

## Partitioning of organic production in marine plankton communities: The effects of inorganic nutrient ratios and community composition on new dissolved organic matter

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### *Abstract*

We investigated the partitioning of carbon, nitrogen, and phosphorus between particulate and dissolved production using 11-m<sup>3</sup> marine mesocosms (bags) in a Norwegian fjord with a salinity of 28.3, a chlorophyll concentration of 0.6 µg L<sup>-1</sup>, an even biomass among five algal groups, and nitrogen limitation as the initial conditions. The experiment lasted 21 days in August. Addition of silicate (+Si) resulted in diatom dominance, while a more diverse community was present in treatments with no added Si (-Si). Addition of inorganic nutrients in a N:P gradient from 64 to 4 either conserved the initial N limitation or forced the plankton communities to P limitation. Per added limiting nutrient, the diatom-dominated bags produced more particulate (POC) and dissolved organic carbon (DOC) than the other bags. However, the relative partitioning of net production to POC and DOC did not differ as a function of the plankton communities. Between 22% and 33% of the net production accumulated as new DOC. The higher values were found in the N-limited bags. The production of new dissolved organic nitrogen (DON) was variable over time, and short periods of positive production were followed by removal (negative production). Between 6% and 22% of the assimilated N was

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### *Acknowledgments*

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recovered as new DON in the N-replete bags, while the DON production was very low during N limitation. The community structure had no effects on nitrogen partitioning. Diatom dominance (+Si bags) resulted in P sequestration to particles and a constant low net production of dissolved organic phosphorus (DOP) across the nutrient gradient. The production of DOP was low in the P-limited (-Si) bags; however, with a surplus of inorganic P, most of the assimilated P (74% to 85%) was recovered as new DOP. The consequence was a huge range in stoichiometric ratios for newly produced dissolved organic matter (DOM). With N limitation, the C:N ratio of new DOM was from 40 to 100, but it was below 40 under N-replete conditions. The C:P ratio of new DOM in the -Si bags traced the P availability, and values approached 500 in P-deficient bags to values between 17 and 58 in the P-replete bags. The C:P ratio of new DOM in the +Si bags was about 300 at all dosing regimes. Consequently, the range in N:P ratios was also large, with values from below 1 to about 30. Carbon-rich DOM in oceans and coastal waters is not necessarily a function of a slow diagenetic "maturation" process but can be produced almost immediately. Both the nutrient regime and phytoplankton community composition affected the production and composition of new DOM in this experiment.

Dissolved organic matter (DOM) has a key position in aquatic ecosystems, where it quantitatively dominates the organic chemical environment (Wetzel 2001; Hansell and Carlson 2002) and is central to organic production recycling in the microbial loop (Williams 2000). One major outcome of a global DOM research effort over the past fifteen years is the change in perception of DOM from a recalcitrant and largely inert pool to a view of DOM as variable in time and space and of global importance in the biogeochemical cycle(s) of nutrients and carbon (Hedges 2002). Many studies concerning the production, role, and fate of allochthonous and autochthonous DOM in both freshwater and marine systems have recently been reviewed in Findlay and Sinsabaugh (2003) and Hansell and Carlson (2002). For analytical reasons, DOM is often chemically separated into three groups; dissolved organic carbon (DOC), nitrogen (DON), and phosphorus (DOP), and it is apparent that our knowledge decreases in the sequence  $DOC > DON > DOP$ .

In open oceans, coastal waters, and lakes not influenced by large terrestrial inputs, most DOM has an autochthonous origin, and phytoplankton primary production is at the base but many different biotic and abiotic processes participate in the production. Of these, direct exudation from algae, particle solubilization, and losses caused by grazing and viral lysis have been identified as quantitatively important (Karl et al. 1998; Nagata 2000). Bacterial assimilation and photochemical decomposition/transformation are the important DOM removal processes (Carlson 2002).

The concentration and composition of DOM species are a function of a complex set of processes with different timescales that create an organic pool most often dominated by carbon-rich compounds with long turnover times. However, seasonal and episodic short-term changes in the concentration of DOM show that production and removal are not always balanced in time and space (Carlson et al. 1994; Williams 1995; Søndergaard et al. 2000). Furthermore, microbial decomposition studies with samples from the photic zone have shown that a measurable but also variable fraction of the standing pool can be decomposed within a few weeks with DOP more available than DON, which is in turn more available than DOC (Hopkinson et al. 2002). These features are part of the explanation why the marine DOM pool is enriched in C over N and even

more depleted in P compared with the particulate sources often close to the canonical Redfield C:N:P ratio (106:16:1). Oceanic DOM typically averages about 300:25:1, but it is variable (Hopkinson et al. 1997; Benner 2002).

The N and P depletion of DOM has been linked with age, where N and P are conserved in the biota during recycling in the microbial loop, leaving the standing DOM pool carbon rich (Williams 1990). However, an analysis of seasonal DOM accumulation (Williams 1995) and experimental studies (Norrman et al. 1995; Søndergaard et al. 2000) have confirmed that relatively new DOM can have rather high C:N ratios approaching 20 or more. Nutrient-deficient algae, especially diatoms (Fajon et al. 1999; Børshheim et al. 2005), and their high production of carbohydrates and subsequent accumulation have often been used to explain the high C:N ratio. Thus, it can be suggested that the age of the DOM pool is not the only reason for N and P depletion. Carbon-rich DOM may be produced directly by the algae, or bacteria and algae almost immediately remove nutrient-rich compounds during periods with low availability of inorganic sources. The induction of high-alkaline phosphatase activity during P limitation is an example (Middelboe et al. 1995). How limitation by different nutrients (N or P) affects the production and possible accumulation of different DOM species is less clear, although experimental evidence with diatoms suggests that P limitation as opposed to N limitation results in higher loss of compounds more resistant to bacterial utilization (Obenosterer and Herndl 1995). While there is a reasonable knowledge regarding carbon partitioning and the behavior of DOC (Carlson et al. 1998; Søndergaard et al. 2000; Carlson 2002, among many), empirical evidence with respect to the production and fate of DON and DOP is scarce (Bronk 2002; Karl and Björkman 2002).

The purposes of this study were to investigate (1) if changes in the ratios of inorganic nitrogen and phosphorus availability would change the partitioning between particulate and dissolved production and among specific DOM species and (2) if and how different algal communities under such circumstances affected the partitioning. We hypothesized that N and P would be conserved in the biota and would not accumulate as DON and DOP under N or P limitation, respectively, and that DON and/or DOP could

Table 1. Initial conditions in the mesocosms with respect to water chemistry before addition of nutrients.

S (%)	pH	Alk. (meq L <sup>-1</sup> )	Chl. (μg L <sup>-1</sup> )	TDN (μmol L <sup>-1</sup> )	DON (μmol L <sup>-1</sup> )	NO <sub>3</sub> +NO <sub>2</sub> +NH <sub>4</sub> (μmol L <sup>-1</sup> )	TP (μmol L <sup>-1</sup> )	DOP (μmol L <sup>-1</sup> )	PO <sub>4</sub> (μmol L <sup>-1</sup> )	DOC (μmol L <sup>-1</sup> )	Si (μmol L <sup>-1</sup> )
28.3	7.78	2.06	0.6	10.8	10.6	0.06	0.74	0.5	0.08	128	0.23

accumulate during nutrient-replete situations. The consequence would be a stoichiometry of newly produced DOM related to the nutrient regime.

The hypothesis was tested by creating a plankton bloom in ten marine mesocosms followed by dosing with inorganic nutrients in a N:P gradient creating both N and P limitation. Silicate was added to five bags to induce a phytoplankton community development toward dominance of diatoms.

## Material and methods

**Mesocosm design and treatments**—Ten 11-m<sup>3</sup> mesocosms (closed bags with a conical lower part) were deployed on 02 August 2002 at the European Union (EU) Large Scale Facility in Raunefjord close to Bergen, Norway, where they were filled with water from the fjord. The design of the mesocosms was as described in Søndergaard et al. (2000). The water in the mesocosms was continuously renewed with water from the fjord at 10% per day and circulated with an air-lift. All measured variables were sampled from the bags and the fjord inlet, which made it possible to calculate net production values in time steps of 24 h based on the difference in concentrations and accounting for the amount coming from the fjord. Thus, summation of these daily production values over time provided a value for the total net production during a specified period, and the slope of the regression line for cumulative production versus time gave the average daily net production. The formula used to calculate net production in μmol L<sup>-1</sup> d<sup>-1</sup> between two days was:

$$NP \approx \frac{(B_1 + B_2)/2 - [(F_1 + F_2)/2 \times 0.1]}{0.9} - B_1,$$

Table 2. Total amount of nutrients added during each experimental phase (μmol L<sup>-1</sup>) to the different mesocosms and the signatures used to identify each bag. Phase I was 7 days and the same amount of N and P was added to all bags but only silicate to five Si bags. Phase II was 10 days and with a N:P treatment gradient. Signatures: R = additions with the Redfield ratio (16), 4N and 4P have four times more N and P than Redfield, respectively, and Si is the label to signify addition of silicate. Nitrogen was added as NaNO<sub>3</sub>, phosphorus as K<sub>2</sub>HPO<sub>4</sub>, and silicate as Na<sub>2</sub>SiO<sub>3</sub>.

Signatures	4N	2N	R	2P	4P	4NSi	2NSi	RSi	2PSi	4PSi
Additions										
Phase I										
N	—	—	11.2	—	—	—	—	11.2	—	—
P	—	—	0.7	—	—	—	—	0.7	—	—
Si	—	—	0	—	—	—	—	12.6	—	—
Phase II										
N	32	16	8	8	8	32	16	8	8	8
P	0.5	0.5	0.5	1	2	0.5	0.5	0.5	1	2
Si	—	—	—	—	—	18	18	18	18	18
N:P gradient	64	32	16	8	4	64	32	16	8	4

where  $B_1$ ,  $B_2$ ,  $F_1$ , and  $F_2$  were the concentrations measured at day 1 and day 2 in one specific bag ( $B$ ) and the inlet water ( $F$ ), respectively. As we only had one sampling per day and did not know the concentrations between the sample times or whether a diurnal signal was present, we chose a linear dilution model and not an exponential model. In other words, we changed all inflowing water once every 24 h.

The initial conditions with respect to inorganic nutrients and other environmental variables are presented in Table 1. The temperature increased from about 18°C to 21°C during the experiment. A phytoplankton bloom was created over 7 d starting 04 August by one daily addition of inorganic nutrients at Redfield ratio (N:P = 16, Table 2); this was called phase I. The daily amount added to each bag increased the concentration by 1.6 μmol N L<sup>-1</sup> and 0.1 μmol P L<sup>-1</sup> followed by a loss depending on the inflow. Silicate was also added to five of the mesocosms to increase the concentration by 1.8 μmol Si L<sup>-1</sup> and to initiate dominance of diatoms. Phase I was followed by 10 d with daily additions of inorganic nutrients with N:P ratios of 64, 32, 16, 8, and 4 (phase II), and continuation of the Si dosing. The nutrient treatments and bag signatures are summarized in Table 2. The experiment was continued for another 5 d in phase III, where glucose was added to six bags. Here we report on the first two phases. The effects and fate of glucose are reported separately (F. Thingstad et al. unpubl. data).

It can be very difficult to know if a specific nutrient is limiting. Here we used the criterion that our detection of either inorganic nitrogen (NH<sub>4</sub> + NO<sub>2</sub> + NO<sub>3</sub> > 0.02 μmol L<sup>-1</sup>) or soluble reactive phosphate (SRP > 0.01 μmol L<sup>-1</sup>) and not the other species made it likely that this nutrient was limiting or at least created a situation with

strong competition for the resource. Accordingly, during phase II of the experiment P became limiting in the bags labeled 4N, 2N, 4NSi, and 2NSi, and nitrogen was limiting in 2P, 4P, 2PSi, and 4PSi. Most probably, N was also limiting in R and RSi (see following). Metal speciation and other chemical properties in the bags RSi, 2PSi, and 4PSi have been published elsewhere (Muller et al. 2005).

Sampling was performed with clean technique mid-morning each day before the addition of nutrients. All subsamples were from one batch of water from each bag and the fjord inflow. Subsampling was organized with respect to contamination risks, so samples for carbohydrates, proteins, and other DOM species had priority.

**Biological properties**—Pigments were conserved in liquid nitrogen on Avante GF 75 filters, extracted in ethanol, and separated and quantified by high-performance liquid chromatography (HPLC) (Wright et al. 1991). Marker pigments were recalculated to comparable chlorophyll *a* biomasses (Mackey et al. 1996). Bacteria were enumerated by flow cytometry (Marie et al. 1999). Rate measurements included bacterial production measured with the  $^3\text{H}$ -leucine method (Kirchman 1993). Respiration was measured in selected bags as oxygen consumption in darkness over 24 h (Williams and Jenkinson 1982). A respiratory quotient of 1 was used to compare with carbon units. Samples for meso- and macrozooplankton ( $>200\ \mu\text{m}$ ) were collected the last day of the experiment and identified to the level of group and genera. The biomass was estimated from enumeration, size measurements, and converting these to carbon biomasses. Bacterial growth efficiency was measured in short-term (72 h) batch cultures based on increases in particulate organic carbon (POC), decreases in DOC, and oxygen consumption (see Søndergaard et al. 2000).

**Biogeochemical variables**—Ammonium was measured fluorometrically (Holmes et al. 1999) and nitrate, nitrite, phosphate, and silicate were measured with standard methods (Grasshoff et al. 1999). Samples for POC measurements were captured on precombusted GF/F filters and measured as  $\text{CO}_2$  after combustion (Søndergaard and Middelboe 1993). The coefficient of variation (CV) for POC was about 15% for these samples. DOC and DON were measured in GF/F filtered samples by high-temperature combustion and chemoluminescence detection of nitrogen followed by subtraction of the inorganic N species (Cauwet 1999). CV for the DOC measurements was  $<3\%$ , while CV for the calculated DON typically was between 15% and 25%. Particulate organic N (PON) was not measured but calculated as the difference between the measured N species and the amount added to a specific bag. These calculated PON concentrations were only used to approximate the N partitioning. Particulate organic phosphorus (POP) was analyzed on  $0.2\text{-}\mu\text{m}$  pore-sized polycarbonate membranes, and total P (TP) was analyzed in unfiltered samples by wet oxidation (Grasshoff et al. 1999). DOP was calculated as  $\text{TP} - (\text{POP} + \text{SRP})$  with a CV at about 30%. Organic species in the DOC and DON fraction were further characterized by analyses of dissolved combined neutral sugars (DCNS) after hydrolysis and

detection of specific neutral aldoses with HPLC-PAD (pulsed amperometric detector) (Borch and Kirchman 1997) and combined amino acids (DCAA) after hydrolysis, reaction with orthophthaldialdehyde, and fluorometric detection (Roth 1971). The absorption and fluorescence properties of the produced DOM are reported in Stedmon and Markager (2005).

**Presentation and statistical analyses**—The results are mostly presented as cumulative production curves over time; i.e., a positive slope signifies a net production (accumulation) of the chemical species in question. Total bacterial production and total community respiration for phase II are summed values from the daily measurements. The design of the experiment excluded replication; however, many of the bags evolved similarly with respect to a number of variables. These results are presented as pooled values from two or more bags. The criterion for pooling was that the slopes of linear regressions versus time were not significantly different ( $p < 0.05$ , *t*-test) and/or the coefficients of variation for at least 60% of the pooled data points were  $<15\%$ . Some of the measurements were rather “noisy” with fluctuating time developments. These included DON, DCAA, and partly DOP. Pooling of these variables was based on a subjective judgment viewing all curves together. All data from the regression analyses of production versus time are presented in Table 3 together with standard error (SE) calculated for 95% confidence intervals. Slopes significantly different from zero are accepted at  $p < 0.01$  (*t*-test), unless otherwise stated.

## Results

**Biotic properties**—The addition of nutrients created an increase in phytoplankton biomass from about  $0.4$  to  $8.8\ \mu\text{g Chl L}^{-1}$  and a cumulative production of  $11\ \mu\text{g Chl L}^{-1}$  in phase I (Fig. 1A). The dosing gradient during phase II resulted in higher net production of chlorophyll in the N-replete bags, and the +Si bags had a higher net production of chlorophyll per added nutrient than the -Si bags (Fig. 1A). The six bags dosed at Redfield or low N:P ratios (P replete) had a lower net production of chlorophyll during phase II and were close to steady state.

The algal community in the bags initially had a rather even biomass distribution among dinoflagellates, chlorophytes, prymnophytes, diatoms, and cyanobacteria. Adding silicate changed the community within a few days to be dominated by diatoms (50–70% of the biomass) and chlorophytes (20–30%). The community in the -Si bags maintained an even biomass distribution among five algal groups. The major change was that chlorophytes were substituted with prasinophytes. Despite no silicate being added, diatoms were still present and accounted for 20–30% of the biomass.

**Carbon production and partitioning**—All bags had a positive net production of POC during phase II, but the rates tended to slow after day 14, most probably due to nutrient limitations (Fig. 1B). High dosing with N and P and +Si resulted in high accumulation of particulate

Table 3. Linear regressions of cumulative production versus time during Phase II. Slopes with SE values of  $\pm 95\%$  confidence limits are shown. The slopes are net production rates in  $\mu\text{mol C, N or P L}^{-1} \text{ day}^{-1}$ . The results are pooled according to criteria mentioned in Materials and methods. POC = particulate organic carbon, DOC = dissolved organic carbon, DCNS = dissolved combined neutral sugars, DON = dissolved organic nitrogen, DCAA = dissolved combined amino acids, POP = particulate organic phosphorus, DOP = dissolved organic phosphorus.

Bags	POC	SE	n	DOC	SE	n	DCNS	SE	n
4N and 2N	12.6 $\pm$ 2.5		10	6.35 $\pm$ 0.48		22	0.48 $\pm$ 0.1		22
R, 2P and 4P	4.1 $\pm$ 1.4		15	2.07 $\pm$ 0.37		33	0.48 $\pm$ 0.09		33
4NSi and 2NSi	30.7 $\pm$ 3.4		10	8.62 $\pm$ 0.92		20	2.36 $\pm$ 0.14		22
RSi	15.8 $\pm$ 1.1		5	8.62 $\pm$ 1.01		11	0.89 $\pm$ 0.11		11
2PSi	19.9 $\pm$ 2.0		5	8.62 $\pm$ 1.12		10	1.62 $\pm$ 0.08		11
4PSi	30.7 $\pm$ 1.4		5	8.62 $\pm$ 1.51		11	1.42 $\pm$ 0.18		11
				DON	SE	n	DCAA	SE	n
4N				0.14 $\pm$ 0.1		11	0.06 $\pm$ 0.04		10
2N				0.35 $\pm$ 0.1		11	0.08 $\pm$ 0.02		10
R, 2P and 4P				0.06 $\pm$ 0.06		33	-0.07 $\pm$ 0.04		30
4NSi and 2NSi				0.25 $\pm$ 0.09		22	0.14 $\pm$ 0.04		20
RSi, 2PSi and 4PSi				0.09 $\pm$ 0.06		33	0.01 $\pm$ 0.03		30
	POP	SE	n	DOP	SE	n			
4N and 2N	0.025 $\pm$ 0.01		12	0.013 $\pm$ 0.01		10			
R	0.025 $\pm$ 0.01		6	0.036 $\pm$ 0.01		5			
2P	0.025 $\pm$ 0.02		6	0.072 $\pm$ 0.04		5			
4P	0.025 $\pm$ 0.01		6	0.121 $\pm$ 0		5			
4NSi, 2NSi and RSi	0.028 $\pm$ 0.01		18	0.029 $\pm$ 0.01		15			
2PSi	0.054 $\pm$ 0.01		6	0.029 $\pm$ 0.01		5			
4PSi	0.152 $\pm$ 0.02		6	0.029 $\pm$ 0.03		5			
	CHL	SE	n						
4N and 2N	1.23 $\pm$ 0.16		22						
R, 2P and 4P	0.21 $\pm$ 0.08		33						
4NSi	2.6 $\pm$ 0.18		11						
2NSi	1.34 $\pm$ 0.11		11						
RSi, 2PSi and 4PSi	0.44 $\pm$ 0.11		33						

carbon; the POC : Chl ratio moved from about 126 in phase I to above 200  $\mu\text{g C} (\mu\text{g Chl})^{-1}$  in phase II. The ratio remained constant at about 126 in all -Si bags (see Table 3).

The background concentration of DOC in the fjord water varied from 125 to 140  $\mu\text{mol L}^{-1}$ , and the treatments resulted in a net production of DOC in all bags, increasing the concentrations to between 165 and 240  $\mu\text{mol L}^{-1}$ . As with POC, the DOC production rates decreased or even went to zero after day 14 (Fig. 1C). However, all slopes calculated from day 7 to 17 were significantly different from zero during phase II ( $p < 0.01$ ; Table 3). The nutrient gradient did not affect the production of new DOC in the +Si bags, while the net production differed among the -Si bags, with significantly higher production in the P-deficient bags. The net DOC production in all -Si bags and RSi and 2PSi averaged 50% of the POC production, i.e., 33% of the total net production, but were relatively lower at about 22% in bags 4NSi, 2NSi, and 4PSi due to the high POC production.

During the first 3–4 days of the experiment, dissolved combined neutral sugars (DCNS) were consumed at low rates in all bags followed by a positive net production (Fig. 1D). During phase II, the production of DCNS was not statistically different among the -Si bags, and the daily

rate was  $0.48 \pm 0.1 \mu\text{mol C L}^{-1} \text{ d}^{-1}$  (Table 3). However, as the production of DOC was about 3-fold higher in the two P-limited bags (4N and 2N), DCNS only accounted for about 7% of the DOC production compared with 23% in the bags dosed at Redfield ratios and with N limitation (Table 3). In the +Si bags, the production of DCNS was higher than in the -Si bags and differed systematically across the nutrient gradient (Fig. 1D). The highest production rates were found in the two P-limited bags at  $2.36 \mu\text{mol C L}^{-1} \text{ d}^{-1}$ , followed by the N-deficient bags 2PSi and 4PSi at 1.6 and  $1.4 \mu\text{mol C L}^{-1} \text{ d}^{-1}$  (not statistically different). The lowest production rate ( $0.89 \mu\text{mol C L}^{-1} \text{ d}^{-1}$ ) was measured in RSi. The rates were significantly different ( $p < 0.05$ ) among these three groups. DCNS explained from 10% to 27% of the DOC production in the +Si bags.

Measurements of bacterial production (BP), bacterial growth efficiency (BGE), bacterial abundance, total community respiration, and zooplankton biomass made it possible to calculate carbon partitioning during phase II. Bacteria cells did not accumulate, thus the net production was removed in the microbial loop. We calculated the respiration in the microbial loop by assuming that bacterial production was respired in the microbial food web and using the measured BGE at  $20 \pm 8\%$  (average  $\pm$  SD,  $n = 9$ ,

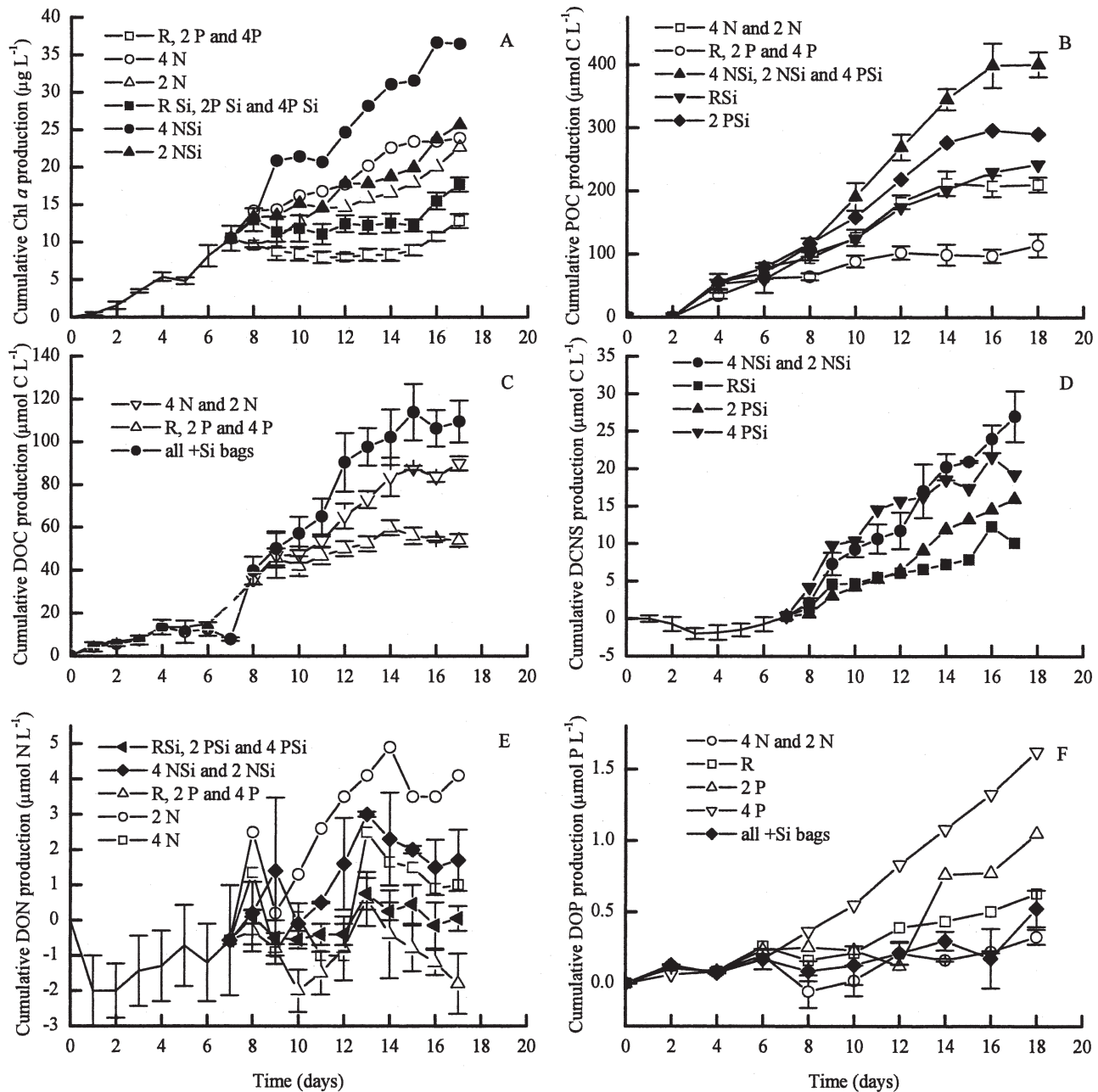


Fig. 1. Cumulative production of (A) chlorophyll *a*, (B) POC, (C) DOC, (D) DCNS, (E) DON, and (F) DOP as a function of treatments in 10 mesocosm bags. The criteria for pooling data for some bags are explained in the Material and methods section. For these, the means  $\pm$  standard deviations are shown. Legends refer to the dosing of nutrients after day 7; R = Redfield ratio dosed bags; in the bags 2P, 2N, 4P, and 4N, phosphorus and nitrogen was dosed at 2 or 4 times the Redfield ratio, respectively. Si indicates that silicate was included in the dosing; see Table 2. Phase I ended at day 7.

data not shown). Subtraction from total plankton respiration provided an estimate of the nonbacterial respiration, i.e., the respiration by algae and zooplankton.

Meso- and macrozooplankton biomass was only measured when the experiment was terminated, so the production over time was not known. Since POC was measured on samples filtered from small volumes, the larger zooplankton were probably not represented. Zooplankton biomasses ranged from 3 to 14  $\mu\text{mol C L}^{-1}$ , and the higher values occurred at high N dosing. Thus,

zooplankton contributed  $<10\%$  to the POC production. The zooplankton biomass in the  $-Si$  bags was dominated by adult and various copepodit stages of *Centropages*, with some *Acartia* and very few cladocerans. The biomass in the  $+Si$  bags was more evenly distributed among *Centropages*, *Acartia*, and *Oithona*, with the cladoceran *Podon* representing about 2% of the biomass.

Diatoms and chlorophytes dominated the  $+Si$  bags, and more POC was produced per added limiting nutrient than in the  $-Si$  bags at all dosing regimes (Fig. 2). It was also

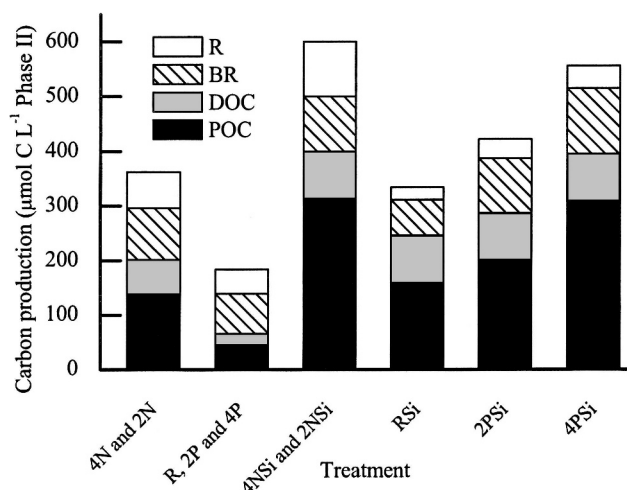


Fig. 2. Carbon partitioning among respiration by phytoplankton and larger heterotrophs (R), microbial respiration (BR), dissolved organic carbon (DOC), and particulate organic carbon (POC, including larger zooplankton) during phase II.

clear that the low N dosing in R, 2P, and 4P resulted in a much lower total C production than in the bags 4N and 2N. This result supports the assumption of N limitation in R, 2P, and 4P. Among the +Si bags, RSi had the lowest production and 4PSi had a POC production similar to 4NSi and 2NSi, which showed that the production of POC as opposed to chlorophyll was apparently not strongly controlled by N. Thus, carbon accumulated in cells subjected to nutrient limitation. In the +Si bags, POC was about 50% of the total production (POC + DOC + BR) compared with 20% to 30% in the -Si bags. The respiratory losses were consequently relatively higher in all -Si bags as the bacterial production only varied a few  $\mu\text{mol L}^{-1}$  among all bags. Across all bags, between 21% and 33% of the total net carbon production (POC + DOC) accumulated as DOC. The lower values were found in 4NSi, 2NSi, and 4PSi. Microbial respiration was between 50% and 75% of total plankton respiration (Fig. 2).

**Nitrogen production and partitioning**—DON and DCAA showed a systematic pattern in net production rates over the nutrient ratio gradient, albeit with much variability and higher uncertainties and consequently larger relative confidence limits than most other variables (Table 3). During the first day of the experiment, about  $2 \mu\text{mol L}^{-1}$  DON was apparently removed from all bags, lowering the in situ concentration of “old” DON from about  $10.5$  to  $8.5 \mu\text{mol L}^{-1}$  (Fig. 1E). Nitrate increased by about  $0.4 \mu\text{mol L}^{-1}$ , so most of the lost DON can only be explained by an increase in PON; however, this is speculative. The decrease in DON was followed by a low, but positive net production in all bags during phase I. During phase II, the production of new DON fluctuated between positive and negative values. Viewed over the entire phase II, most of the N-replete bags had a positive and statistically significant production ( $p < 0.05$ ); however, 4N was only marginally positive ( $p = 0.1$ ). For all other bags, a positive net production for the entire phase II could

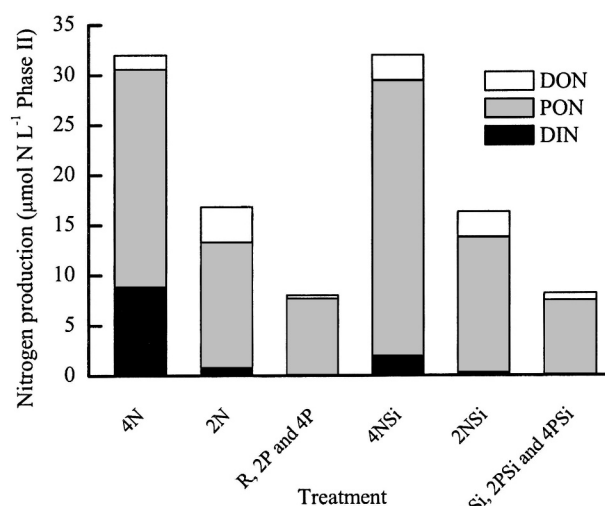


Fig. 3. Nitrogen partitioning among DON, particulate organic nitrogen (PON), and inorganic nitrogen (DIN) during phase II.

not be detected at  $p = 0.1$  (Fig. 3). All bags had negative DON production at some stage between day 8 and 11, followed by a positive period. These systematic fluctuations over time are the reason for low or no correlations of new DON production versus time during phase II (Table 3).

Dissolved combined amino acids (DCAA) could explain between 23% and 40% of the DON production at high N dosing. Net production of DCAA was not detected at Redfield dosing or in the N-deficient bags (Table 3). As for DON, the net production of DCAA was higher in 2N than in 4N. These results provide credibility to the observed pattern of a positive DON production in the N-replete bags.

Nitrate was detected in 4N from day 9 and increased to about  $8 \mu\text{mol L}^{-1}$  on day 17. A low concentration in 2N (about  $0.5 \mu\text{mol L}^{-1}$ ) was observed from day 8 but without any further increase over time. In 4NSi, between  $0.5$  and  $1 \mu\text{mol L}^{-1}$   $\text{NO}_3^-$  was measured from day 11, while nitrate in 2NSi never increased above  $0.3 \mu\text{mol L}^{-1}$ . Thus, with some confidence, we concluded that 4N, 2N, and 4NSi were N-replete and P-limited and, with less confidence, 2NSi (Fig. 3).

A nitrogen-partitioning budget for phase II showed that most added N must have moved to PON (Fig. 3). PON was not measured directly but calculated as the difference between added nitrogen and the measured DON and inorganic N. Between 6% and 22% of the assimilated N was recovered as DON in the N-replete bags (6%, 22%, 8%, and 16% for 4N, 2N, 4NSi, and 2NSi, respectively) and an uncertain 4% to 8% in the N-deficient bags. Statistically significant amounts of new DON during phase II were only produced in the bags dosed with at least twice the Redfield N:P ratio.

**Phosphorus production and partitioning**—The nutrient ratio gradient resulted in distinct DOP production patterns. In all +Si bags, the DOP production was constant and rather low at an average of  $0.029 \mu\text{mol L}^{-1} \text{d}^{-1}$  (Fig. 1F;

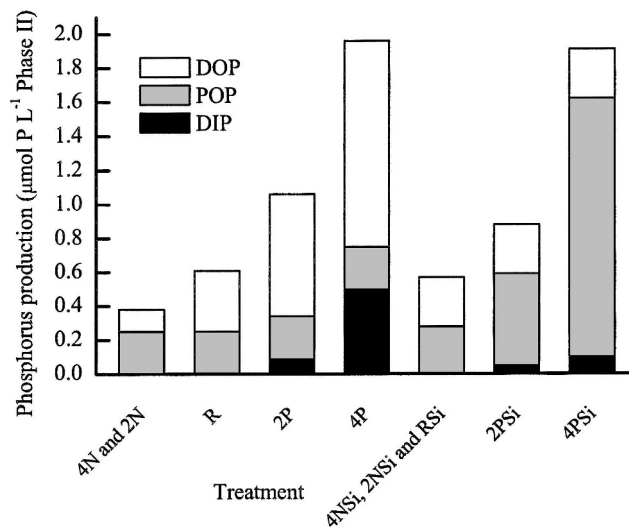


Fig. 4. Phosphorus partitioning among dissolved organic phosphorus (DOP), particulate organic phosphorus (POP), and inorganic phosphate (DIP) during phase II.

Table 3). The DOP production in the  $-Si$  bags reacted to the nutrient regime and was low at P limitation and high when P was replete (Fig. 1F). The positive net DOP production over time was significant for all  $-Si$  bags at  $p < 0.01$ , except for 2P, which was only significant at  $p = 0.1$ . Contrary to DOP, the POP production in all  $-Si$  bags was of the same magnitude, while most excess P was recovered in particles in the  $+Si$  bags (Fig. 4). Of the assimilated P, the fraction of DOP increased from 30% in 4N and 2N to 50%, 75%, and 85% in R, 2P, and 4P, respectively. Due to the dominant assimilation of P into particles in the  $+Si$  bags, the partitioning toward DOP decreased when P became replete.

Inorganic P began to accumulate during phase II at the highest P dosing and more so in the  $-Si$  bags than in the  $+Si$  bags (Fig. 4). We therefore concluded that P was replete and N limiting in 2P, 4P, 2PSi, and 4PSi.

*Stoichiometry of new DOM*—The stoichiometry of new DOM should not be confused with the ratios measured in situ including the background of DOC, DON, and DOP in the fjord water. The calculations of new production exclude this background. At the start of the experiment, the C:N:P ratio of the DOM pool in the fjord was 245:18:1, as expected from, e.g., Hopkinson et al. (1997). The new DOM produced during phase I had a ratio of 170:6.5:1 and became N depleted due to the decrease of DON (negative production) in the beginning of phase I.

During phase II, DON fluctuated systematically between positive and negative production periods with low correlations over time as a consequence. All bags had a significant positive production of DON until day 13, followed by negative production rates. The decrease in DON production after day 13 was most pronounced in the Redfield dosed and the N-deficient bags. New DON production in R, RSi, 2P, 2PSi, 4P, and 4PSi over the entire phase II was close to zero and thus resulted in DOC: DON ratios of new DOM approaching infinity. For comparative reasons, we

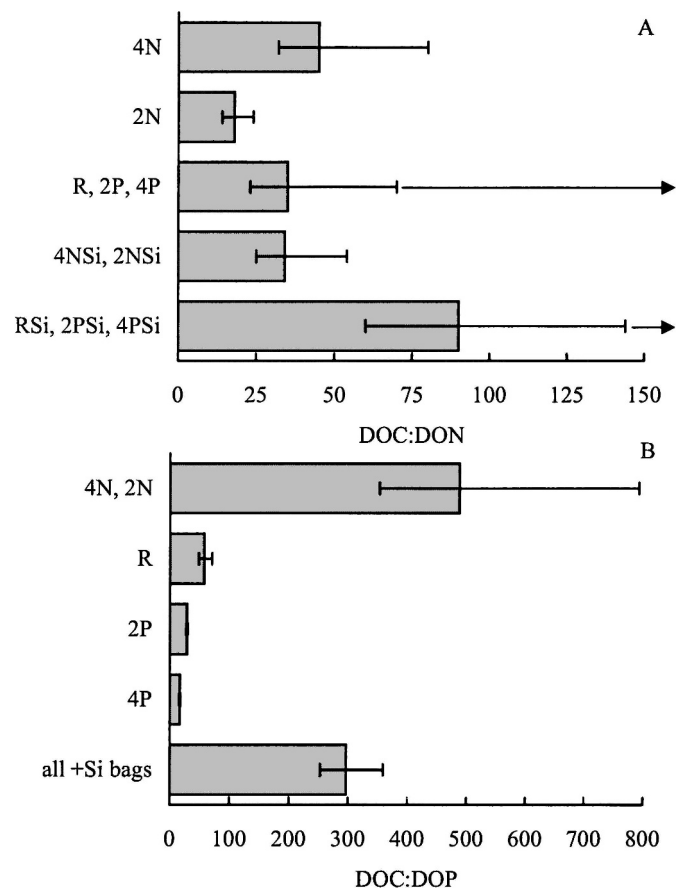


Fig. 5. (A) C: N and (B) C: P stoichiometry of new DOM produced during phase II. The rates are found as the slopes in Table 3. The C: N ratios in the N-limited bags are uncertain and can be much higher (indicated by arrows), but not much lower. Means  $\pm$  standard deviations are shown for pooled bags.

have here chosen to use the positive but uncertain slopes of DON production calculated for the entire phase II (Table 3); however, we realize that the ratios can be much higher, but not much lower.

The C: N ratios of new DOM in the N-deficient bags were between 35 and 90 and probably higher in the  $+Si$  bags than in the  $-Si$  bags (Fig. 5A). With inorganic nitrogen in surplus, the ratio was still higher than Redfield, but it was constrained between 20 and 44. The almost constant production of new DOC and DOP in all  $+Si$  bags and conservation of most added P in particles resulted in a constant DOC: DOP ratio of 300 (Fig. 5B). The communities in the P-deficient  $-Si$  bags also produced new DOM depleted in P, with a C: P ratio of 488:1, while the N-deficient and P-replete bags had a gradient in ratios (58, 29, and 17), which corresponded to the availability of surplus phosphate (Fig. 5B).

Most N: P ratios for new DOM were accordingly depleted in N (or enriched with P). In all N-replete bags, the ratios were around 10; however, the ratio was 27 for bag 2N due to a relatively high DON production. In all N-deficient bags, the N: P ratios were very low, between 0.5 and 3. The high DOP and low DON production in bag 4P

resulted in the lowest ratio of 0.5. The N:P ratios were uncertain as the relative confidence values were high (Table 3).

## Discussion

*Mesocosm management*—The applied nutrient dosing strategy with inorganic N and P was chosen to induce a strong competition for either N or P in two types of phytoplankton communities. The addition of silicate to one set of bags resulted in the expected outcome with diatom dominance; however, diatoms still accounted for about 20% of the biomass in the  $-Si$  bags. The meso- and macrozooplankton developed in accord with the amount of added nitrogen; thus, grazing by large zooplankton was higher in the bags with most added N (Thingstad et al. unpubl. data). There were no systematic changes in the phylogenetic composition of bacterioplankton and the abundance of picoalgae related to the dosing gradient or Si addition (Larsen, pers. comm.). These observations led us to conclude that any differences in DOM production between the  $\pm Si$  treatments most likely were linked to the composition of the phytoplankton.

The dosing strategy was designed to give identical biomass development in all bags if N and P were available close to the Redfield ratio in the fjord water and, if phytoplankton cells “obey” Redfield, which they might not do (Klausmeier et al. 2004). The production of more biomass in the bags with added surplus N compared with R and RSi strongly suggests that the plankton community in the fjord is limited by N. Thus, we “lost” our intended Redfield-controls (R and RSi), which most probably were N limited, like all bags dosed at low N:P ratios. The detection of inorganic N and P proved the dosing to result in N- and P-replete and N- and P-deficient communities, respectively, and no community was at any time replete with both nutrients in measurable quantities 24 h after addition. It was also found that nutrient uptake and particulate production, especially in the diatom-dominated bags, were extremely flexible with respect to C:N:P ratios.

The results of course have to be interpreted with some caution in a global oceanic and coastal water context. We forced a plankton bloom by adding nutrients at different N:P ratios over relatively few days, the addition of which deviated substantially from the initial conditions. Thus, the algal communities were perturbed each day. Grazing by large zooplankton most probably never reached a level in accord with the algal resources; however, high microzooplankton biomass and herbivory may have had a substantial impact on both diatoms and smaller algae (Verity and Vernet 1992), but this impact was not measured. Furthermore, the level of nutrients and their N:P ratios were higher and more extreme than normal for this Norwegian fjord. We might have forced the communities to respond more dramatically, both with respect to nutrient uptake stoichiometry and competition, than can be expected in a “normal” situation. The observed patterns among the different nutrient regimes and between the two principal communities ( $\pm$  dominance of diatoms) with respect to POC and DOM production were real, but they

cannot be quantitatively translated to other systems. We argue that these are qualitative patterns that show how a coastal summer plankton community can respond to nutrient competition and produce DOM with widely different chemical composition.

*Partitioning of carbon*—The algae in  $+Si$  bags produced more carbon biomass (POC) per added limiting nutrient than their  $-Si$  counterparts, whether N or P became limiting. Furthermore, the highest carbon production was found in the  $+Si$  bags with replete N and P; i.e., at the most severe P and N limitations, the dominance of diatoms resulted in similar production of POC. To the contrary, N limitation in the  $-Si$  bags showed very low POC production compared with P limitation (Fig. 2), most probably caused by N limitation in the fjord water. It is obvious that the composition of the community had a major influence on the POC production at identical nutrient supply.

It has been shown both experimentally and in situ that a relatively large fraction of primary production by various biotic processes is lost from the particulate phase to the DOC pool (Norrman et al. 1995; Carlson et al. 1998; Søndergaard et al. 2000, among many). It has also been suggested that nutrient deficiency and high grazing in postbloom situations enhance the dissolved route (Norrman et al. 1996; Olsen et al. 2002; Børsheim et al. 2005), although high DOC losses by growing algal populations can also be found (Søndergaard et al. 2000). Culture studies with diatoms and other algae have convincingly demonstrated that nutrient deficiency is often accompanied by high relative release rates (Myklesstad 1977; Obernosterer and Herndl 1995; Olsen et al. 2002).

Between 37% and 67% of the total carbon production (POC + DOC + BR, where DOC+BR is the gross loss) was lost to the dissolved phase, and between 57% and 22% of gross DOC was recovered as new DOC. The range of partitioning to the dissolved phase was in accord with previous observations (Carlson et al. 1998; Søndergaard et al. 2000). The lowest relative losses (37–40% gross DOC) were found in the  $+Si$  bags most replete in either N or P (4NSi, 2NSi, and 4PSi). The bags that stood out with respect to high relative DOC loss were the three N-depleted  $-Si$  bags (R, 2P, and 4P), where gross DOC production averaged 67%, of which only 22% accumulated (Fig. 2). These bags also had the highest ratio of BP:POC production. The relatively high BP could be explained by high microzooplankton grazing on the dominant small-sized phytoplankton, resulting in high organic recycling of labile compounds (Nagata 2000). Overall, there was no relationship between either the relative or the absolute DOC production and whether N or P was limiting.

Accumulation of dissolved combined carbohydrates has often been linked with nutrient-deficient diatoms (Myklesstad 1977; Ittekkot et al. 1981). Despite the fact that the absolute accumulation rates of DCNS in the  $-Si$  bags were much lower than the rates in the  $+Si$  bags (3- to 5-fold), the contribution of DCNS to new DOC ranged similarly from 7% to 25% across the nutrient treatments and did not depend on diatom dominance. It is interesting to observe that N deficiency in the  $-Si$  bags, and P deficiency in the

+Si bags resulted in the highest DCNS contribution to new DOC, with N-deficient diatoms not much lower. Accordingly, high relative losses and accumulation of DCNS are not only a function of diatom dominance, but seem more related to nutrient deficiencies or imbalances. In a mesocosm study comparable to the present one, Børsheim et al. (2005) showed that N-limited diatoms store large amounts of carbohydrates, which are released to the water during termination of a bloom. However, we did not have a massive decay of cells releasing carbohydrates to support high bacterial production as observed by Børsheim et al. (2005). Concerning DCNS, our results are comparable to those by Meon and Kirchman (2001), where DCNS explained between 24% and 30% of new DOC produced by two experimental phytoplankton blooms, both dominated by diatoms. A previous mesocosm study in Raunefjord, although not at the same time of the year, showed that combined carbohydrates could explain from 50% to 70% of new DOC; this was measured, however, with a different method (MBTH [3-methyl-2-benzothiazolinone hydrazone hydrochloride], Søndergaard et al. 2000), which probably included more compounds in the carbohydrate pool than the highly specific HPLC method.

*Partitioning of nitrogen*—Most of the added nitrogen was recovered in particles and less so in new DON. The working hypothesis was that N limitation in the bags dosed with high P should result in low or no new DON production due to an efficient recycling of organic N. If a small fraction of the produced DON became resistant due to their bacterial origin (McCarthy et al. 1998; Ogawa et al. 2001), some DON would accumulate. The overall conclusion is that the hypothesis can be accepted without including any production of new resistant DON. Significant amounts of new DON were only detected in the bags with measurable concentrations of inorganic N, i.e., during P limitation (Fig. 3). It is not possible to judge if the two types of algal communities and their accompanying heterotrophs produced different amounts of DON. This avenue of evaluation is excluded by the low sensitivity (high noise) of the DON measurements.

The time courses of the cumulative DON production showed a cyclic pattern with distinct periods of net production and removal in all bags. New DON was apparently produced in all bags in the beginning of phase II, which was then followed by a period of removal. In the N-limited bags, all or most new DON was removed between day 13 and 17, leading us to accept the stated hypothesis, with the precautionary note that the time-scale of observation is of importance. The short-term dynamics between periods of net production and removal in all N-replete bags indicate that at least part of the newly produced DON was not resistant to removal by bacteria or other organisms. We cannot account for the actual fate of the removed DON because a detailed mass balance cannot be constructed. However, the combination of our results that show both high DON and high DCAA production in the N-replete bags with the analytical precision around 20% (CV) indicates that the variability is not noise.

Most previous studies on DON production have been carried out in situ and with stable isotopes where the uptake of  $^{15}\text{NH}_4^+$  or  $^{15}\text{NO}_3^-$  and the subsequent release of  $^{15}\text{DON}$  were measured. Such measurements are highly sensitive but are most often short-term (hours), do not “catch” variations in DON behavior over days, and most often knowledge concerning nutrient limitation is lacking. Bronk (2002) reviewed the literature on DON production and removal and found that on average 40% of the inorganic N uptake in oceans moved to the DON pool (data with a huge variability), along with 38% in coastal areas, and about 23% in estuaries. In our study, the range for new DON production relative to inorganic N uptake was from 6% to 22%, which is in the lower range of published values. The relative DON production would be about 50% higher if calculated from the day 13 values.

During P limitation and N sufficiency, plankton communities can produce new DON, but new DON apparently does not accumulate during N limitation or at least only small amounts that escaped the sensitivity of our chemical measurements.

*Partitioning of phosphorus*—Both the nutrient regime and the algal community structure affected the partitioning of P. Despite the somewhat extreme mineral nutrient N:P ratios of 8 and 4 in the N-deficient bags, most of the added P was recovered in particles in the +Si communities. Across the nutrient gradient, the same amount of new DOP was produced (Fig. 4), leaving only low concentrations of inorganic P. In contrast, all the -Si communities had a very constant POP production. The production of new DOP was very low at P deficiency, but the production traced the gradient when P was in surplus. The reason(s) for this difference between communities is not obvious, but the simplest explanation is a higher ability of diatoms (and green algae) to store excess amounts of P compared with the other algae. In the P-replete -Si bags, the excess P was either excreted directly as DOP by the algae, or it was indirectly produced by grazing and viral activity (Middelboe et al. 2003). The DOP most probably accumulated due to low alkaline phosphatase activity (data not shown). Our hypothesis of low or no production of new DOP at P deficiency is only partly supported by the results. The hypothesis is rejected for the diatom-dominated communities because the same amount of new DOP was produced across P availability. However, in the -Si bags, the gradient of DOP production showed the predicted pattern of low new production at P deficiency to very high production at P-replete and N-deficient conditions.

As for DON, the production and turnover of DOP have mostly been studied in short-term (hours) experiments using the stable isotope  $^{33}\text{P}$  or with  $^{32}\text{P}$  (Karl and Björkman 2002), except for the long-term (weeks) studies by Olsen et al. (2002) and Vadstein et al. (2003), which used the partitioning of  $^{33}\text{P}$  in P-limited chemostats (slow dilution at 1% per day) with microbial food webs of different complexity. It is therefore difficult to compare our results directly with a broad range of other results. The production of DOP in relation to assimilation of inorganic P ranged from a low 16% in the most P-replete +Si bag (4PSi) due to

storage of P in particles to a maximum of 74–85% in the P-replete –Si bags (4P and 2P). During P limitation, between 34% and 50% of the uptake was recovered as DOP. Our results are comparable with those reported by Olsen et al. (2002), who, at P-limited conditions, found that between 16% and 39% of the P uptake were partitioned to DOP apparently independent of food web complexity. Björkman et al. (2000) found that 10% to 40% of net inorganic P uptake in a plankton community in the North Pacific subtropical gyre was recovered in DOP. The conclusion must be that even at severe P limitation, a relatively large fraction of the available P is partitioned to DOP. Thus, DOP production seems both dependent on the nutritional environment and the composition of the plankton community. To the best of our knowledge the present experiment is the first to show this pattern.

*DOM stoichiometry*—The particulate source of DOM in open marine areas has on average C:N, C:P, and N:P ratios of 7, 106, and 16, respectively. In general, marine DOM has C:N, C:P, and N:P ratios about 15, 300, and 25 (Hopkinson et al. 1997; Benner 2002) and no obvious relationship to the stoichiometry of the particulate sources. Thus, DOM is either produced under N- and P-depleted conditions, as would be the case if dominated by algal release of carbohydrates or the depletion could be explained by preferential recycling of N- and P-rich DOM species back to particles via inorganic compounds or by direct uptake. The in situ DOM stoichiometry could also be a combination of both processes.

We have not presented the POM stoichiometry of the present experiment in detail, but it can be calculated from Figs. 2, 3, and 4 with the caveat that PON was calculated and not measured. This might overestimate PON because some N would be lost to wall growth and sedimentation. Previous experience with these mesocosms has shown this type of loss to be relatively low due to the aeration system and the duration of the experiment. There is no relationship whatsoever between the stoichiometry of new POM and new DOM.

At N-limited conditions, the newly produced DOM in phase II had a high C:N ratio (>40), maybe even approaching infinity (Fig. 5A). With N repletion, most bags, except 4N, produced DOM with a C:N ratio < 40. Accordingly, DOM depleted in N can be newly produced, and a slow diagenetic sequence is not necessary. This result is in agreement with previous findings by Norrman et al. (1996) and Søndergaard et al. (2000), which showed that new DOM had C:N ratios from 22 to close to infinity. Subtraction of DCNS would not decrease the C:N ratios below 20. Thus, the remaining non-DCNS compounds are also carbon rich. Using the average C:N values of DOM by Benner (2002) and Bronk (2002) for oceans (15) and coastal waters (17), DOC would have to be removed preferentially to decrease our values toward these end points. Decomposition studies (Kragh and Søndergaard, unpubl. data) have shown that most of the accumulated DOC could be degraded microbially within 50 d, and the background C:N ratio at about 15 would be reached if new DOC was more degradable than new DON. This conclu-

sion is somewhat surprising given that the relative degradability of marine DOM most often is viewed in the sequence DOC < DON < DOP (Hopkinson et al. 2002).

The C:P ratio of the newly produced DOM ranged from 17 in the most P-replete –Si bag (4P) to about 500 in the P-deficient bags (4N, 2N). No variation in the C:P ratio was present across the nutrient gradient with dominance of diatoms, where the ratio was constant at about 300. The conclusion is that new DOM can be depleted in P to the oceanic background levels without a long diagenetic process, and, with dominance of diatoms, the P depletion is apparently not related to the mineral nutrition ratio. Olsen et al. (2002) and Vadstein et al. (2003) used a dual isotopic labeling technique to estimate DOC and DOP production in chemostat microcosms with a prasinophyte as the primary producer and combinations of other trophic elements (flagellates and rotifers) and different degrees of P limitations. They observed a wide range of C:P ratios, between 103 and 600. Due to the different techniques used to measure and calculate DOC and DOP, the results of Olsen et al. (2002) and Vadstein et al. (2005) cannot be directly compared with our results; however, they also observed accumulation of new and biodegradable DOC that resulted in the production of P-depleted DOM. Accordingly, carbon can be removed selectively from DOM by bacteria when the competition for P between algae and bacteria changes and thus bring the DOM stoichiometry back to “normal” marine background levels.

At present we do not know whether the high C:P ratio in the –Si bags was due to a fast removal of DOP or due to a direct algal loss of P-depleted and carbon-rich DOM (carbohydrates). The C:P ratio in the N-replete bags was between 525 and 1000. We therefore suggest that some of the DOP must have originated from more P-dense organisms than the dominant algal component of POM. Bacteria and other heterotrophs would be possible candidates.

With a P surplus, the +Si bags stored most of the excess P in particles, while the –Si bags produced DOP in high concentrations. It is apparent that there is a fundamental difference in how different communities dominated by different algal types behave with P in excess or depletion at these experimental conditions.

Earlier speculations on the regulation of particulate and dissolved organic production and how planktonic biota affects the partitioning have been justified and experimentally proven by our results. We found that newly produced DOM can be as depleted in N and P as the standing stock of old oceanic DOM. Furthermore, both the production and stoichiometric composition of new DOM were a function of the availability of inorganic nutrients and the composition of the phytoplankton community. New DOC accumulated at all nutrient regimes, whether N or P was replete or limiting. The accumulation of new DOC ranged from 22% to 33% of the POC production, independent of the composition of phytoplankton although with very different absolute values. The communities dominated by diatoms produced much more organic carbon (both POC and DOC) per available limiting nutrient. During N-limited conditions, the production of

new and accumulating DON was very low or even zero, and no difference between the two types of communities was found. The consequence was very N depleted new DOM. New DON accumulated during N-replete conditions, and the partitioning ranged from 6% to 22% of the inorganic uptake.

Production and partitioning of new DOP deviated substantially between the two phytoplankton communities. The production of new DOP was independent of the nutrient regime in diatom-dominated communities, while the new DOP production by more diverse phytoplankton communities traced the P availability and produced very small amounts of DOP in P-limited conditions, but large amounts under P-replete and N-limiting conditions. Before a more global and general theory can be developed, it remains to be tested how initial conditions of the plankton communities and their nutritional environment may shape the chemical composition of newly produced DOM and how this DOM develops into the known oceanic “background” composition.

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