

Net accumulation and flux of dissolved organic carbon and dissolved organic nitrogen in marine plankton communities

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Abstract

Marine mesocosms were manipulated with inorganic nutrients over a period of 22 d to investigate organic carbon partitioning under a variety of nutrient regimes. The chemical analyses and biotic measurements included inorganic nutrients, pigment signatures, particulate and dissolved organic species, bacterial production, and community respiration. The biodegradability of dissolved organic carbon (DOC) was investigated with in vitro decomposition experiments.

The net particulate organic carbon (POC) production was 50% of the total organic production during the initial 6 d of nutrient-replete growth and during a major diatom bloom. In all other situations the carbon partitioning was strongly in favor of DOC, which accounted for 82 to 111% of the total production. The production of new DOC preceded new DON by about 1 week. Thus, the new dissolved organic matter (DOM) initially had an infinite C:N ratio, which fell to 11–20 when DON started to accumulate. The highest C:N ratio was measured during a nutrient-replete diatom bloom. Dissolved polysaccharides accounted for 50 to 70% of the new DOC, and the lowest relative amount was produced during a diatom bloom. The chemical analyses unequivocally demonstrated that carbon partitioning in favor of carbon-rich DOM can take place during an active diatom bloom and not only during the decay of a bloom. The DOC-producing mechanisms cannot be fully identified. However, during the different growth phases the DOC production varied, as did the speciation of DOM with respect to the C:N ratios. When net

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production of dissolved organic nitrogen (DON) was detected after 11 d, the DON production accounted for 25 to 50% of the daily added and assimilated inorganic nitrogen. The measurements of community respiration made it possible to calculate the maximum carbon recycling by bacteria and bacterial net DOC assimilation. These calculations showed the estimates of carbon partitioning to be very sensitive to bacterial growth yield values and the factors used to convert leucine and thymidine isotope incorporation to bacterial production.

Decomposition experiments showed that at least 35% of the new DOC was biodegradable over 10–12 d and that inorganic nutrients only marginally affected use. The calculated turnover times of new DOC were between 15 and 25 d. The semilabile nature of new DOC with respect to microbial attack is suggested as the main reason for the medium-term accumulation of new DOC.

Dissolved organic carbon (DOC) in the oceans is one of the largest exchangeable organic reservoirs on the planet and plays a major role in the biogeochemical cycles of C, P, and N (Hedges 1992). The deep-water DOC pool is characterized by old and very refractory compounds, which persists at concentrations of about 40 to 50 μM in the deep oceans (Hansell and Carlson 1998). In the photic zone, a dynamic fraction of newly produced dissolved compounds overlies the refractory background and is taken to be susceptible to microbial decomposition aided by photochemical processes (Mopper et al. 1991).

Recent surveys of the temporal and spatial distribution of DOC in many different marine areas have shown accumulation of new DOC in the photic zone and over the growing season (Copin-Montégut and Avril 1993; Carlson et al. 1994; Børsheim and Mykkestad 1997; Carlson et al. 1998). Concentrations about double those in deep water have often been observed in surface waters not influenced by terrestrial runoff. Williams (1995) concluded that the seasonal increase of carbon-rich dissolved organic matter (DOM) could exceed accumulation in the particulate fraction, which would have important consequences on the biogeochemical control of carbon export from the upper ocean. The observation that DOC and dissolved organic nitrogen (DON) vary seasonally has reinforced interest in the biotic and abiotic mechanisms that control this seasonal cycle and the extent to which newly produced DOC is biodegradable. Several experimental studies have shown that at least part of the accumulated DOC is biodegradable over time scales of days to weeks (Ogura 1972; Ittekkot et al. 1981; Carlson and Ducklow 1996; Fry et al. 1996) and averages 19% of the total DOC (Søndergaard and Middelboe 1995). Because the DOC pool on a global scale is similar in size to CO_2 in the atmosphere, it is essential for the reliability of global carbon models to include realistic values for the turnover of DOC and to understand the processes involved in carbon cycling.

The production of autochthonous DOC ultimately relies on autotrophic production within the water column, and accumulation of DOC can only take place if the production is decoupled from consumption in time. Accumulation has most often been related to algal blooms and their subsequent decay, where nutrient deficiency can give rise to a high production of carbohydrates by diatoms (Mykkestad 1977; Ittekkot et al. 1981; Norrman et al. 1995; Obernosterer and Herndl 1995). However, as DOC accumulates over the growing season (see Williams 1995), and not only during bloom situations, food web processes other than algal exudation and lysis can be involved. Particle solubilization by bacterial enzymes (Smith et al. 1992) and DOC release by the activity

of grazers are other DOC-producing processes. Although photochemical oxidation (Mopper et al. 1991; Lindell et al. 1995), coagulation, and adsorption to sinking particles (Chin et al. 1998) all participate in the removal of DOC from the photic zone, it seems to be the current view that bacterial activity is responsible for the bulk of the decomposition of new DOM (Carlson et al. 1998; Williams 2000). However, photochemical transformations may facilitate certain critical steps—particularly in the case of the more resistant material (Anderson and Williams 1998).

Mechanistic explanations of the reasons for DOC accumulation take two not mutually exclusive routes. The conventional explanation is that a part of the new DOC has a high biochemical resistance to bacterial degradation or becomes resistant as a result of biotic or abiotic transformation. A high resistance to microbial attack is in accordance with the results of Fry et al. (1996): that DOC released during the decay of a diatom bloom was not completely mineralized even over a number of weeks, but apparently was transformed into refractory compounds by processes occurring in the microbial food web (Toggweiler 1989) or, alternatively, protected by membrane fragments in submicron particles (Borch and Kirchman 1999). Photochemical reactions may also take part in the transformation to resistant compounds (Benner and Biddanda 1998). It has also been found that DOC harvested from different algal species and growth phases decomposes at very different rates (Chen and Wangersky 1996). A difference in the quality of DOC related to food web structures and algal species was also found in a comparison between the Ross Sea and the Sargasso Sea. DOC produced during a *Phaeocystis* bloom in the Ross Sea did not result in any substantial DOC accumulation; however, this did occur in the microbial food web dominated Sargasso Sea (Carlson et al. 1998).

Alternative, but not exclusive, hypotheses for accumulation of biodegradable DOC have been proposed. Williams (1995) suggested that the assimilation of carbon-rich DOM was controlled by the availability of nitrogen. Thingstad and Lignell (1997) argued that trophic interactions in the microbial food web could keep the bacterial capacity for decomposition low, with a combination of grazing control of the biomass and nutrient limitation of growth rates. The latter could be caused by either nitrogen or phosphorus. The bacteria–protist model was substantiated in a later study (Thingstad et al. 1999). The consequence of nutrient limitation would be the accumulation of otherwise biodegradable DOC (Rivkin and Anderson 1997).

In the present study we aimed to explore the production, assimilation, and accumulation of DOC and DON. The nu-

trient regime of eight marine mesocosms was manipulated to produce a range of phytoplankton bloom situations. State and rate measurements were made in order to determine organic carbon partitioning and the relative role of the parameters that may control DOC accumulation.

Materials and methods

Study site, dosing strategy, and sampling—The study was carried out in July 1997 at the EU Large Scale Facility in Espesrend close to Bergen, Norway. Eight 11 m³ mesocosms (4-m deep bags) made of 0.15-mm polyethylene with a 90% photosynthetically active radiation (PAR) transmission were moored alongside a raft in the fjord. The mesocosms were filled with fjord water 5 d before the experiment was initiated. An airlift system ensured homogenous water masses within the bags. A continuous flow of water from the fjord at 1,100 L d⁻¹ provided a hydraulic renewal of 10% d⁻¹. Accordingly, we operated a pulsed chemostat-type system by direct additions of the nutrients to the mesocosms. The water renewal made it necessary to correct for the different dilutions of each measured variable in order to compare changes in concentrations and production rates over time. The variables measured as concentration (POC, chlorophyll, DOC, etc.) were recalculated to net production values taking account of the dilution and the eventual increases or decreases in concentration over time. The rate measurements, e.g., bacterial production, were also corrected for the continuous loss of productive volumes replaced with water from the fjord. All corrections were made in time steps of 1 or 2 d depending on the frequency of measurements. Most results are presented as cumulative net production over time, where the slopes of the curves represent production rates. We present the measured chlorophyll concentrations for comparative reasons (Fig. 1A). The decision to pool the results from different bags or to present the results from individual bags and treatments was made by comparing the production rates by linear regressions and acceptance of slope differences at $p < 0.05$. When the results from more than two bags were pooled, the calculated standard deviations are presented, whereas the range is used if the results from two bags were pooled.

The dosing strategy (presented in Table 1) was devised to generate initially nutrient-replete and subsequently replete and deficient growth regimes of the plankton. During phase I (6 d) all bags received equal dosing of inorganic nutrients at the Redfield ratio for N and P and with an excess of Si in order to initiate a diatom bloom. During phase II (days 6 to 15) the bags were treated in pairs. Addition of either inorganic nitrogen or phosphorus was terminated in two pairs of replicate bags. In phase III a new set of manipulations included further changes in the inorganic nutrient additions to single mesocosms. Glucose was added to selected bags during phases II and III (Table 1), but the results from the glucose-dosed bags are only presented if they do not differ from their parallel undosed bags. In vitro DOC degradation experiments were carried out with water from two of the bags dosed with glucose during phase III (bags 3 and 5). However, as an increase in monomeric carbohydrates and

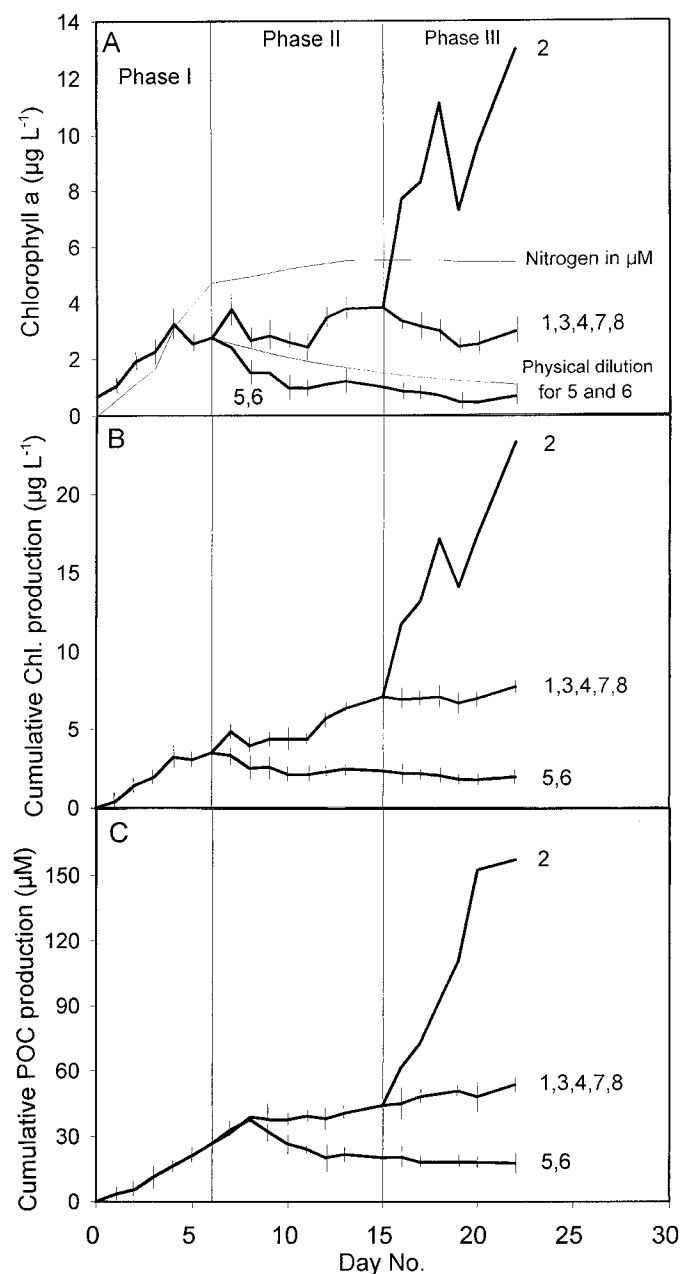


Fig. 1. (A) Average chlorophyll *a* concentrations, the calculated increases in added nitrogen (μM), and the physical dilution of chlorophyll *a* in bags 5 and 6. The different treatments are identified by bag number, and the different dosing phases (see Table 1) are shown by the vertical lines. (B) Average and cumulative net production of chlorophyll *a* separated with respect to treatments. (C) Average and cumulative net production of particulate organic carbon (POC). Error bars signify 1 standard deviation for $n > 2$ and range for $n = 2$.

bacterial production could not be detected in the mesocosms, no effects of glucose were carried into the degradation experiments. The results of the glucose-dosing experiment are presented elsewhere (Thingstad et al. pers. comm.).

Sampling was carried out early morning to midmorning before the daily nutrient additions employing clean techniques (acid-washed Nalgene containers, teflon tubing,

Table 1. Summary of nutrient dosing of the mesocosms. The daily additions in μM element: nitrate 0.6; silicate 1; phosphate 0.04. At days 3, 4, and 5 during phase I nutrients were added threefold, twofold, and twofold, respectively. Glucose was added at $6 \mu\text{M d}^{-1}$ (as C) to the bags indicated by italics. 5N and 5P refer to fivefold daily additions.

Bags	Phase I (days 0–5)	Phase II (days 6–15)	Phase III (days 16–22)
1	N + P	N + P	N + 5P
2	N + P	N + P	5N + P
3	N + P	N	<i>N + Glu</i>
4	N + P	N	N
5	N + P	P	<i>P + Glu</i>
6	N + P	P	P
7	N + P	<i>N + P + Glu</i>	<i>N* + P + Glu</i>
8	N + P	<i>N + P + Glu</i>	<i>N + P + Glu</i>

* Added as NH_4^+

gloves, etc.). Water samples from each of the eight bags and from the inlet were transported to the laboratory and redistributed for the various analyses within 20–30 min of sampling. The inlet water to the mesocosms was sampled from a 2 m³ darkened container on the mooring raft.

Chemical analyses—Inorganic nutrients were analyzed with a 5 channel Technicon AAII, segmented flow autoanalyzer for the micromolar nutrients and by chemiluminescence and fluorescence techniques for the nanomolar analyses, and these followed the micromolar and nanomolar methods previously published (Woodward et al. 1999). A brief summary report of the results is given here; silicate was found in the range 0.5 to 5 μM in all bags at all dates. The cessation of phosphate addition resulted in nitrate concentrations $>0.1 \mu\text{M}$ in bags 3 and 4 during phases II and III. The fivefold increased dosing with N in bag 2, during phase III, also left an unused pool of nitrate and undetectable phosphate ($<0.08 \mu\text{M}$). Phosphate at concentrations higher than 0.1 μM occurred at day 13, i.e., 7 d after nitrogen addition to bags 5 and 6 ceased. Nitrate was also detected in bag 1 phase III.

Table 2. Slopes and coefficients of determination (r^2) in linear regression calculations of net production of DOC, DON (μM), DPCHO, and DMCHO (μM glucose carbon) versus time. Bag 2 with high addition of nitrogen is treated separately. Relationships between DOC and chlorophyll *a* ($\mu\text{g L}^{-1}$) and DPCHO and DOC are also presented.

Variables	Bags	Period (days)	Slope	r^2	n	Units
DOC vs. time	1, 4, 6*	4–22	3.1 ± 0.11	0.98	57	$\mu\text{M d}^{-1}$
DON vs. time	1, 3, 4, 5, 6, 7, 8	11–22	0.28 ± 0.04	0.88	77	$\mu\text{M d}^{-1}$
DMCHO vs. time	1, 2, 4, 6	5–22	0.28 ± 0.03	0.88	14	$\mu\text{M d}^{-1}$
DPCHO vs. time	1, 3, 4, 5, 6, 7, 8	5–22	2.2 ± 0.12	0.96	53	$\mu\text{M d}^{-1}$
DPCHO vs. DOC	1, 4, 6	5–22	0.70 ± 0.06	0.89	41	$\mu\text{M } \mu\text{M}^{-1}$
DOC vs. time	2	15–22	14.5 ± 1.0	0.98	6	$\mu\text{M d}^{-1}$
DON vs. time	2	15–22	0.74 ± 0.08	0.89	6	$\mu\text{M d}^{-1}$
DPCHO vs. time	2	15–22	7.4 ± 0.08	0.99	5	$\mu\text{M d}^{-1}$
DPCHO vs. DOC	2	15–22	0.48 ± 0.06	0.96	5	$\mu\text{M } \mu\text{M}^{-1}$
DOC vs. Chl.	2	15–22	85.2 ± 8.4	0.96	6	$\mu\text{mol } \mu\text{g}^{-1}$

* Includes bags 3, 5, 7, 8 and 2 before addition of glucose and extra nitrogen, respectively.

Dissolved organic carbon and total dissolved nitrogen were measured with an integrated online system including a Shimadzu TOC-5000 and a Sievers NO analyzer. Triplicate samples from each mesocosm were filtered (precombusted Whatman GF/F filters) using an all-glass filtering system. The filtrates were collected in acid-cleaned and burned glass tubes before acidification and bubbling with CO_2 -free carrier gas. Analysis was made by injection of 100- μl samples into the combustion tube. Calibration, subtraction of blank, and calculations followed the procedures described by Cauwet (1994). Dissolved organic nitrogen (DON) was calculated by subtraction of inorganic nitrogen species, which always was a very small fraction of the total nitrogen.

Dissolved carbohydrates were measured with a slight modification of the MBTH (3-methyl-2-benzothiazolinone hydrazone hydrochloride) method (Johnson and Sieburth 1977). Filtered samples were poisoned with HgCl_2 , stored, and analyzed within 2 months. Dissolved monosaccharides (DMCHO) were measured directly, whereas total dissolved carbohydrates were determined after hydrolysis with 1.8 M HCl for 3.5 h at 100°C (Senior and Chevolut 1991). Dissolved polysaccharide (DPCHO) concentrations were then calculated after subtraction of the DMCHO. All values are presented as glucose carbon equivalents.

Biological analyses—Algal pigments collected on a GF/F filter were extracted for 12 h at 1–2°C with 90% acetone. Chlorophyll *a* (Chl *a*) concentrations were calculated from readings of excitation at 440 nm and emission at 665 nm in a Perkin Elmer LS5 Luminescence Spectrometer. A detailed analysis of diagnostic phytoplankton pigments was carried out by high pressure liquid chromatography (HPLC) separation (Wright et al. 1991) after ethanol extraction on ice and then recalculated to Chl *a* concentrations for each identified algal group by the algorithms developed by Mackey et al. (1996). The samples (GF/F filters) were stored in liquid nitrogen until analysis. Particulate organic carbon (POC) was measured on precombusted GF/F filters by dry combustion in a solid sample module connected to the Shimadzu TOC-5000/Sievers NO coupled instrument.

Bacterial activity was measured in triplicate by ^3H -thy-

midine and ^{14}C -leucine incorporation during 1-h separate incubations at in situ temperatures. Thymidine was added to a final concentration of 10 nM and leucine at 50 nM. The procedures followed the standard protocols for cold TCA extraction (Bell 1993; Kirchman 1993). Production was calculated from leucine incorporation into bacterial proteins using a conversion factor of $3.1 \text{ kg C mol}^{-1}$ (Kirchman 1993). Thymidine incorporation was converted to cell production by the factor $2 \times 10^{18} \text{ cells mol}^{-1}$ (Bell 1993) and to carbon with a constant biomass per cell of 20 fg C. The molar incorporation of the two substrates correlated positively and linearly with a coefficient of 0.98. The molar leucine:thymidine incorporation ratio was 38.7 and within the range of published values (Shiah and Ducklow 1997).

Bacterial abundance was determined by epifluorescence microscopy after 4'-6-diamidino-2-phenylindole (DAPI) staining (Porter and Feig 1980). Samples were immediately fixed with glutaraldehyde and the slides were prepared within a few hours and kept frozen until counting.

Whole community respiration was measured 10 times both as oxygen uptake and carbon dioxide production during 24-h dark-bottle incubations at in situ temperatures. Oxygen was measured with a high-precision Winkler titration (Williams and Jenkinson 1982) and CO_2 with an automated coulometric titration system (Robinson and Williams 1991).

DOC decomposition experiments—Mineralization of the DOC pools in bags 1, 3, 5, and 7 was measured six times as the time-dependent decrease of oxygen in GF/F filtered water, dispersed into duplicate 250-ml acid-cleaned glass-stoppered bottles. Bacterial growth in the seawater cultures was measured as the increase in cell abundance and POC in parallel samples. Inorganic nutrients were added at $10 \mu\text{M N}$ (NaNO_3) and $2 \mu\text{M P}$ (KH_2PO_4) to establish carbon-limited growth conditions in a second series of bottles and allowed for the assessment of the effects of inorganic nutrients. All samples were incubated underwater, in darkness, at a constant temperature (20°C) and then subsampled over the following 10 to 12 d. Oxygen was measured with a Clark-type electrode (YSI Model 58 and a 5905 probe), which was calibrated daily against Winkler titration (Williams and Jenkinson 1982). The overall precision of the method was $2 \mu\text{M}$. After each measurement the BOD bottles were refilled with about 1 ml of water from the bottles used to sample POC; this was in order to prevent the formation of air bubbles while replacing the stoppers. POC was measured after filtration of 25 ml of sample onto a 13-mm GF/F filter, dried at 40°C , and stored frozen until analysis by dry combustion (Søndergaard and Middelboe 1993). Bacteria were counted after DAPI staining (*see above*).

The use of oxygen was translated to carbon equivalents with a respiratory quotient of 0.8, and the change in the concentration of POC was added to the oxygen use to reach a value of consumed DOC. DOC consumed after 3 d of incubation was defined as the most biolabile DOC fraction (BDOC_{0-3}) according to Pitter and Chudoba (1990), whereas the DOC used between days 3 and 12 of the incubations is considered less labile, but biodegradable (BDOC_{3-12}). Based on this definition, we present the BDOC as two different pools. Bacterial carbon growth yield (Y) was calculated for

the initial 3 d of incubation from production of POC, oxygen use, and a respiratory quotient of 0.8; thus $Y = \text{POC}/(\text{POC} + \text{oxygen} \times 0.8)$. The appearance of flagellates and recycling of organic carbon hindered calculation of growth yield beyond 5 d of incubation. After 5 to 7 d of incubation, the POC production accounted for less than 10% of the carbon mineralization calculated from O_2 consumption. Bag 7 and bags 3 and 5 were dosed with glucose during phases II and III, and phase III, respectively (Table 1). Here we only present the results gained from samples where the concentrations of monosaccharides did not differ from their undosed parallel bags. Because a strong glucose signal was present for BDOC_{0-3} in bag 7, these results are not included.

Results

The biotic environment—The addition of inorganic nutrients during phase I resulted in a minor algal bloom reaching 3 to $4 \mu\text{g Chl } a \text{ L}^{-1}$ in all bags (Fig. 1A). Further changes were only found in those bags where the nitrogen loading was changed. Cessation of nitrogen along with the continuation of phosphate additions in bags 5 and 6 was followed by a continuous decrease in chlorophyll approaching that of the concentration in the inlet water ($0.7 \mu\text{g L}^{-1}$). The decrease was somewhat faster than explained by dilution (Fig. 1A). The fivefold increased nitrogen dosing of bag 2 in phase III increased the chlorophyll concentration from 3.5 to about $13 \mu\text{g Chl } a \text{ L}^{-1}$ (Fig. 1A), whereas the fivefold increase of P in bag 1 had no effect. These results indicate that nitrogen was controlling the development of the algal biomass. The calculated development in the concentration of added nitrogen is inserted in Fig. 1A.

The cumulative net production of chlorophyll clearly showed that the daily addition of nutrients (nitrogen and nitrogen + phosphate) resulted in a continuous positive production of chlorophyll, although at decreasing rates over time (Fig. 1B). The cessation of nitrogen addition to bags 5 and 6 (phase II) resulted in negative chlorophyll production after day 6.

The net production of POC revealed a time sequence for the production of chlorophyll. In the bags with continuous nitrogen additions (1, 3, 4, 7, and 8), the rate of particulate carbon production was constant at about $4.9 \mu\text{M d}^{-1}$ until day 8 and thereafter decreased to $1.2 \mu\text{M d}^{-1}$ over the remaining period (Fig. 1C). After day 8 the net POC production in bags 5 and 6 (cessation of nitrogen addition) was negative. The response to the fivefold higher nitrogen addition to bag 2 (phase III) was an immediate and higher rate of POC production (Fig. 1C). The relationship of POC to chlorophyll for nutrient-replete growth during phase I and in bag 2 phase III was strong ($r^2 = 0.94$) and with a conversion of $85 \mu\text{g C} (\mu\text{g Chl})^{-1}$, although the relationship decreased to $42 \mu\text{g C} (\mu\text{g Chl})^{-1}$ in bags 1, 3, 4, 7, and 8 during phases II and III. These values are within the range of most published results (Malone and Ducklow 1990). However, after day 8 the POC:chlorophyll value for bags 5 and 6 was consistently higher [about $189 \mu\text{g C} (\mu\text{g Chl})^{-1}$] than in the other bags. This indicates a fast removal of chlorophyll compared with other particulate organic fractions.

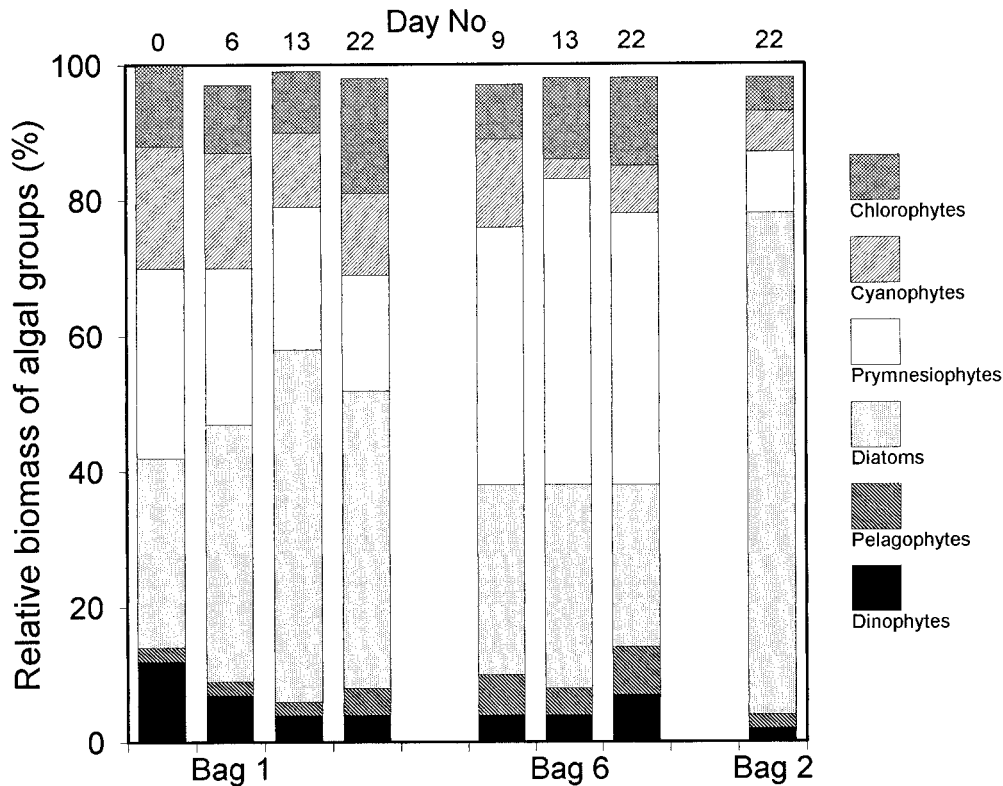


Fig. 2. Relative distribution of algal groups calculated from the conversion of group specific pigments to chlorophyll *a*. Results are from bags 1, 2, and 6, which represent the different dosing treatments. Day of sampling shown above each column.

The phytoplankton community was initially composed of diatoms, prymnesiophytes, cyanophytes, chlorophytes, and dinophytes in almost equal quantities (Fig. 2). Diatoms became more dominant in the constantly nitrogen-dosed bags (1, 3, 4, 7, and 8), whereas prymnesiophytes became more dominant after nitrogen was no longer added (bags 5 and 6). Elimination of phosphate additions had no measurable effect. The addition of extra nitrogen to bag 2 (phase III) resulted in the dominance of diatoms, accounting for 75% of the total chlorophyll when the experiment was terminated (Fig. 2).

The net production of bacteria cells was negative in all bags without regard for the different treatments, except for the slight accumulation of cells in bag 2 during phase III (Fig. 3A).

On the other hand, bacterial production measured by the radioisotopes increased immediately in response to the increases in algal biomass (Fig. 3B). After nitrogen was no longer added to bags 5 and 6, the bacterial production remained at the rate achieved during phase I. The fivefold higher nitrogen loading of bag 2 (phase III) resulted in an increase in bacterial production coincident with the increase in chlorophyll (Fig. 1B). Total bacterial production (leucine method) for the experimental period of 22 d was 45 μM carbon in bags 5 and 6; 64 μM in bags 1, 3, and 4; and 97 μM in bag 2 (Fig. 3B). The production values calculated from leucine and thymidine correlated linearly and perfectly ($r^2 = 0.98$, $n = 62$); however, the leucine values were three-

fold higher than the thymidine values and suggest a threefold discrepancy in the conversion factors used.

The organic environment—DOC and DON accumulated in all bags, but with distinctly different time sequences. From day 4 onward the net production of DOC in bags 1, 2, 4, and 6 proceeded at a constant rate of 3.1 $\mu\text{M d}^{-1}$ (Fig. 4). The concentrations in these bags increased from 112 to 155 μM . The cessation of nitrogen addition to bag 6 and of phosphate to bag 4 did not affect the rate of net DOC production. The addition of extra nitrogen to bag 2 from day 15 immediately increased the DOC production rate to 14.9 $\mu\text{M d}^{-1}$ (Fig. 4), and the concentration in bag 2 approached 200 μM when the experiment was terminated.

DON did not accumulate during the initial 11 d of the experiment. From day 11 onward the average rate of DON production was 0.28 $\mu\text{M d}^{-1}$ and the molar DOC: DON production ratio was 11. In bag 2 (phase III) the net DON production increased to 0.74 $\mu\text{M d}^{-1}$ (Fig. 4), and the new DOM was by comparison carbon rich, with a C:N ratio of 20. The linear regression parameters for DOC and DON production versus time are summarized in Table 2, and the C:N ratios of the new DOM are summarized in Table 3. The scaling in Fig. 4 shows the clear departure in newly produced DOM from the canonical Redfield value.

From day 5 a detectable net production of dissolved polymeric carbohydrates (DPCHO) was measured in all bags. The average production rate was 2.2 $\mu\text{M d}^{-1}$ (as glucose

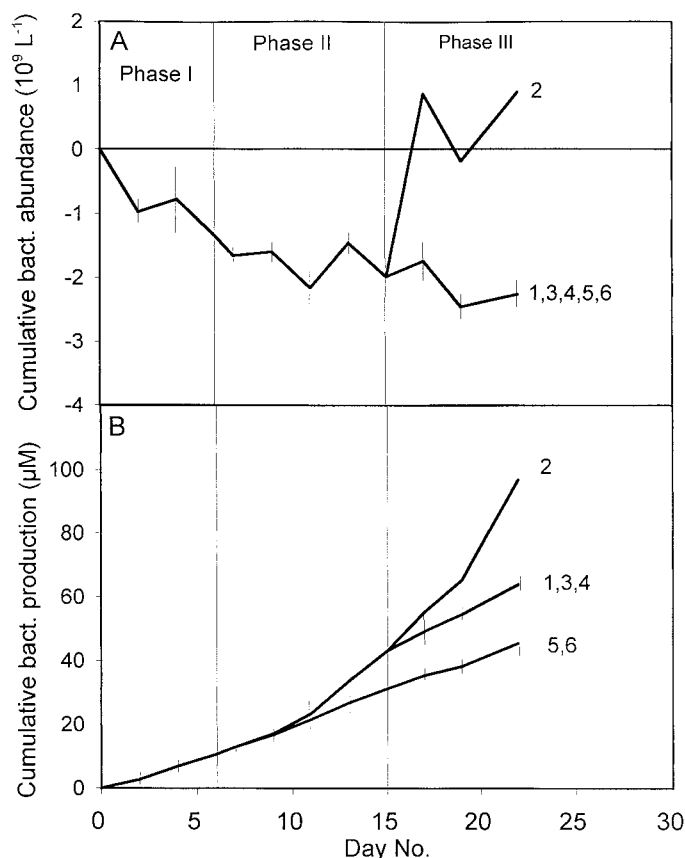


Fig. 3. (A) Average and cumulative net production of bacterial cells (\pm SD, $n = 7$). (B) Average and cumulative bacterial net production measured with the leucine method. Bags 3 and 5 with added glucose are included as no effect on production could be detected compared with their undosed parallel bags. Error bars signify 1 standard deviation for $n > 2$ and range for $n = 2$.

carbon equivalents) without any significant effects of treatment (Fig. 5). The only exception was an enhanced production in bag 2 during phase III, where the DPCHO production rate increased to $7.4 \mu\text{M d}^{-1}$. A linear regression of DPCHO versus DOC for bags 1, 4, and 6 showed a good correlation ($r^2 = 0.89$) and DPCHO accounted for 70% of the new DOC (Table 2). About 50% of the DOC production in bag 2 phase III was DPCHO (Table 2). Under the assumption that the measured DPCHO was nitrogen free, it can be calculated that new and accumulating nonpolysaccharide DOM had a very low C:N ratio of 3.2 in bags 1, 4, and 6 and a C:N ratio of 10 in bag 2 (phase III) (Table 3). The inclusion of residues with nitrogen in the carbohydrate measurements (e.g., glucosamine, muramic acid) would increase the C:N ratios of the calculated non-DPCHO compounds.

Over the course of the experiment, the concentration of dissolved monosaccharides (DMCHO) increased from about 4.5 to $7 \mu\text{M}$ (glucose carbon equivalents). Taking account of the dilution with fjord water, the increased concentration was equivalent to a net production of about $5 \mu\text{M}$ during the experiment (Fig. 5) and an average production rate of $0.28 \mu\text{M d}^{-1}$ (Table 2). The diatom bloom in bag 2 phase III did not result in higher net production of DMCHO, and

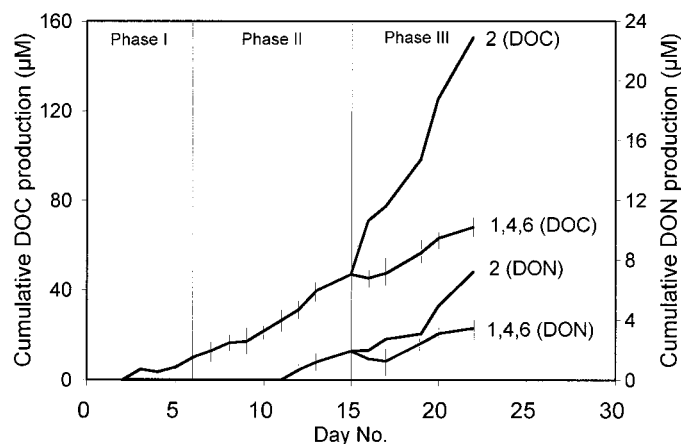


Fig. 4. Average and cumulative net production of DOC and DON. The bags are separated with respect to treatment in the different phases of dosing. Error bars are \pm SD, $n = 3$. The scaling of the y-axes approximates to the Redfield C:N ratio.

the added glucose to bags 3 and 5 during phase III was undetectable and accordingly must have been assimilated during the 22 h between addition and the next sampling.

Partitioning of the organic carbon production—The calculated production from the chemical measurements represents the ecosystem net organic carbon production partitioned into particulate and dissolved fractions. POC dominated the production during phase I (Fig. 6). The lag of about 4 d in the DOC production compared with POC had the consequence that only 21% of the total organic carbon (TOC = POC + DOC) accumulated in the dissolved fraction. In the bags dosed continually with nitrogen (1 and 4), the distribution changed during phases II and III to favor the production of DOC, which accounted for 68% of TOC (Fig. 6). The increased nitrogen dosing of bag 2 partly favored POC production. However, the production of DOC was still high, and about 30% of the TOC accumulated as DOC (Fig. 6).

The cessation of nitrogen addition to bag 6 (and 5) created the most dramatic change in favor of DOC production. Although the net production of POC became negative TOC production remained positive, and during phase II and III the net DOC production was 118% of the cumulated TOC production (Fig. 6). A DOC production $>100\%$ occurred

Table 3. Molar DOC:DON ratios of new DOM accumulating in the bags and the C:N ratios of the nonpolysaccharide DOM. The calculated non-DPCHO ratios assume that nitrogen is not included in the polysaccharide measurements (see text).

Bags	Period (days)	C:N of "new" DOM	C:N of new non-DPCHO DOM
All	0–6	infinite	infinite
1, 2, 3, 4, 5, 6	6–11	infinite	infinite
1, 4, 6	11–22	11	3.2
2	15–22	20	10

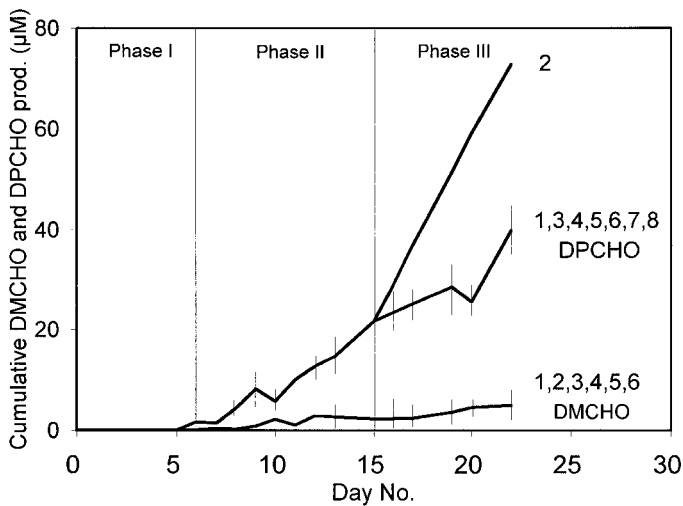


Fig. 5. Average (\pm SD, $n = 4$ to 8) and cumulative net production of dissolved polysaccharides (DPCHO) and dissolved mono-saccharides (DMCHO).

because the production of DOC was larger than the loss of POC.

To account for the partitioning of total net primary production (i.e., excluding algal respiration) and reach an estimate of the gross release of DOC, the heterotrophic respiration must be known. Heterotrophic respiration was not measured directly, hence it is not possible to establish an exact carbon budget. Calculation of gross DOC production is not trivial. The unknown variables are in situ bacterial respiration and the amount of bacterial biomass recycled via grazing and viral lysis and reentering bacterial production. The measurements of bacterial production should make it possible to estimate the amount of DOC used by bacteria and not detected by the chemical measurements of POC and DOC. An estimate of the gross DOC production must include the total amount of organic carbon assimilated and metabolized by the bacteria, including respiratory losses, and this amount has to be added to the net DOC production to reach gross DOC production. Here we approximate the total DOC flux through the bacterioplankton with the constraint given by the measured community respiration. Hence, bacterial respiration cannot be larger than the total community respiration. The values for the estimated net amount of DOC assimilated by the bacteria are presented in Fig. 6 and Table 4, and the detailed arguments to reach an estimate of the net DOC assimilation are presented in the discussion.

During phase I, the calculated gross production of DOC (new DOC + net DOC assimilation) accounted for about half of the net system production, which is TOC + net DOC assimilation. During phase II and III, gross DOC production accounted for 82% in bags 1 and 4 and 111% in bag 6 (Fig. 6). In bag 2 (phase III), the gross DOC production was 52% of the net system production. Except for phase I, the net production (accumulation) of DOC and the supply of dissolved organic substrates to fuel the bacterial production totally dominated the carbon partitioning. The smallest percentage of labile DOC calculated as net DOC assimilation divided by the gross DOC production was 38% and pro-

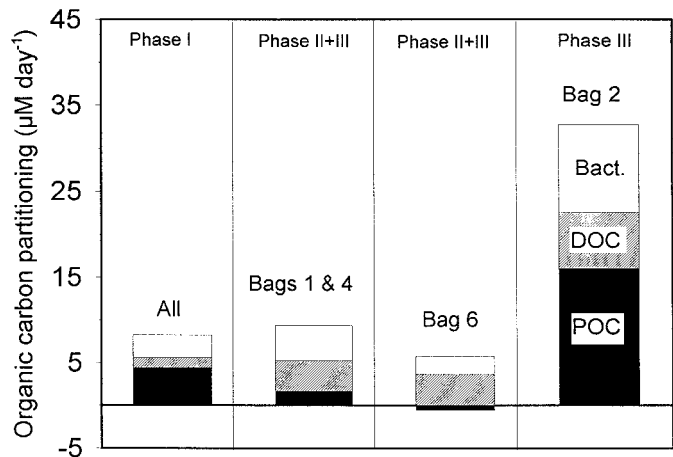


Fig. 6. The partitioning of net production of particulate organic carbon (POC) and dissolved organic carbon (DOC) and bacterial net assimilation of DOC (Bact.). The values of bacterial net assimilation were calculated with a growth efficiency of 0.43 and have been corrected for organic carbon recycling. See Table 4 and the discussion.

duced in bag 6, whereas 61% of the DOC produced by the diatom-dominated community in bag 2 (phase III) was used by the bacteria and escaped accumulation and chemical detection. The lability of DOC in bags 1 and 4 during phase II and III was 53%. The most labile DOC (70% uptake of the gross production) was produced during phase I.

Biodegradability of DOC measured in vitro—We characterized the ambient DOC pools as a labile fraction used within 3 d (BDOC_{0-3}) and a semilabile but still biodegradable pool mineralized during the subsequent 8 to 9 d of incubation (BDOC_{3-12}). The ambient concentration of BDOC_{0-3} was between 5 and 10 μM (Fig. 7A) with no major changes with respect to time or treatments. During the first 3 d of the in vitro incubations, the bacterial carbon growth yield averaged $43 \pm 10\%$ (\pm SD, $n = 32$), and addition of inorganic nutrients did not affect the result.

The pool size of BDOC_{3-12} gradually increased from 5 μM to 20–25 μM in bags 1, 3, 5, and 7 (Fig. 7B) along with the in situ accumulation of DOC. All values for bag 5 (the N-deficient bag) were lower than in the other bags. Total BDOC (BDOC_{0-12}) thus ranged from 10 to 30 μM and accounted for 10 to 22% of the DOC. A linear regression of total BDOC versus the concentration of new DOC ($f(x) = -0.32 + 0.35x$, $r^2 = 0.49$, $n = 23$) showed that about 35% of the accumulated DOC was used by bacteria within 10 to 12 d.

The time courses for DOC use are exemplified with the results from bag 1 (Fig. 8). When we initiated the experiment (day 0), the endpoint in DOC use was approached within 1 week and averaged 14 μM . Likewise, the DOC use endpoint was reached for the samples collected on day 8. Although the rate of DOC use increased as a function of the higher concentrations of DOC, the endpoint was not reached in the samples from the remaining dates, and the degradation continued linearly beyond our last sampling (Fig. 8).

The use of bacterial cultures to measure BDOC has the

Table 4. Comparison of community dark respiration and bacterial production measured with the leucine and the thymidine methods. All values are in μM and are cumulative with respect to phases of treatment. The derived bacterial respiration (R) was calculated from growth (G) as $R = G(1 - Y)/Y$, where Y is the bacterial growth yield. $Y = 0.43$ is taken from our in vitro decomposition experiments and 0.28 from del Giorgio and Cole (1998). The value of G was derived from estimates of bacterial production, *see text*. The determined community respiratory quotient was 0.71 ± 0.03 , $r^2 = 0.97$, $n = 18$ and used to convert oxygen respiration to carbon equivalents. The weighted average of $0.27 \mu\text{mol O}_2 \mu\text{g Chl}^{-1} \text{d}^{-1}$ from Langdon (1993) was used to calculate algal respiration. Protozoan respiration was estimated as $G \times 0.7$, and the calculated bacterial respiration was by subtraction of algal and protozoan respiration from the average community respiration. Total DOC assimilation is thus bacterial production plus the calculated respiration. Net DOC assimilation is calculated from total DOC assimilation by iteration of bacterial carbon recycling with efficiencies of 42 and 28%. The recycled DOC is thus the difference between total and net DOC assimilation. A detailed explanation is presented in the text.

Variable	All bags (phase I)	Bags 1 and 4 (phases II and III)	Bag 2 (phase III)	Bag 6 (phases II and III)
Bacterial production (leucine, G)	11	53	50	35
Derived respiration (R) $Y = 0.43$	14.6	70		46.4
Derived respiration (R) $Y = 0.28$	28	136		90
Bacterial production (thymidine, G)	2	18	19	13
Derived respiration (R) $Y = 0.43$	2.7	23.9		17.2
Derived respiration (R) $Y = 0.28$	5	46		33
Community CO_2 respiration	28	117	—	43
Community O_2 respiration in carbon equivalents	30.5	102	—	61
Mean community CO_2 respiration	29	110		52
Calculated algal respiration	3.9	19.5		4.1
Calculated protozoan respiration	7.7	37		24.5
Calculated bacterial respiration by difference	17.4	53.5		23.5
Total DOC assimilation	28.4	106.5		58.5
Maximum net DOC assimilation				
Assuming $Y = 0.43$	16.2	60.9	74*	33.4
Assuming $Y = 0.28$	20.4	76.6		42.1

* Assuming conditions as during phase I.

implicit assumption that the bacteria are carbon limited. This assumption was tested by addition of inorganic nutrients (N and P). The comparison between experiments with and without addition of inorganic nutrients showed that nutrients only had a marginal effect: $f(x) = 3.1 + 0.96x$, $r^2 = 0.74$, $n = 26$. The small positive intercept and the $f(x) = x$ line show that the addition of inorganic nutrients in most cases very slightly, but not significantly, increased the measured amounts of BDOC; neither was the rate of use affected.

Discussion

Carbon partitioning—Previous studies in oceanic and coastal environments have shown DOC to accumulate over the productive season (Copin-Montégut and Avril 1993; Carlson et al. 1994; Williams 1995; Børsheim and Mykkestad 1997) and after episodic bloom events (Ittekkot 1982; Billen and Fontigny 1987). Carbon partitioning of planktonic primary production in favor of DOC and an uncoupling between DOC production and degradation therefore must be a common phenomenon in marine waters.

The net production of POC and DOC in the mesocosms clearly showed that the carbon partitioning was dominated by DOC production except during phase I (Fig. 6). The most pronounced result was found in the community with terminated nitrogen dosing, where the net DOC production was 118% of the net TOC production. Such a high value can only be explained by the conversion of POC to DOC at a high yield and only if most of the phytoplankton production

was lost as DOC. The nutrient-replete and fast-growing diatom-dominated community in bag 2 (phase III) and the growing but more complex phytoplankton communities in bags 1 and 4 had a net DOC production of 30 and 70% of TOC, respectively. These results are in general agreement with Williams (1995), who showed that accumulation of DOC often surpasses the accumulation of POC in coastal water. However, any analysis of organic carbon partitioning not only relies on the chemical measurements, but must also include the amount of DOC immediately assimilated by the bacterioplankton and sequestered to biomass and respiration.

An estimate of gross DOC use by the bacteria can be made based on our measurements of bacterial production and the community respiration as determined by O_2 and CO_2 measurements (Table 4). We need to meet the constraint that bacterial respiration must be less than community respiration, as some part of the respiration will be algal and protozoan. We may assume (*see* Williams 1982) that metazoan respiration is unlikely to be a major component of overall measured respiration.

Given our determined bacterial growth yield of 43%, we can derive bacterial respiration from bacterial production. There is caution to note in addition to the obviously limited nature of the data. No allowance is made for recycling of DOC from bacterial biomass reentering the DOC pool via mortality by grazing and viral lysis. Recycling has the consequence that a fraction of the organic carbon can enter bacterial production measurements more than once and thus provides an overestimate of the net amount of carbon assim-

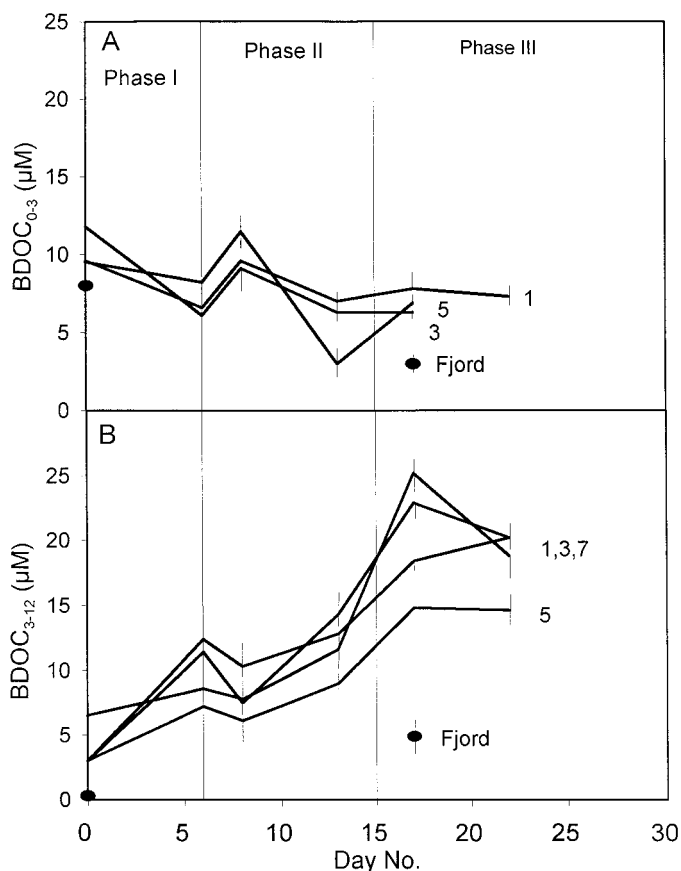


Fig. 7. Results of DOC decomposition in samples from mesocosms and presented as the means of duplicate samples with inserted range bars. Sampling was done on days 0, 6, 8, 13, 17, and 22. (A) The amount of DOC used during the first 3 d of incubations (BDOC₀₋₃). (B) The amount of DOC used from day 3 to 12 (BDOC₃₋₁₂) of the decomposition experiments.

ilated by bacteria. Because the sum of bacterial production and derived respiration was either very close to or higher than the community respiration, i.e., without subtraction of respiration by other organisms, organic recycling seems very likely (Table 4). Algal respiration may be estimated from chlorophyll concentrations (Fig. 1A), the relative biomass contribution of algal groups (Fig. 2), and the coefficients given by Langdon (1993). Protozoan respiration is more difficult to estimate directly. However, as no bacterial biomass accumulated, all bacterial production must be consumed in the microbial food web—this gives a mechanism to estimate protozoan respiration. A minimum value is calculated from presumed grazing of the bacterial production by the protozoans and a protozoan growth yield of 30% (Riemann and Christoffersen 1993).

Bacterial respiration can also be calculated from the community respiration by subtraction of algal and protozoan respiration (see Table 4), and much of this estimate is contained within that calculated from leucine production and more than contained within that calculated from the thymidine measurements. This may be taken to suggest that leucine uptake provides the most accurate of the two measurements of bacterial growth. However, if we take the median bacterial

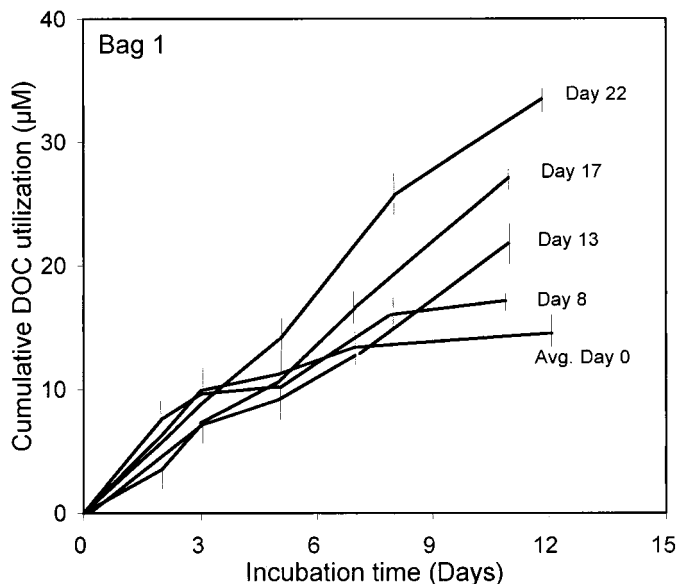


Fig. 8. Time courses of cumulative DOC use during the decomposition experiments with water from bag 1. The values from day 0 are averaged from all bags (\pm SD, $n = 4$ to 8).

growth yield value for oceanic and estuarine populations of 0.28 (del Giorgio and Cole 1998), then from the leucine technique we get overestimates of bacterial respiration (i.e., they are greater than community respiration) and more acceptable estimates of respiration from the thymidine observations (Table 4).

We can sum up the calculated bacterial respiration and measured production to obtain total DOC use. This will contain a component of recycled DOC, which we may attempt to correct for. The maximum recycled DOC may be estimated from the bacterial growth yield as $1/(1 - Y)$, where Y is the fractional growth yield [$Y^0 + Y^1 + Y^2 + Y^3 \dots = 1/(1 - Y)$]. This gives a maximum figure as it assumes a single step in recycling where all produced bacterial biomass is recycled back to the bacteria at each iteration. Subtraction of bacterial biomass fueling protozoan respiration and growth would lower recycling. However, such a calculation would involve a new series of uncertain assumptions and become very speculative. Given the above equation, our observed value of 0.43 for Y , the leucine incorporation as an estimate of bacterial growth, and bacterial respiration calculated from the community respiration, we can derive an estimate for net DOC use. Net DOC assimilation is total TOC assimilation divided by the maximum estimate of fractional recycling of 1.75. These calculations are presented in Table 4. Using 0.28 for Y (del Giorgio and Cole 1998) would give a maximum fractional recycling of 1.39. Because of the assumptions made, the calculated net DOC flux is most likely a minimum figure. Hence, our estimate of carbon partitioning is conservative with respect to DOC assimilation. The calculations showed respiration by bacteria and protozoans grazing their biomass to be from 82 to 92% of the community respiration (Table 4). The calculated net DOC assimilations ($Y = 0.43$) are the values entering the bacterial carbon compartment in Fig. 6. The gross DOC production

is the sum of net DOC accumulation and bacterial net assimilation. A detailed analysis of carbon partitioning at the community level can only be carried out given the constraint from our measurements of community dark respiration.

Although many measurements of bacterial production and comparisons with primary production are present for various marine areas (Ducklow and Carlson 1992), only the study by Carlson et al. (1998) is available for a direct comparison with the present results, where the production of POC, DOC, and bacteria was quantified. The low DOC production and high relative lability of DOC during the autotrophic phase I was similar to the carbon partitioning during a *Phaeocystis* bloom in the Ross Sea, where Carlson et al. (1998) calculated the gross DOC production (*see definition above*) to be about 30% of the net system primary production. In their study, the bacterial DOC assimilation was calculated from bacterial production measurements (thymidine method) using a 14% growth efficiency and without compensation for organic recycling. Thus, most of the estimated bacterial carbon uptake was immediately converted to CO₂. Only 9% of the total primary production in the Ross Sea accumulated as new DOC. Our equivalent values for phase I were 46% gross DOC production and 20% accumulation. By contrast, high net DOC production and low lability dominated the carbon partitioning over the growing season in the Sargasso Sea, where the gross DOC production was about 90% of the cumulative net system production and about half accumulated as new DOC (Carlson et al. 1998). In our study, the partitionings between POC and DOC and between bacterial uptake and accumulation of DOC during phases II and III were rather similar to the situation reported for the Sargasso Sea. Gross DOC production was between 51 and 111% of the total production (lowest in bag 2 phase III), and the DOC accumulation was between 30 and 62% of the gross DOC production (Fig. 6). The most labile DOC was produced during nutrient-replete growth in phase I and by the nutrient-replete diatom-dominated community in bag 2 (phase III). The least labile DOC was produced by the declining community in bag 6. In accordance with Carlson et al. (1998), we can conclude perhaps not surprisingly that autotrophic communities with accumulating phytoplankton biomass have the lowest partitioning of production into DOC and apparently produce the most labile DOC.

DOC production and the biotic environment—DOC-producing processes include release from phytoplankton, losses from grazing and viral-induced lysis, and bacterial solubilization of particles (Søndergaard and Middelboe 1995). The quantitative importance of each process under in situ conditions remains unclear, and mechanistic explanations for the production of new DOC are partly speculative. High production and accumulation of DOC have been observed in cultures of nutrient-deficient diatoms (Myklestad 1977; Guerrini et al. 1998) and during the decay of natural and experimental diatom blooms (Ittekkot 1982; Norrman et al. 1995; Fajon et al. 1999). There is no doubt that release from phytoplankton, and especially from diatoms, in the stationary growth phase can be a dominant DOC source. However, the more gradual seasonal accumulation of new DOC in picoplankton-dominated systems like the Sargasso Sea (Carl-

son et al. 1994) and in other marine areas (Williams 1995) indicate that formation of new DOC can take place in different types of plankton communities. These observations are similar to the present results.

The dosing strategy of the mesocosms created four different plankton communities with respect to production and composition. During phase I all of the phytoplankton groups responded to the nutrient additions and increased their biomass at equal and constant rates (Figs. 1B and 2). Bag 2 (phase III) was characterized by rapidly growing diatoms, where the presence of inorganic nitrate and silicate above 1 μM indicated a nutrient-replete situation, although the phosphate concentration was comparatively low at the last sampling day. The cessation of nitrogen addition was followed by a negative POC production and represented the decline of the plankton community. The effect of stopping the nitrogen addition and the immediate and vigorous algal growth in bag 2 phase III, when the nitrogen dosing was increased, suggested that nitrogen was the controlling factor for the development of the algal biomass. In the bags with constant nitrogen additions (1 and 4), the rate of POC production decreased after day 8 (Fig. 1C), but the TOC production proceeded linearly at a rate of about 5.7 $\mu\text{M d}^{-1}$ during the entire experiment. The decreased rate of POC production could be an indication of competition and partial nitrogen limitation, since a constant amount of added nitrogen had to be distributed among an increasing amount of algae and other organisms. Development of increased grazing could also have contributed to a decrease in the rate of POC production and the subsequent transfer of particulate nitrogen to higher trophic levels.

All communities produced DOC at high but different rates and of varying lability. The DOC-producing processes cannot be identified in detail, but, being nutrient replete, the exponential growth of different algae during phase I and the diatoms in bag 2 phase III showed that exudation from healthy algae is the most likely process to have dominated. Thus, high production of DOC takes place during an active diatom bloom and not only during the decay of a bloom. The high DOC production by the nitrogen-limited and declining community is in agreement with previous findings (Ittekkot 1982; Norrman et al. 1995; Fajon et al. 1999).

Relative accumulation of DOC and DON—After the introduction of nutrients in phase I, the POC production increased immediately, whereas the net production of DOC and DON had a time lag of about 4 and 11 d, respectively. The consequence was an infinitely high C:N ratio of the new DOM for 7 d, followed by a ratio of 11 (Table 3), a value similar to that (14.2) estimated for the C:N ratio of DOM accumulation in the English Channel by Williams (1995). The new DOM produced during the vigorous growth of diatoms in bag 2 (phase III) was carbon-rich with a C:N ratio of 20. This result is similar to the result by Norrman et al. (1995), where new DOM accumulating after the sedimentation of a diatom bloom had a C:N ratio of 22. Hence, a high release of carbon-rich DOM is not solely related to decaying diatoms, but evidently also linked with nutrient-replete and growing diatoms.

From 50 to 70% of the new DOC was polysaccharides,

which accordingly were the most important products released and not immediately metabolized by bacteria. The production of polysaccharides by phytoplankton has most often been associated with diatoms and nutrient deficiencies (Mykkestad 1977; Ittekkot et al. 1981; Eberlein et al. 1983; Guerrini et al. 1998; Fajon et al. 1999). However, the production of polysaccharides was almost constant in all the bags (Fig. 5 and Table 2), and Prymnesiophytes became dominant in bag 6 as opposed to diatoms in all other bags. It would appear that different communities of phytoplankton with different nutrient regimes can release polysaccharides at high rates.

The measures of polysaccharide production made it possible to calculate the C:N ratio of the new non-DPCHO DOM. The new non-DPCHO DOM in bag 2 (phase III) had a ratio of 10 (Table 3) and was obviously dominated by other carbon-rich organic compounds, possibly lipids, which are major storage products in diatoms. The new non-DPCHO DOM in bags 1, 4, and 6 had a much lower C:N ratio of 3.2 (Table 3). This suggests that proteinaceous material, or, less likely, nucleic acids (C:N ratios of 3.8 and 2.6, respectively; Anderson 1995) dominated the new non-DPCHO DOM in the bags with slow growing (bags 1 and 4) and declining (bag 6) phytoplankton communities. The calculated C:N ratios are minimum values because we assume that nitrogen-containing compounds are not included in the polysaccharide analyses. The ratios are therefore biased to the low side by the possible inclusion of N-containing compound like glucosamine, muramic acid, and others. The concentration of such compounds is not known and cannot be corrected for.

The production of DON in bags 1 and 4 was $0.28 \mu\text{M d}^{-1}$ and half the inorganic nitrogen dosing of $0.6 \mu\text{M d}^{-1}$. The partitioning of the added inorganic nitrogen to DON was 25% in bag 2 phase III. These values are in agreement with those found by Bronk et al. (1994). In oceanic, coastal, and estuarine environments they found an average of 25 to 41% of the inorganic nitrogen uptake to be released as DON. However, the net release rate in our study was about fourfold higher than the highest rate observed in the study by Bronk et al. (1994).

Biodegradability of new autochthonous DOC—Most time-course studies on the biodegradation of DOC have shown the decomposition rate to decrease over time, where the most labile compounds initially are metabolized at high rates, followed successively by less available compounds at lower rates (e.g., Ogura 1972; Connolly et al. 1992; Chen and Wangersky 1996; Fry et al. 1996). In accordance with the general view that labile substrates like amino acids and monosaccharides at most times are kept at low concentrations in the nM range (Fuhrman and Ferguson 1986; Jørgensen and Jensen 1994) the BDOC_{0-3} pool did not systematically change in our experiment, but was rather low and constant in the range 3 to $10 \mu\text{M}$ (Fig. 7A). In contrast, the concentration of BDOC_{3-12} increased over time (Fig. 7B), concomitant with the accumulation of new DOC.

At the start of the experiment the average concentration of total BDOC (BDOC_{0-12}) was $14 \mu\text{M}$ (Fig. 8) with a turnover time of about 7 d. Any value above $14 \mu\text{M}$ therefore

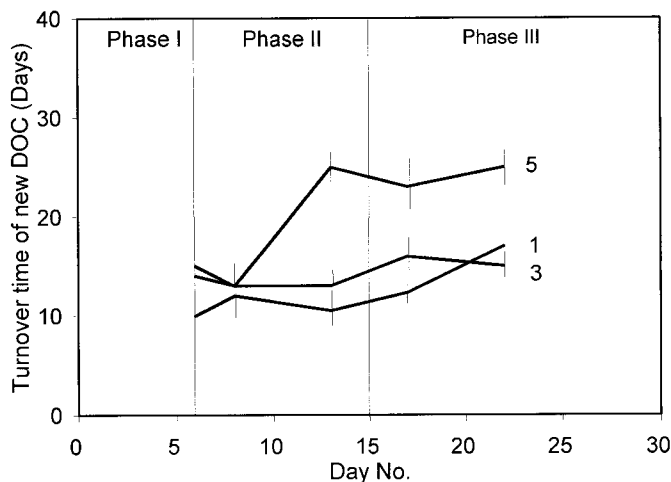


Fig. 9. Development in calculated turnover times for accumulated DOC. Samples were collected in the indicated bags over the course of the mesocosm experiment. Range of duplicate samples inserted.

must represent the newly produced pool of DOC. The use rates of new DOC (see Fig. 8) were used to calculate the theoretical turnover times for the accumulated DOC, assuming that all new DOC was available for decomposition. Preferential use of new DOC from a decaying diatom bloom as opposed to older DOC was found by Fry et al. (1996) and gives support to our approach relating the BDOC_{0-12} values with new DOC. The calculated turnover times ranged from 9 to 25 d (Fig. 9), with the longest turnover times in bag 5, due to very low rates of use. The turnover times of the new DOC were relatively constant despite the continued accumulation of DOC. The measured use rates increased fivefold from about $0.6 \mu\text{M d}^{-1}$ at day 8 to $3 \mu\text{M d}^{-1}$ at day 22. If the turnover time for the biodegradable DOC at day 0 is added to those calculated for the new DOC, the turnover time for the total biodegradable DOC increased from about 7 to 22–32 d during the experiment.

It can be argued that the calculated turnover times from the in vitro decomposition experiments are not those present in the mesocosms. The experiments were carried out in the dark at 20°C , which was about 5 to 7°C higher than in the mesocosms, and the endpoint of decomposition was not reached in all of the incubations. On average only 35% of the new DOC was used during the 10 to 12 d incubations and with constant rates (Fig. 8). An asymptotical decrease in use rates as the endpoint is approached would increase the turnover times. The higher temperature would enhance decomposition, whereas the absence of UV radiation could have had either a positive or negative effect. Depending on the origin of DOC, photochemical processes can both enhance and retard DOC use. Benner and Biddanda (1998) showed that DOC from the photic zone was more resistant to radiation than DOC from deep waters. The relatively short incubations could not determine whether a part of the new DOC was refractory to microbial use. However, the constant rates of use, even after 12 d of incubation (Fig. 8) make it likely that much more than 35% could in fact be degraded. Fry et al. (1996) found that about 30% of the new DOC of

diatom origin was resistant to microbial attack and remained so even after 2.5 yr of incubation. However, the turnover time of the biodegradable fraction in their experiment was in accordance with the present results. Like our experiments, those of Fry et al. (1996) were carried out in the dark, so an eventual effect of UV radiation cannot be evaluated. We conclude that the degradation rates and turnover times in our experiments are comparable with other results from in vitro experiments of DOC decomposition, but we cannot rule out UV effects in the mesocosms, which could change the in situ decomposition.

The long turnover times of new DOC compared with the BDOC when we started the experiment suggest the new products to be less labile to microbial attack and can explain the accumulation and the potential role of photochemical processes. Most of the new DOC was polysaccharides, and it is tempting to suggest that the semilabile nature of new DOC is linked with the chemical structure of these polymeric compounds. It is clear that inorganic nutrients did not limit the bacterial degradation, as their addition only marginally enhanced the degradation. Another explanation for the relatively long turnover times could be the protection of polymeric material by liposome submicron particles as suggested by Borch and Kirchman (1999). We put forward the suggestion that the seasonal accumulation of oceanic DOC is mainly a consequence of its semilabile nature.

Conclusions—The sequestration of oceanic primary production into POC and DOC has major implications for the functioning of the biological pump and food web structure (Karl et al. 1998). Although production in the form of particles can directly enter grazer food chains and participate in the vertical flux, DOC must reenter the food web via bacterial use. The vertical communication between the photic and aphotic zone with respect to DOC occurs by mixing processes inserting a delay, and a spatial separation, between primary production and decomposition by the heterotrophs.

The development of different biotic communities during this mesocosm experiment showed that carbon partitioning in favor of DOC production is not associated only with decaying algal communities or microbial food web dominated communities. High DOC partitioning at the level of 46 to 82% of the cumulative carbon production was found during autotrophic phases with increasing algal biomass. The highest partitioning of 111% was found in a nitrogen-deficient community with negative net POC production. The high DOC production might be linked to cell lysis, POC solubilization, or zooplankton grazing. Between 39 and 70% of the produced DOC was assimilated by the bacterioplankton. The most labile DOC was produced by exponentially growing phytoplankton communities, whereas the least labile DOC was produced by a declining and nitrogen-deficient community. Between 30 and 60% of the gross DOC production accumulated as new carbon-rich DOM composed primarily of polysaccharides (50 to 70% of the pool).

Decomposition experiments showed that at least 35% of the new DOC was biodegradable over 12 d and the degradation was not influenced by the mineral nutrient regime of the bacteria. Turnover times of 3–4 weeks for the new DOC

show its semilabile nature and explain the observed accumulation.

The DOC-producing mechanisms cannot be fully elucidated. However, during different growth conditions the DOC production varied, as did the C:N ratios of the net DOM production. The varying lability showed that different dissolved organic compounds were produced. We have from this study created a platform for more detailed studies, where the polymeric nature of new DOM might be compared with the enzymatic potential of the bacterioplankton to investigate and explain DOM accumulation.

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