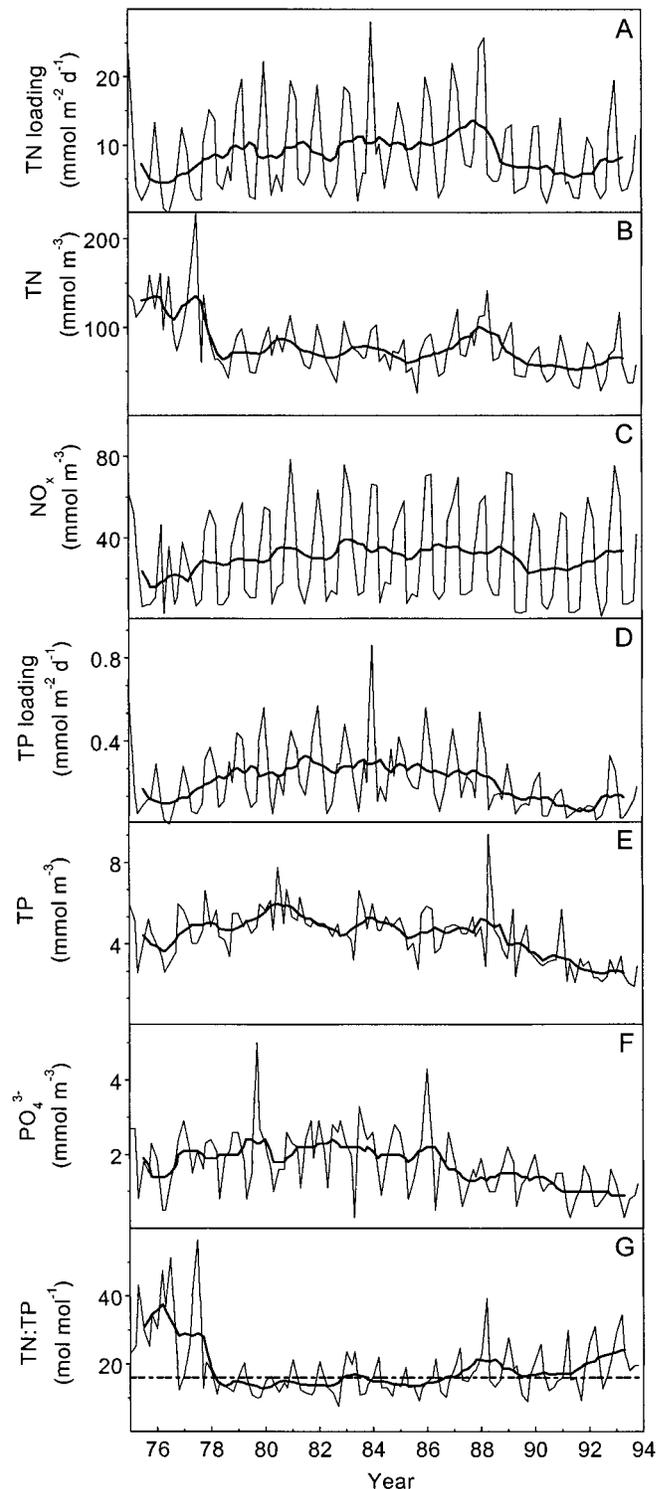
Fig. 6. Bimonthly averaged time series and 12-month running means in the western Wadden Sea from 1975 to 1993. (A) Total nitrogen loadings (mmol m^{-2} d^{-1}). (B) Total nitrogen concentrations

(mmol m^{-3}). (C) Summed nitrate and nitrite concentrations (mmol m^{-3}). (D) Total phosphorus loadings (mmol m^{-2} d^{-1}). (E) Total phosphorus concentrations (mmol m^{-3}). (F) Phosphate concentrations (mmol m^{-3}). (G) Ratios between total nitrogen and total phosphorus concentrations (mol mol^{-1}). The dotted line represents the Redfield ratio (TN : TP = 16 mol mol^{-1}). Time series on nutrient loadings and concentrations were taken from the water quality monitoring database (DONAR) of the Dutch Ministry of Transport and Public Works.
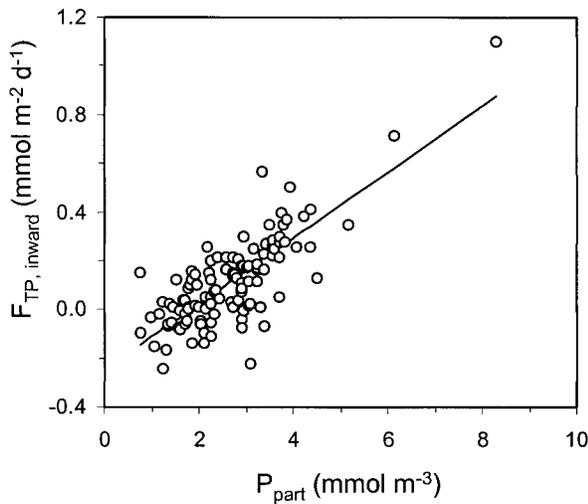
Fig. 7. Empirical linear relationship between bimonthly averaged concentrations of particulate phosphorus P_{part} (mmol m^{-3}) in the Marsdiep tidal inlet (Sta. b in Fig. 1) and bimonthly residual fluxes of total phosphorus $F_{TP,inward}$ (mmol m^{-2} d^{-1}) as derived from the phosphorus budget of the western Wadden Sea between 1975 and 1993 ($n = 114$, $r^2 = 0.62$, $P < 0.001$).

summer (Fig. 3C), which may point at other limiting factors than N or P. Growth conditions reshifted towards P-limitation after 1987, as shown by the increase of TN:TP ratios (21 ± 3) in most recent years.

Nutrient budgets—The residual TP-fluxes ($F_{TP,inward}$; mmol m^{-2} d^{-1}) proved to be significantly linearly related to the bimonthly averaged concentrations of particulate phosphorus (P_{part}; mmol m^{-3}) in the Marsdiep tidal inlet (Fig. 7), which agrees with the expected contribution of inward transport of particulate matter to the residual fluxes (Van Raaphorst and Van der Veer 1990):

$$F_{TP,inward} = -0.246 + 0.135 \cdot P_{part} + \epsilon$$

Here, 0.135 m^3 m^{-2} d^{-1} represents the average inward transport rate of particulate matter and ϵ the bimonthly variation in these rates.

For the phosphorus budget (Table 4), both the summed inputs and outputs were higher during period B1 (1978–1987) than during period A (1974–1977) and period B2 (1988–1993). The residual flux of the phosphorus budget calculations ($F_{TP,inward}$ in Table 4) was, however, more or less constant during the entire study period. The average flux of 0.11 mmol TP m^{-2} d^{-1} between 1975 and 1993 is similar to the inward transport of particulate matter retaining 2,400 kg P per tide as estimated by Postma (1961) for the early 1950s.

For the nitrogen budget (Table 4), both the summed inputs and outputs declined from the period between 1974 and 1977 to period B1 (1978–1987) and period B2 (1988–1993), despite the increase in nitrogen loadings from land. From 1975 to 1977, the exchange flux to the North Sea ($K_1 \cdot (N_a - N_b)$) in Table 4 was more than four times the magnitude of this flux thereafter. Budget calculations imply that the high TN concentrations in the western Wadden Sea during period A

Table 4. Annual averages ($\pm$ SD) of phosphorus and nitrogen mass fluxes (mmol m^{-2} day^{-1}) of the western Wadden Sea. The abbreviations of the nutrient mass fluxes are presented in Fig. 1B.

Nutrient	Fluxes	1975–1977 ($n = 3$)	1978–1987 ($n = 10$)	1988–1993 ($n = 6$)
Phosphorus	Inputs			
	$Q_d \times P_d + Q_e \times P_e$	0.15±0.05	0.26±0.03	0.13±0.05
	$Q_2 \times P_c$	0.15±0.01	0.16±0.02	0.11±0.02
	sum	**0.30**	**0.42**	**0.24**
	Outputs			
	$K_1 \times (P_a - P_b)$	0.12±0.07	0.18±0.05	0.07±0.07
	$Q_1 \times P_b$	0.31±0.06	0.37±0.04	0.25±0.08
	sum	**0.43**	**0.55**	**0.32**
	Residuals			
	$F_{TP,inward}$	0.13±0.09	0.13±0.06	0.08±0.09
		0.13	**0.13**	**0.08**
Nitrogen	Inputs			
	$Q_d \times N_d + Q_e \times N_e$	6.47±1.82	9.87±1.56	7.71±2.22
	$Q_2 \times N_c$	5.16±0.49	2.60±0.38	2.21±0.33
	$F_{TN,inward}$	9.05±4.11	0.42±1.19	0.53±1.51
	F_{atm}	0.19	0.19	0.19
	sum	**20.87**	**13.08**	**10.64**
	Outputs			
	$K_1 \times (N_a - N_b)$	14.48±2.30	3.18±2.41	2.07±3.03
	$Q_1 \times N_b$	8.59±1.28	5.53±0.84	4.84±1.52
	sum	**23.07**	**8.71**	**6.91**
	Residuals			
	$F_{TN,res}$	2.20±3.41	−4.37±2.27	−3.73±1.27
		2.20	**−4.37**	**−3.73**

were accomplished by a high rate of accumulation of partic-ulate matter originating from the open sea or trapped in the Wadden Sea basin (positive $F_{TN,inward}$ in Table 4) and a rela-tively small gain from an unknown source (positive $F_{TN,res}$ in Table 4). After 1978 (periods B1 and B2) the inward trans-port of particulate nitrogen from the open sea decreased. At the same time, the residual nitrogen flux ($F_{TN,res}$ in Table 4) shifted from net gain into net loss (Fig. 3D).

Discussion

Phytoplankton species composition—Based on the ob-served changes in species composition, particularly during the nutrient-controlled months of the growing season (July–August), we conclude that the phytoplankton community structure in the western Wadden Sea changed drastically be-tween 1977 and 1978 and between 1987 and 1988. The com-munity structure was relatively stable during the periods be-tween these shifts, viz. from 1974 to 1976 (period A), from 1978 to 1987 (period B1), and 1988 to 1994 (period B2).

The major changes in phytoplankton biomass and species composition coincided with changes in absolute and relative (TN:TP) nutrient concentrations. A combination of the time series on trophic state (using Chl *a* as a proxy) and limiting resources (TN:TP ratio) shows the same distinctive periods as phytoplankton community structure (Fig. 8C). The in-crease in phytoplankton abundance between 1976 and 1978 (Fig. 8A) corresponded with a decrease in TN:TP ratio (Fig. 8B). Thereafter, the phytoplankton biomass remained high (Fig. 8A), but the community structure changed again be-tween 1987 and 1988 upon the reshift to phosphorus-con-trolled conditions (Fig. 8B).

Colimitation effects—It may be questioned whether nutri-ent control is relevant at all in the Marsdiep since summer values of nutrients showed minimum thresholds of 1 mmol m^{-3} for nitrate plus nitrite (Fig. 6C) and 0.2 mmol m^{-3} for phosphate (Fig. 6F). Due to tidal mixing, the levels of de-tritus are relatively high in the Dutch coastal area. It has been calculated that at least 50% of the light absorption dur-

$\rightarrow$
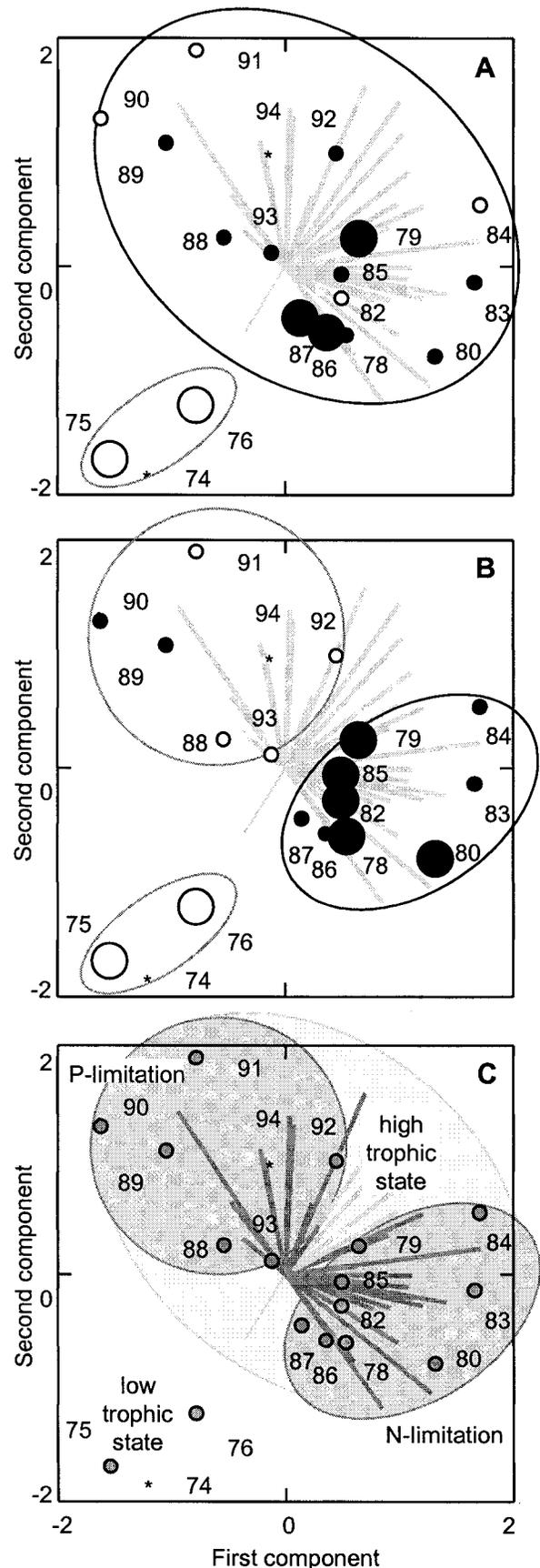
Fig. 8.   Covariance biplots (1st and 2nd axis) based on Principal Component Analysis of the species composition of the phytoplank-ton community during the nutrient-controlled periods of the grow-ing season (July–August) between 1974 and 1994. Relative phyto-plankton species abundances are represented by grey vectors and years by numbered dots. (A) Trophic state (indexed as the average annual Chl *a* concentrations: large white dots <5.5 mg m^{-3}; small white dots 5.5–7.5 mg m^{-3}; small black dots 7.5–9.5 mg m^{-3}; large black dots >9.5 mg m^{-3}). (B) Average TN:TP ratios during the growing season from March to August are indicated for each year (large white dots <16 mol mol^{-1}; small white dots 16–20 mol mol^{-1}; small black dots 20–30 mol mol^{-1}; large black dots >30 mol mol^{-1}). (C) Indications of different growing circumstances for phytoplankton during the study period as derived from the trophic state and the ambient nutrient ratios. The indications of annual tro-phic state and TN:TP ratio are restricted to the period for which the nutrient budgets could be estimated (1975–1993); the remaining years (1974 and 1994) are indicated with asterisks.

ing the spring bloom is due to particulate matter such as silt and clay (Gieskes and Kraaij 1975). During summer periods the amount of these particles is not higher than during spring whereas the incident irradiance levels are, and the Chl *a* concentrations are lower. This implies that the average irradiance levels in the water column are higher during summer than during the spring bloom maximum. Consequently, the phytoplankton biomass must be limited by factors other than only light during summer.

The strong correlation between phytoplankton community structure and TN:TP ratios strongly suggests that, despite the relatively high concentrations of these nutrients, they do have a regulatory effect with respect to species composition. This apparent contradiction can be explained on the basis of phytoplankton ecophysiology. During nutrient-limited growth at nonsaturating irradiance levels, algae enhance their nutrient quota and growth is sustained at higher substrate concentrations.

This physiological acclimation, mainly studied in freshwater species, has been observed in N-limited (Zevenboom et al. 1980) and P-limited (Riegman et al. 1985; Riegman and Mur 1985) continuous cultures of the cyanobacterium *Oscillatoria agardhii*, in N- and P-limited chemostats of the cyanobacterium *Synechococcus linearis* (Rhee and Gotham 1981; Healey 1985), and in N-limited continuous cultures of the green alga *Scenedesmus* and the diatom *Fragilaria crotonensis* (Rhee and Gotham 1981). More recently, this colimitation effect of a nutrient and a physical factor (i.e., light) on the physiology and growth response of algae has been described for marine phytoplankton species such as *Phaeocystis pouchetii* and coastal diatoms (Cloern et al. 1995; Hegarty and Villareal 1998).

From these studies, it can be concluded that in coastal areas where suboptimal light conditions prevail during summer periods due to high suspended matter levels, nutrients can still be limiting the specific growth rate at higher concentrations compared to waters with lower turbidity, as a consequence of colimitation effects. Indeed, physiological studies (only occasionally performed) on summer phytoplankton in the Marsdiep area, do indicate the occurrence of N or P limitation despite elevated dissolved inorganic nutrient concentrations (Riegman et al. 1990).

Size structure—Our results further imply that a shift toward a eutrophic environment particularly favored flagellates such as colony-forming *Phaeocystis* spp. (Cadée and Hegeman 1986; Riegman et al. 1992), and diatoms >1,000 μm^3 such as *Rhizosolenia* and *Thalassiosira* spp. This latter development confirms the theoretical forecast by Stolte and Riegman (1995) stating that eutrophication results in a dominance of large phytoplankton species. Theory further predicts an extensive overflow of these large phytoplankton species via sedimentation to benthic communities (Thingstad and Sakshaug 1990; Riegman et al. 1993).

Most of the large diatom species that increased in the Marsdiep inlet do not appear as single cells but form chains (Table 1). High densities of chain-forming diatoms rapidly coagulate (Jackson 1990) which may have further intensified the downward flux of these species (Smetacek 1985; Logan and Alldredge 1989; Kiørboe 1993), and thus the deposition

of phytoplankton cells at the sediment after 1978. Although the phytoplankton composition shifted between 1987 and 1988, the total biovolume and the proportion of large diatoms remained high.

Nutrient budgets and denitrification—We applied budget calculations to better understand the response of the western Wadden Sea to changing nutrient supply. While trends in TP loadings from land were reflected in phosphate and TP concentrations, the gradual increase in TN loadings from 1975 to 1988 was reflected in NO_x concentrations but not in those of TN. This disparity suggests a nonlinear system response to enhanced nutrient inputs, thus questioning the often-assumed one-to-one relationship between loadings and availability of nutrients. The drop in TN concentrations after 1978 can be explained by, first, a decreased input of particulate N ($F_{TN,inward}$ in Table 4) from the open sea, and second, an increased removal of nitrogen in the basin itself. Apparently, the trapping efficiency of particulate N decreased upon increased N loading and decreasing TN:TP ratios.

The residual flux of the nitrogen budget ($F_{TN,res}$ in Table 4) was negative after 1978. Because this flux is N specific, i.e., not accounted for in the P-budget, the most plausible explanation for this loss is denitrification, the reduction of nitrate to dinitrogen-gas. Nitrogen loss in coastal marine systems has been considered proportional to the TN input (25–75%; Seitzinger 1988; Borum 1996) or concentration (Borum 1996). Our calculations indicate that nitrogen removal in the western Wadden Sea was 35% of the total nitrogen inputs and 45% of the input from land from 1978 onwards, corresponding well to the budgets reviewed by Borum (1996).

The calculated annual mean residual losses of approximately 4 mmol N m^{-2} d^{-1} are high, but still within the range of directly measured denitrification rates reported for a suite of coastal marine sediments (Seitzinger 1988; Christensen 1994; Borum 1996). Additionally, this rate agrees well with the relationships described for two Danish fjords at an average TN concentration of about 70 mmol m^{-3} (Borum 1996). Recently published mean denitrification rates measured in sediments in the German Wadden Sea (Jensen et al. 1996) and Danish coastal waters (Nielsen et al. 1994) with the ^{15}N isotope pairing technique, point at values of about 0.4 mmol N m^{-2} d^{-1}. Similarly, winter measurements with acetylene-blockage techniques at high nitrate concentrations indicated low rates for the western Wadden Sea (Kieskamp et al. 1991). The discrepancy between budget estimates and experimental data of different origin cannot easily be explained. Enhanced sediment-water exchange due to wave action in the actual field situation compared to the different experimental setups may play a role, in particular for permeable sandy Wadden Sea tidal flats (Rutgers van der Loeff 1981; Shum 1993; Hüttel et al. 1998).

Denitrification and phytoplankton—Rates of benthic denitrification are related to water column concentrations of nitrate and to fluxes of labile organic matter to the sediment following phytoplankton blooms (Jensen et al. 1988; Middelburg et al. 1996). Subsequently, the increase in nutrient loadings and coinciding higher phytoplankton biomass probably explains the major part of the increase in denitrification

in the western Wadden Sea after 1978. Standardized time series of chlorophyll and denitrification rates showed very similar patterns in the long-term trends between 1978 and 1993 (Fig. 3E). This resemblance in trends strongly suggests phytoplankton biomass to be an important factor determining the interannual variation in nitrogen loss of the western Wadden Sea.

As for the phytoplankton biomass, major changes in phytoplankton community structure occurred around 1977 such as the observed increase of larger cells (Fig. 5A) and the suggested increase in nitrate storage capacity (Fig. 3D). These additional responses of the phytoplankton community to changes in nutrient resources may have contributed to the observed increase in denitrification. Assuming that the annual average specific growth rate of phytoplankton is about 0.5 day^{-1} (Parsons et al. 1977), and that 25–50% of the phytoplankton production sinks out to the sediment with the nitrate pools still intact (Lomstein et al. 1990) and is subsequently mineralized by the denitrifying community (Jørgensen 1996), the maximum denitrification of the pools may have been 0.1–0.2 mmol N m^{-2} day^{-1}. This implies that even if such a mechanism existed, its contribution to the total estimated residual fluxes of the nitrogen budget would have been very small (<5%).

Conclusions

The major conclusions of our long-term field observations are that: (1) Shifts in TN : TP ratios have had a strong effect on the species composition of the marine phytoplankton community in the western Wadden Sea. (2) Eutrophic and nitrogen-controlled conditions result in a disproportional increase of large diatom species (individual cell size >1,000 μm^3). (3) Long-term trends in Chl *a* concentrations were positively correlated with those in nitrogen loss rates, suggesting enhanced benthic denitrification through increased deposition of phytoplankton biomass.

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