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Production and decomposition of new DOC by marine plankton communities: carbohydrates, refractory components and nutrient limitation

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Abstract The accumulation and biodegradation of dissolved organic carbon (DOC) and dissolved and particulate combined neutral sugars (DCNS, PCNS) were followed during a period of 22 days in experimental marine phytoplankton incubations. Five different growth regimes were established in 11 m^3 coastal mesocosms to test whether an alternate Redfield ratio with either N or P depletion and ±diatom dominance would induce accumulation of refractory DOC (RDOC) and DCNS. The highest accumulation of DOC, DCNS and PCNS was found in the diatom dominated mesocosms. Sixteen percent of the newly accumulated DOC in the mesocosms with diatoms dominating could be explained by DCNS, while only 6% was explained in the mesocosms with few diatoms. PCNS composition was similar in all mesocosms and with dominance of glucose and mannose, while DCNS were more evenly distributed with the following mole percentages fucose 15, rhamnose 14, arabinose 6, galactose 27, glucose 20 and mannose 18%. The DCNS composition did not reflect the PCNS composition at any time during the experiment. Accumulated DCNS were quickly degraded and only 1% of the new RDOC was explained by DCNS. RDOC accumulated after day # 17 in the two mesocosms driven towards the most severe P limitation both with and without silicate. This shows that RDOC can be produced directly by the phytoplankton or indirectly in food web processes during the later stages of a bloom where the phytoplankton is P limited.

Keywords Dissolved organic carbon · Autochthonous DOC · Biodegradability · Mineralisation · Refractory DOC · Dissolved combined neutral sugars (DCNS)

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Introduction

It is well established that phytoplankton blooms contribute significantly to the over all production of DOC (Eberlein et al. 1985; Billen and Fontigny 1987; Williams 1995) and several studies have investigated accumulation and degradation of autochthonous produced dissolved organic matter (Norrman et al. 1995; Søndergaard et al. 2000a; Meon and Kirchman 2001; Kragh and Søndergaard 2004). Decoupling of production and degradation during bloom events supplies new potentially labile DOC in an intermittent fashion. However, we know little of the specific processes and mechanisms that enable the production of refractory dissolved organic carbon (RDOC).

Some of the processes to explain the formation of RDOM from autochthonous DOM sources are microbial processes that alter the molecular structure of DOM, making it resistant to further degradation and

thereby preserving fixed carbon in the ocean (Ogawa et al. 2001). Protection of proteins in submicron particles (Stoderegger and Herndl 1998) as well as photochemical transformations (Tranvik and Kokalj 1998) are other processes proposed to explain the production of RDOM. As a result, the RDOM pool is a product of a vast amount of different and complex processes; mostly composed of carbon rich compounds. N and P depletion of DOM has mainly been linked to the diagenetic age of the material, as nutrients are selectively removed from DOM over time, leaving the RDOM relatively rich in carbon (Williams 1990). However, Williams (1995) found in a seasonal study the C/N ratios of POC and DOC to be about twice the Redfield value of 6.6. This have further been supported by experimental studies by Norrman et al. (1995) and Søndergaard et al. (2000b), which shows that relatively new DOC can have C/N ratios of 20 or more. Thus, three competing hypothesis emerge from the literature: the first is that RDOC is produced over long periods of time through bacterial-mediated remineralization, the second is that RDOC is produced directly during blooms releasing high C:N DOC, and the third is that DOC is transformed to a refractory state relatively quickly through one of the processes mentioned above.

Phytoplankton cells typically contain from 13 to 35% carbohydrates (Parsons et al. 1961; Biddanda and Benner 1997) and extracellular release from nutrient deficient algae, especially diatoms, can be a major source of carbohydrates (Fajon et al. 1999). Søndergaard et al. (2000b) has further found that dissolved polysaccharides accounted for 50–70% of the newly produced DOC during an active bloom and not only during the decay of the bloom. Carbohydrates makes up the largest identified fraction of the DOC pool (Pakulski and Benner 1994) and total carbohydrates (TCHO) can account for up to 47% of the consumed DOC during summer (Burney et al. 1981). Chromatographic separation of neutral sugars provides a direct and sensitive measurement at the molecular level for an important and very dynamic subset of the entire carbohydrate pool. Dissolved combined neutral sugars (DCNS) have been reported to account for about 54% of bulk carbohydrates during bloom events (Biersmith and Benner 1998). Thus, DCNS can possibly make up to 30% of the DOC pool during bloom events and therefore likely to play an important role in the DOC dynamics.

While there is a relatively good understanding of how DOC and DCNS can accumulate during blooms and sequentially degrade (Norrman et al. 1995; Biddanda and Benner 1997; Fajon et al. 1999; Søndergaard et al. 2000a), very little information is available about which factors causes DOC moieties to enter the pool of RDOC with very long turnover times. Skewed Redfield ratio and thereby limitation by nutrients (N or P) could be one factor that influence the accumulation and degradation of DOC and DCNS and herein the production of RDOC. Experimental evidence with diatom cultures show that P limitation yields higher accumulation of DOC resistant to bacterial degradation, than does N limitation (Obernosterer and Herndl 1995). In this study we investigated how DOC and DCNS accumulated during different nutrient ratios in a plankton community and how they subsequently were degraded. Ten different growth regime scenarios were established in large coastal mesocosms to test whether an alternate Redfield ratio with either N or P depletion or ±diatom dominance would induce accumulation of RDOC and of DCNS.

Materials and methods

The experiment was carried out in ten mesocosms (closed bags with a conical lower part) with a volume of 11 m^3 each placed in Raunefjord at an EU large scale facility close to Bergen, Norway. The mesocosms were filled with water from the surrounding Raunefjord on 02 August 2002 and 10% of the volume was exchanged with fjord water continuously per day. During the filling of the ten 11 m^3 mesocosms larger metazoans and other particles were removed with a 100 μm mesh to minimize the effect of larger grazers. Nutrients were added once a day in the morning. Two sets of five mesocosms were deployed, and one set had silicate added throughout the experiment. A phytoplankton bloom was created during the first 7 days (Phase I) with daily addition of 1.6 μmol N-NO_3 l^{-1}, 0.1 μmol P-PO_4 l^{-1} (Redfield ratio) and 1.8 μmol Si l^{-1} in five bags (Table 1). During the next 14 days (phase II) the N to P ratio was altered within the mesocosms to 64, 32, 16, 8 and 4, and supplied in quantities so the limiting nutrient should allow similar biomass to develop in each mesocosm if the uptake were to obey the Redfield ratio. Furthermore, in Phase II the dilution with fjord

Table 1 Summary of nutrient dosing

Mesocosms	Phase I (days 0–7)	Phase II (days 8–21)
4N	2N + 2P	4N + P
2N	2N + 2P	2N + P
R	2N + 2P	N + P
2P	2N + 2P	N + 2P
4P	2N + 2P	N + 4P
4NS	2N + 2P + Si	4N + P+Si
2NS	2N + 2P + Si	2N + P+Si
RS	2N + 2P + Si	N + P+Si
2PS	2N + 2P + Si	N + 2P + Si
4PS	2N + 2P + Si	N + 4P + Si

The daily additions in μmol l^{-1} were: nitrate 11.2, silicate 12.6 and phosphate 0.7, which equivalents R addition. 2N, 4N, and 2P, 4P refer to two- and fourfold daily addition of N and P, respectively

water would bring each bag to a steady state with respect to biomass, i.e. the loss of limiting nutrient equals the addition. Naming of the ten bags is therefore as follows: 4N, 2N, R, 2P, 4P, 4NSi, 2NSi, RSi, 2PSi and 4PSi. The nutrient addition was, respectively, 0.05, 0.05, 0.05, 0.1 and 0.2 μmol P l^{-1} and 3.2, 1.6, 0.8, 0.8 and 8 μmol N l^{-1}. Si addition was held constant at 1.8 μmol Si l^{-1} in phase II. More details about the nutrient stoichiometry during the experiment is reported in Conan et al. (2007).

Water was sampled each day at mid morning from all the bags and the fjord using a clean technique and before addition of nutrients. This procedure enabled us to track the background input from the fjord. All sub-sampling was carried out from one batch of water in sequence determined by risk of contamination, with sampling for neutral sugars and DOC first as these measurements are most prone to contamination due to handling. The chlorophyll, POC, inorganic nutrients, and DOC in the mesocosms were measured each day for a period of 22 days.

Sampling for carbohydrates was divided into intensive days and extensive days. At intensive days duplicate samples were taken for dissolved free and combined neutral sugars (DFNS and DCNS) and particulate combined neutral sugars (PCNS) from all mesocosms and the inlet water. On extensive days, samples for DCNS were taken from the inlet water and mesocosms 4N, R, 4P, 4NSi, RSi and 4PSi.

The biodegradation experiments were performed at day # 0, 4, 7, 12, 17, 22 with water from bags 4N, R, 4P, 4NSi, RSi and 4PSi and the fjord. Prior to incubation the water (approx 1 l) was filtered through pre combusted GF/F filters to remove any eukaryotic organisms and left a subsample of the indigenous bacteria. The incubations were carried out in darkness and 18°C and samples for DOC and DCNS were taken with decreasing frequency at times 0, 5, 10, 20 and 150 days. Labile DOC (LDOC) in Table 3 is defined as DOC degraded at rate higher than 0.05 day^{-1}. We defined the recalcitrant endpoint as the concentration in the sample taken after 150 days of incubation, but realise that a 'true' endpoint is probably never obtained in most experiments (Fry et al. 1996). Semi labile DOC is defined as DOC-(LDOC + RDOC).

Analytical methods

Replicates of GF/F filtered water for dissolved neutral sugar analysis were sampled in pre-combusted glass vials and analysed immediately. DFNS was analysed directly, while a sample for DCNS was hydrolyzed for measurement as described in Borch and Kirchmann (1997). The particulate fraction was collected on a combusted GF/F filter (450°C, 5 h), dried and subsequently hydrolysed in 3 ml of Milli Q water and sulphuric acid. Samples were measured on a HPLC (Dionex DX500) microbore system with a PA10 column. The detection limits were <10 nM for individual species of the six aldoses separated. The concentrations of total carbohydrates are expressed in carbon units using the carbon content of each hexose or pentose.

DOC samples were collected each day and analyzed accordingly to Conan et al. (2007).

DOC measurements from the degradation experiments were sampled and analyzed according to Kragh and Søndergaard (2004) except that measurements were performed on a Shimadzu TOCV instead of a TOC5000.

Results

The results of chlorophyll *a*, POC and inorganic nutrients have been presented by Conan et al. (2007). The most prominent outcome with respect to algal biomass was that the diatom dominance in the +Si bags resulted in about twice the carbon biomass per

limiting nutrient than in the −Si set of bags. The six bags dosed at Redfield or low N: P ratios (P replete) had a lower net production of chlorophyll during phase II than those dosed with a higher N:P ratio. Thus, Conan et al. (2007) concluded that the plankton community in the fjord was most likely limited by N.

The algal community in the bags was initially with 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. Meso- and macrozooplankton biomass was only measured when the experiment was terminated. Zooplankton biomasses were from 2, 5 to 12 μmol C l^{-1} in the 4P, R and 4N mesocosms, respectively. Thus, zooplankton contributed <10% to the POC production. The zooplankton biomass in the −Si bags was dominated by adult and different copepodit stages of *Centrophages* with some *Acartia* and very few cladocerans. The biomass in the +Si bags was more evenly distributed among *Centrophages*, *Acartia* and *Oithona* and with the cladoceran *Podon* representing about 2% of the biomass.

During phase I (days 0–6) all bags in the ±Si set of bags were pooled with regard to DOC, PCNS and DCNS, respectively. The criterion for pooling each set of five bags was that the slopes of the linear regression of measured variables versus time were not significantly different ($p < 0.05$, *t*-test). Initial DOC concentration in the bags was 128 ± 2.3 μmol C l^{-1}, while the fjord had a DOC concentration of 133 μmol C l^{-1} at day # 0. Independent of treatment DOC accumulated at a rate of 2 μmol C l^{-1} day^{-1} during the first 5 days of the experiment (Fig. 1). At day 6 the mesocosms with added silicate started to accumulate DOC at a slightly higher rate of 3 μmol C l^{-1} day^{-1}.

At day # 7 (phase II) the nutrient regimes were altered, as described previously. From this time the DOC accumulation in the mesocosms started to diverge according to their individual treatment (Fig. 1). The lower rates of DOC accumulation were found in the mesocosms without addition of silicate and low diatom biomass. The accumulation during phase II in the −Si mesocosms increased 4P < 2P < R < 2N < 4N, and with a daily accumulation from 1.4 ± 0.4 to 4.3 ± 0.4 μmol C l^{-1}. Higher rates of DOC accumulation were found in the mesocosms where diatoms were dominant. The pattern showed that the highest daily accumulation was found in the mesocosms deviating most from the Redfield ratio, though higher in the 2NSi and 4NSi than in 4PSi and 2PSi. The rates calculated during phase II varied from 4.3 ± 0.7 μmol C l^{-1} day^{-1} in the RSi treatment to 7.3 ± 0.4 μmol C l^{-1} day^{-1} in the 4NSi treatment (Fig. 1).

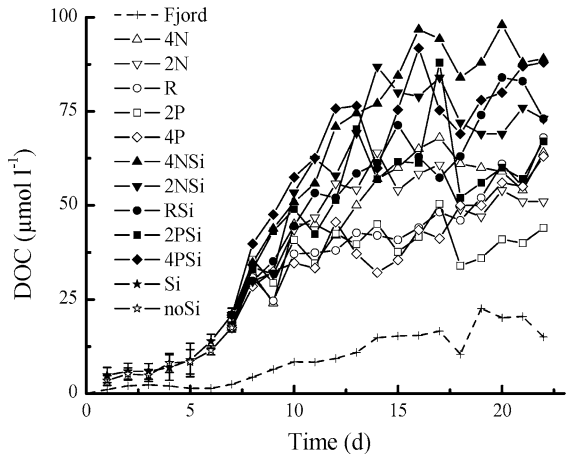

Fig. 1 Accumulation of DOC (μmol C l^{-1}) in the mesocosms and the fjord over the course of the experiment. *Error bars* are 95% confidence intervals

The diatom dominated mesocosms had a higher concentration of PCNS at day # 6 at 4.68 μM ±1.53, compared to the mesocosms without silicate addition at 2.62 μM ±0.97 ($p < 0.05$) (Fig. 2). The lowest accumulation of PCNS over time was found in the mesocosms without diatom dominance. The mesocosms R, 2P and 4P were at several occasions indistinguishable from the fjord despite an accumulation of at least 40 μM POC. No distinct pattern developed in the −Si mesocosms, though the 4N and 2N mesocosms had slightly higher PCNS concentration at day # 12 and 14. The lowest accumulation in the mesocosms with diatom dominance was found in the 2PSi and RSi mesocosms where 20 μmol C l^{-1} had accumulated at day # 14. In the 2NSi and 4NSi treatments a maximum of 29–30 μmol C l^{-1} accumulated at day # 14. The highest accumulation in the

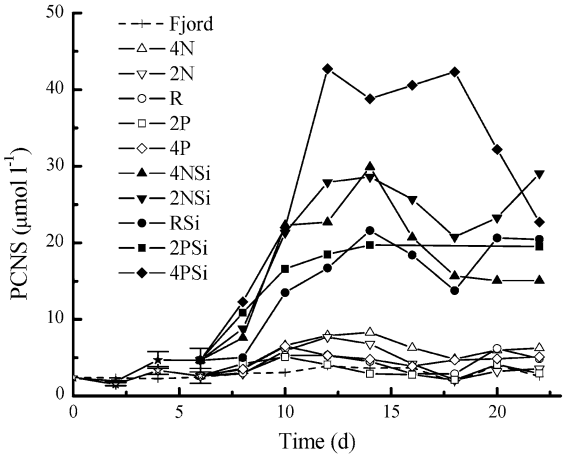

Fig. 2 Concentration of PCNS (µmol C l^{-1}) in the mesocosms and the fjord. *Error bars* are 95% confidence intervals

+Si mesocosms was about 40 µmol l^{-1} for the 4PSi treatment between day # 12 and 18.

Although, the relative proportion of PCNS to POC was small, distinct differences could be found between the mesocosms without and with diatom dominance (POC values shown in Conan et al. 2007). Similar treatments with and without silicate showed different PCNS to POC ratios. The percentage of PCNS to POC in the mesocosms without and with silicate addition were 3.2 ± 0.5 and 12.5 ± 3.7%, respectively ($p < 0.05$). The molecular composition of the PCNS were similar in all mesocosms with a clear dominance of glucose and mannose (Table 2.) and the composition did not change throughout the experiment.

DFNS did not at any time during the experiment reach concentrations above the detection limits for any of the measured aldoses. DCNS remained at a constant concentration during the first 4 days of the experiment (Fig. 3a) thereby not exciding the fjord values. At day # 6 the concentration was about 1 µmol C l^{-1} higher than in the fjord. The nutrient gradient did not result in any systematic pattern of accumulation within the mesocosms without diatom dominance. A slightly higher concentration was found in the 4N mesocosm with a maximum of 5.4 µmol C l^{-1} at day # 15. About 3.5 µmol C l^{-1} were found in the other mesocosms after 14 days. 2P and 2N continued to accumulate to a maximum of 4.1 µmol C l^{-1} at day # 16, while R and 4P decreased to 3.1 and 1.1 µmol C l^{-1} at day # 22, respectively (Fig. 3a).

DCNS in the diatom dominated mesocosms had accumulated 1.6 µmol C l^{-1} at day # 6 and the concentrations were significantly higher than in the fjord (Fig. 3b). After the nutrient regime was altered a treatment dependent pattern became apparent. The mesocosms were grouped with the lowest accumulation in the RSi mesocosm, second lowest in the 2PSi and 4PSi mesocosms and the highest accumulation found in the 2NSi and 4NSi mesocosms, i.e. the P-depleted bags. The accumulation in these five mesocosms was assumed linear (although with variations) during phase II as r^2 values for linear regressions yielded values between 0.94 and 0.99. The daily

Table 2 Mole percentage of particulate combined natural sugars and dissolved combined natural sugars at day # 0 and 22 of the bloom and for DCNS during degradations experiments

	Day	Fucose ± SE	Rhamnose ± SE	Arabinose ± SE	Galactose ± SE	Glucose ± SE	Mannose ± SE
PCNS	0	1 ± 1	3 ± 1	1 ± 1	2 ± 1	16 ± 1	76 ± 2
	22	2 ± 1	3 ± 1	1 ± 1	1 ± 1	18 ± 3	74 ± 6
DCNS	0	15 ± 3	13 ± 5	6 ± 5	27 ± 3	22 ± 4	18 ± 3
	22	15 ± 1	14 ± 5	6 ± 2	27 ± 3	20 ± 4	18 ± 1
Fjord DCNS during degradation	0	9 ± 3	7 ± 1	5 ± 1	27 ± 2	32 ± 4	20 ± 2
	150	10 ± 1	7 ± 4	5 ± 4	20 ± 1	54 ± 6	5 ± 2
Mesocosms DCNS during degradation	0	8 ± 2	8 ± 1	7 ± 2	27 ± 2	29 ± 4	21 ± 3
	5	7 ± 1	5 ± 1	5 ± 1	26 ± 2	36 ± 5	21 ± 4
	10	5 ± 0	8 ± 0	7 ± 0	28 ± 0	37 ± 0	14 ± 0
	20	9 ± 2	11 ± 2	12 ± 3	20 ± 2	40 ± 4	8 ± 4
	150	9 ± 2	11 ± 2	11 ± 2	20 ± 2	40 ± 4	8 ± 2

The percentages are averages for the mesocosms and experiments

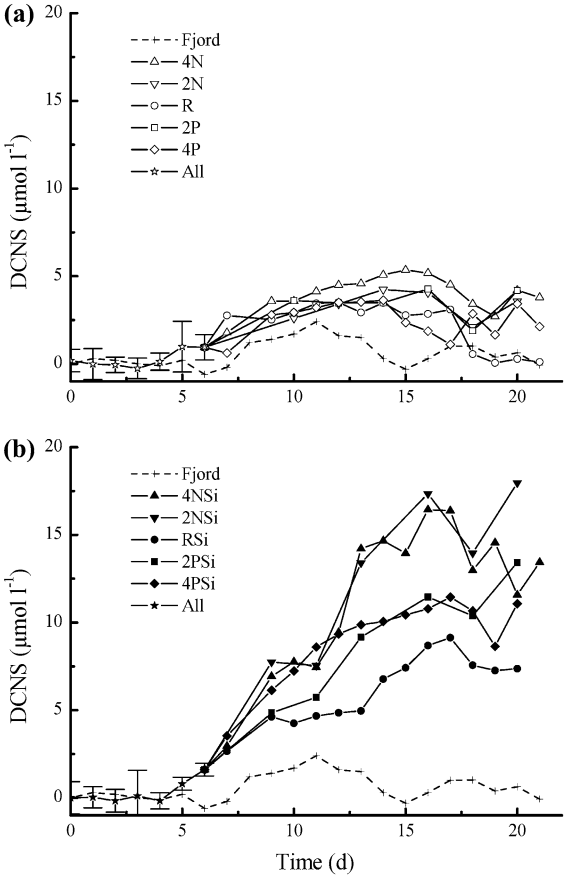

Fig. 3 Concentration of DCNS in mesocosm without added silicate (**a**) and with added silicate (**b**). *Error bars* are 95% confidence intervals

accumulation during phase II in RSi was 0.63 ± 0.06 μmol C l^{-1} day^{-1}. In 2PSi and 4PSi the accumulation rates ranged from 0.98 ± 0.11 to 0.87 ± 0.07 μmol C l^{-1} day^{-1}, respectively. 2NSi and 4NSi accumulated DCNS at rates of 1.59 ± 0.08 and 1.41 ± 0.09 μmol C l^{-1} day^{-1}, respectively (Fig. 3b).

The relative composition of the DCNS species at day # 0 was; fucose: 15 ± 3, rhamnose: 13 ± 5, arabinose: 6 ± 5, galactose: 27 ± 3, glucose: 22 ± 4 and mannose: 18 ± 3 (±SE) (Table 2), with only a small net change during the experiment. Analysis of the DCNS development showed that fucose, arabinose and mannose decreased their fraction at a small but consistent rate of −0.0014 ± 0.0005, −0.0010 ± 0.0003 and −0.0005 ± 0.0003 day^{-1}, respectively. Rhamnose was the only neutral sugar with a significant increasing trend ($p < 0.05$). During the experiment rhamnose accumulated at a rate of 0.0026 ± 0.0007, leading to a small increase in percentage from 13 to 14%. In general, the mole percentage at the end of the experiment appears to be independent of nutrient regime.

The relative contribution of new DCNS to new DOC increased from 0.3% at day # 1 to 10% at the beginning of phase II, though not significant $p > 0.05$ (Fig. 4). The relative high uncertainty in the ratio during the first 7 days of the experiment is caused by the relatively small absolute increase in carbon concentration. From day 7 until the end of phase II a clear pattern developed. The DCNS to DOC percentage in the +Si mesocosms continued to increase to a mean value of 16.4 ± 0.56% at day 22. The mesocosms without silicate decreased in relative percentage of DCNS to DOC from ≈10% at day # 7 to 5.6 ± 1.9% at day # 22.

The water in the mesocosms was renewed at a daily rate of 10% per day to stabilize the created plankton communities. The import of carbon as DOC and DCNS from the fjord was measured to enable us to calculate the accumulation within the mesocosms. Thus, the exchange of water from the mesocosms with fjord water only increased the DOC with some 8 μmol C l^{-1} and the DCNS with 0.7 μmol during the experiment.

Degradation of the DOC from the fjord during 150 days showed an average of 33 μmol C l^{-1} ± 3 ($n = 5$, $p < 0.05$), yielding a refractory background of the fjord DOC of 111 μmol C l^{-1} ±7 ($n = 5$, $p < 0.05$) during the entire period of the experiment (Table 3).

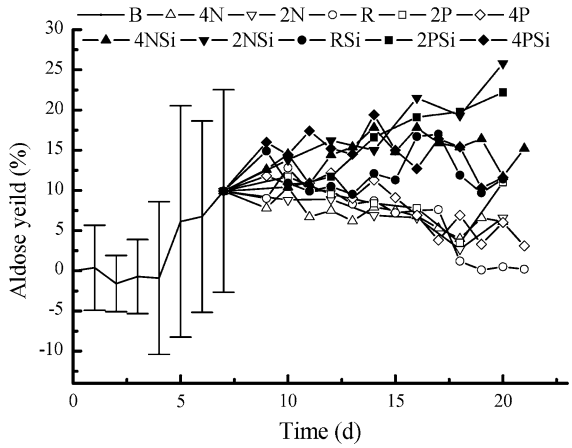

Fig. 4 Aldose yield (% of DOC) during the experiment. *Error bars* are 95% confidence intervals

Table 3 DOC degradability in μmol C l^{-1} shown in bag 4N, R, 4P, 4NSi, RSi, 4PSi and fjord in the six degradation experiments

		Day					
		0	4	7	12	17	22
4N	Labile	N/A	5	30	32	41	22
	Semi-labile	N/A	12	3	16	15	28
	Recalcitrant	N/A	110	113	112	126	136
R	Labile	1	11	33	31	48	64
	Semi-labile	11	19	11	10	9	33
	Recalcitrant	114	114	107	122	106	118
4P	Labile	13	13	21	32	34	78
	Semi-labile	1	7	7	3	18	11
	Recalcitrant	114	114	112	116	110	114
4NSi	Labile	13	13	31	39	35	65
	Semi-labile	1	11	3	24	49	31
	Recalcitrant	114	112	116	115	121	127
Rsi	Labile	13	13	16	65	63	95
	Semi-labile	4	10	16	14	19	39
	Recalcitrant	113	115	108	112	113	112
4PSi	Labile	13	13	11	34	48	80
	Semi-labile	6	6	8	46	31	61
	Recalcitrant	114	114	114	115	123	117
Fjord	Labile	1	N/A	26	0	25	25
	Semi-labile	33	N/A	13	35	7	3
	Recalcitrant	109	N/A	106	111	114	116

Labile DOC is defined as $k1 > 0.05$ day^{-1} and refractory as the fraction left at day # 150. Data not available where marked with N/A

A model with one degradable pool described by 1st order exponential decay provided the best fit for our results with $r^2 > 0.85$. By this approach, the degradable pool is described by the decay coefficient $k1$. We defined the recalcitrant endpoint as the concentration in the sample taken after 150 days, but realise that a "true" endpoint is probably never obtained in these sorts of experiments.

The decay pattern for DOC degradations was tested for similarity by the method in Sokal and Rohlf (1995) in all 18 experimental degradations carried out during phase I, exemplified for day # 4 and 7 in mesocosm 4N (Fig. 5). Initial degradation rates were high during the first 5 days with average degradation rates of 1.2 μmol C day^{-1}, 1.5 μmol C day^{-1} 2.6 μmol C day^{-1} for the experiments at day # 0, 4 and 7, respectively. For the samples collected at day # 12, 17 and 22 a pattern

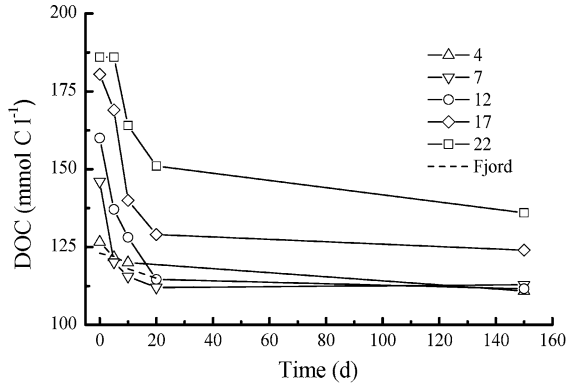

Fig. 5 Degradation of DOC (μmol C l^{-1}) sampled five times during the experiment in mesocosm 4N

developed where initial degradation rates from the mesocosms with added Si were higher than the rates in bags without Si added. In the samples collected at day # 0, 4, 7 and 12 all newly produced DOC was degraded after 150 days and probably faster. Thus, the recalcitrant background of 111 μmol C l^{-1} was reached. Except for the 4N and 4NSi mesocosms sampled at day # 17 and 22 the new accumulated DOC in all the other mesocosms were biodegradable (Fig. 6). The RDOC formation in 4N and 4NSi at day # 17 was 13 μmol l^{-1} and 7 μmol l^{-1} and at day # 22, 25 μmol l^{-1} and 12 μmol l^{-1}, respectively.

The nature of the DCNS composition in the fjord did not alter throughout the experiment nor did the refractory background ($p < 0.05$), so all DCNS degradation data from the fjord were pooled to a common background. The composition of the DCNS in all the experiments performed with the fjord water, showed a significant alteration in composition during the 150 days of incubation. The percentage of galactose, glucose and mannose changed significantly from 27, 32 and 20% at t_0 to 20, 54 and 5% at t_{150}, respectively ($p < 0.05$, t-test, Table 2).

All experiments showed a rapid degradation of all new DCNS within 20 days. The degradation pattern was similar in all incubations and is exemplified in Fig. 7 where all data from the five experiments at day # 22 are shown on a relative scale. The same amount of refractory DCNS was found as in the background DCNS from the fjord in all degradation experiments except for 4N and 4NSi. Approximately 0.3 μmol DCNS l^{-1} had accumulated in 4N and 4NSi on top of the recalcitrant background of 1.25 μmol DCNS l^{-1}.

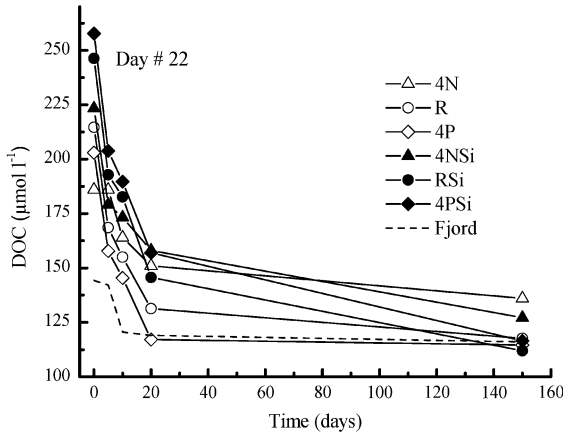

Fig. 6 Concentrations of DOC (µmol C l^{-1}) in biodegradation experiments sampled at day # 22 from the fjord and six mesocosms

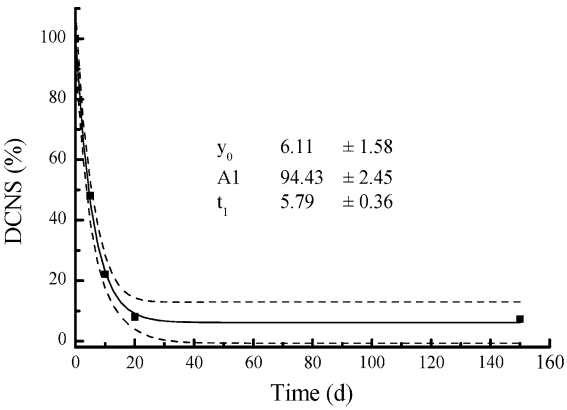

Fig. 7 Degradation of DCNS (% of initial concentration) sampled at day # 22 in 4N, R, 4P, 4NSi, RSi and 4PSi and fitted to a first order exponential decay model with following equation. $y = A1 \exp(-x/t1) + y0$. *Dotted lines* are 95% confidence intervals

Discussion

The reason for any accumulation of new DOC is a decoupling of production and removal. The uncoupling in time and space might be caused by the characteristics of the produced DOC to be inherently highly resistant to microbial degradation and thereby yielding lower rates of degradation than production. Low bacterial activity can also be caused by nutrient limitations or grazer control. The above explanations are not mutually exclusive and have been analysed theoretically by Thingstad and Lignell (1997). DOC accumulated at a higher rate in the mesocosms where silicate was added and diatoms constituted about 70% of the phytoplankton biomass (Conan et al. 2007). The DOC accumulation is in proportion with the POC accumulation showing that addition of silicate increased carrying capacity of algal biomass. Furthermore, P-depletion in the 4N and 4NSi treatments accumulated the highest concentration of DOC within their respective treatments (±Si). This complies well with an earlier study of a single diatom where monomeric carbohydrates, polymeric carbohydrates and dissolved free amino acids as well as bacterial growth yield and α- and β-glycosidase were measured on photosynthetic extracellular release (PER) during 24 h (Obernosterer and Herndl 1995). The diatoms were manipulated with regard to nutrients and P limitation caused an inefficient bacterial uptake of the PER released during the exponential and stationary phase of a bloom.

Accumulation of dissolved combined neutral sugars during diatom bloom events has been observed in several studies (Ittekkot et al. 1981; Biersmith and Benner 1998; Meon and Kirchman 2001) and can contribute quite significantly to the overall DOC accumulation. Biersmith and Benner (1998) found that 20% of newly accumulated DOC could be explained by DCNS. Our findings are comparable as DCNS at day 22 comprised an average of 16.5% (±2.8% $p < 0.05$) of the newly accumulated DOC in the silicate amended mesocosms.

The composition of DCNS in a variety of different environments appears to be rather uniform (Borch and Kirchmann 1997; Aluwihare et al. 1997) and the molecular distribution that we find resembles what Borch and Kirchmann (1997) found for inshore waters. The only deviating mole percentage in our study was arabinose which was slightly lower (2% difference) and mannose which was slightly higher (3% difference). The composition of DCNS only changed slightly during the course of the experiment (less than 2% for any given sugar). This is in contrast to the findings of Meon and Kirchman (2001), where distinct alterations were found in the mole composition of glucose, galactose and fucose. One explanation for this could be that we added nutrients every day and not only in the start of the experiment, which meant that the algal bloom did not reach a senescent phase.

Particulate combined neutral sugars constituted only a minor part of the POC ($\approx$10%) during the

bloom contrary to other published results were carbohydrates made up a larger proportion of the phytoplankton e.g. (Biersmith and Benner 1998). One explanation for this could be that we took out samples in mid morning where the phytoplankton had either respired most of the previous day's production or used it for biosynthesis during the night. The composition of the PCNS was distinctly different from the composition of the DCNS and did not change during the experiment. This could indicate that the carbohydrate loss from the phytoplankton is not passive as the DCNS in the water does not reflect the composition of PCNS. The different composition in the PCNS and DCNS are not caused by selective microbial degradation of newly released DCNS as the DCNS composition did not change towards the PCNS composition throughout 150 days of microbial degradation. One explanation why we do not see concurrence between the PCNS and DCNS composition might be that we do not reach a prolonged senescent phase of the bloom.

The five degradations experiments in each of the five mesocosms carried out during the course of the experimental bloom showed that a major part of the accumulated DOC was readily available for microbial degradation. At day # 4, 7, 12, 17 and 22 an average fraction of 0.51, 0.74, 0.61, 0.66 and 0.68 of the accumulated DOC could be defined as labile ($k1 > 0.05$ day^{-1}). This shows that a major part of the accumulated DOC was not very resistant to microbial degradation and possibly accumulated due to nutrient limitations in the bags. Our study furthermore shows that the accumulated DOC in all mesocosms was fully biodegradable until day 17, where RDOC appeared in 4N and 4NSi (Fig. 6). This indicates that RDOC can be produced directly by the phytoplankton or indirectly in food web processes during the later stages of a bloom where the phytoplankton is P limited. If RDOC was produced by the bacterial assembly in the incubation bottles new RDOC should be present in all degradation experiments and not just in 4N and 4NSi. Although it cannot be excluded, it seems unlikely that photochemical transformation of DOC to RDOC should only occur with P-limitation. Fry et al. (1996) found in one of two mesocosms experiments that a fraction of newly produced DOC with a high C/N ratio was refractory over a time course of 2.5 years, though background DOC values were not measured but calculated by δ^{13}C subtractions. Our findings do not give any evidence that an experimentally induced high C/N ratio should result in production of RDOC. This is argued with the fact that all new accumulated DOC in the N-deficient plankton assemblages was degraded within 150 days. Kragh and Søndergaard (2004) have experimentally showed that zooplankton grazing can decrease the lability of newly produced DOC. They did, however, not find that the production of RDOC was influenced by zooplankton. Our zooplankton data from the end of the experiment shows that zooplankton were present in all mesocoms and the species composition was not affected by the N:P ratio. The zooplankton biomass increased accordingly to the nutrient regime. If RDOC was induced by the zooplankton in the incubation bottles new RDOC should be present in some extend all degradation experiments and not just in 4N and 4NSi.

Previous studies have shown high lability of newly accumulated DCNS submitted to microbial degradation. Kragh et al. (2006) found that 70–80% of the DCNS accumulated during an experimental algal bloom was degraded within 35 days. Another study by Meon and Kirchman (2001) showed that 91% of the accumulated DCNS were degraded within 15 days. The highly reactive nature of DCNS has further been confirmed by Amon et al. (2001) who showed an 80% decrease within 10 days of algal-derived DCNS from an Arctic ice floe. Our findings are therefore in general agreement with the literature as we found that 94% the accumulated DCNS were degraded within 20 days. Thus, DCNS contributes quite significantly to the carbon turnover during bloom events. However, DCNS does not account for more than 1% of the RDOC produced during the later stages of our P limited blooms. Thus, it is not carbon in DCNS that makes up the RDOC.

The composition of the DCNS did change during the 150 days of degradation both for the mesocosms and fjord. The DCNS composition of the fjord was not a global endpoint, such an endpoint was apparently reached during degradation. The endpoints of the microbial degradation of DCNS in the mesocosm water and fjord water produced similar mole percentages. We interpret this as all of the newly produced DCNS are degraded in all but the 4N and 4NSi mesocosms at day # 17 and 22. This is the case as the DCNS concentration returned to the background and the species composition were similar to

that of the fjord after 150 days of incubation. It has previously been suggested that single aldoses can be used as an indicator for the diagenetic age of DOC, through a depletion of glucose over time (Hernes et al. 1996). This study, however, does not support such a conclusion as the mole percentage of glucose increases in both the fjord and mesocosms during the incubation period. Previous results by Kragh et al. (2006) and Meon and Kirchman (2001) indicates as well that strict use of single aldoses as indicators of freshness could lead to erroneous conclusions. We do, however, find that selective degradation occurs so that the DCNS percentage of DOC decreases over time and the global endpoint composition is reached. This implies a steady state between production and removal; at least in a system like Raunefjord.

Our findings contradicts the currently most accepted idea about the formation of refractory DOC being a result of high C:N ratios, which equals old, reworked, nutrient-poor DOC. Our results show that RDOC can be produced directly by the phytoplankton or indirectly in food web processes during the later stages of a bloom where the phytoplankton is P limited.

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