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Changes in particulate and dissolved organic matter in nutrient-enriched enclosures from an area influenced by mucilage: the northern Adriatic Sea

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This study examined the partitioning of organic matter into particulate organic carbon (POC) and dissolved organic carbon (DOC) pools in nutrient-enriched enclosures containing natural plankton from the Gulf of Trieste (northern Adriatic), an area affected by mucilage. The strategy of nutrient additions was to introduce a pulse of new nutrients in concentrations that mimic natural inputs and to survey community structure and organic matter fluxes long enough so that plankton became nutrient-limited. Maximal bacterial biomass attained roughly double the initial value, while autotrophic carbon increased by nearly an order of magnitude. The microflagellate-dominated community released more DOC per unit biomass (5.5 ± 7.2 to 50.6 ± 28.0 μg C μg Chl a^{-1} day^{-1} versus 3.4 ± 3.4 to 10.8 ± 4.6 μg C μg Chl a^{-1} day^{-1} for diatom-dominated phytoplankton), POC increase was modest (~300 μg C l^{-1}) and there was little change in DOC. Organic carbon par-titioning during two experiments in which diatoms prevailed was dominated by POC (>800 μg C l^{-1}) in the exponential growth phase with an increasing contribution of particulate carbohydrates that paralleled gradual nutrient depletion. Transition to the stationary phase and the decay of autotrophic communities were accompanied by the net accumulation of a carbohydrate-rich DOC.

INTRODUCTION

Seasonal accumulation of carbon (C)-rich dissolved organic matter (DOM) recurs in many marine environments (Bjørnsen *et al.*, 1989; Carlson *et al.*, 1994) including some in the Mediterranean and Adriatic Sea (Copin-Montegut and Avril, 1993; Pettine *et al.*, 1999; Tepić *et al.*, 2000). In some cases decoupling of production and utilization with a net accumulation of DOM may be sustained on time scales of months to years (Church *et al.*, 2002), and an ecosystem change of nutrient conditions [a shift from primarily nitrogen (N)-limited environment to phosphorus (P)-limitation] was hypothesized as a driving force (Karl *et al.*, 1998).

Recent analysis of the seasonal cycles of particulate and dissolved organic C and N in coastal waters revealed accumulations of C-rich DOM during productive periods, with C:N ratios well above the Redfield value of 6.6 (Williams, 1995). Reasons for this build-up of C-rich DOM are not clear but it has been hypothesized that lack of mineral N and/or P nutrients in the stratified water column prevents bacteria from taking up stored OM efficiently (Obernosterer and Herndl, 1995; Zweifel *et al.*, 1995). Moreover, OM-degrading bacteria preferentially mineralize P with respect to C, leading to C-enriched OM (Smith *et al.*, 1995). This C-rich OM is exported from the euphotic layer (Carlson *et al.*, 1994) and/or degrades later in autumn. Thingstad and Rassoulzadegan (Thingstad and Rassoulzadegan, 1995) suggested that restricted bacterial degradation of DOM might result from a combination of nutrient limitation and predatory (viral) control.

Seasonal accumulation of C-rich OM is thought to be the precursor of visible mucilage accumulations in the Adriatic Sea. Chemical analyses of mucilage material showed that C:N ratios of mucilage were 9.5–18.6 (Müller-Niklas *et al.*, 1994) and indicated

polysaccharides as its major organic compound (Kovač *et al.*, 1998).

Accumulations of mucilage harm fisheries and tourism, influence pelagic communities, and after settling to the bottom, suffocate benthic organisms. They develop during summer, may affect areas over 10 000 km^2, persist for several months (Vollenweider and Rinaldi, 1995) and locally reach very high mass (Malej *et al.*, 2001). The phenomenon has been noted in the northern Adriatic for at least 250 years; it was first described in 1729 and reappeared several times in the 19th century. In the 20th century, mucilage masses were widespread during the summers of 1905, 1920, 1949, 1988, 1989, 1991, 1997, 2000 and 2002; on 13 additional occasions, mucilage was observed only locally (Vollenweider *et al.*, 1995; Sellner and Fonda Umani, 1999; Flander Putrle, 2003). Although similar phenomena have been recorded in some other coastal areas [(Bhimachar and George, 1950) cited in Jenkinson and Biddanda (Gotsis-Skretas, 1995; Jenkinson and Biddanda, 1995; Lancelot, 1995; MacKenzie *et al.*, 2002)], they have rarely reached such temporal and/or spatial extensions.

Various hypotheses for explaining mucilage development in the northern Adriatic have been proposed. The central role of phytoplankton has been stressed in combination with specific environmental factors and changes in phytoplankton community structure (Degobbis *et al.*, 1995). Nutrient limitation (Obernosterer and Herndl, 1995; Fajon *et al.*, 1999), phytoplankton cell lysis (Baldi *et al.*, 1997) and reduced grazing pressure (Malej and Harris, 1993) are also important. Azam *et al.* (Azam *et al.*, 1999) emphasize sustained bacterial activity and the role of 'slow-to-degrade' OM. Common to all proposed mechanisms are the steps of production and accumulation of DOM resistant to degradation. Understanding the processes that channel carbon flux in favour of DOM which is slow to degrade thus seems crucial for explaining the mucilage phenomenon in the northern Adriatic.

Our experiments were designed to study the partitioning of OM into particulate organic carbon (POC) and dissolved organic carbon (DOC) in relation to different plankton community structures and nutrient conditions with the aim of explaining the mechanisms leading to mucilage formation. We carried out enclosure experiments using plankton from the Gulf of Trieste (northern Adriatic), an area heavily affected by recent mucilage outbreaks. The natural plankton community was used as a model to assay the effects of macronutrient enrichment followed by nutrient limitation. Experiments entailed single additions of different combinations of inorganic N, P and Si nutrients as well as rain and river water additions as natural sources of nutrients. Manipulated plankton communities comprised diverse eukaryotic and prokaryotic autotrophs as well as heterotrophic components of the microbial food web (bacteria and heterotrophic flagellates). Experimental plankton communities were collected from the coastal sea during the spring productive season (April–June), considered the critical period for development of mucilage aggregates in the northern Adriatic (Malej, 1995). The strategy of nutrient additions was to introduce a pulse of nutrients in concentrations that mimic natural inputs in the studied area, and to follow partitioning of OM into particulate autotrophic and heterotrophic plankton and into OM pools. Experiments were designed to survey community structure and fluxes of OM long enough after nutrient additions so that communities became nutrient-limited.

METHOD

Field sampling

Enclosure experiments were undertaken in June 1994 (PALEX 1), April 1995 (PALEX 2) and March/April 1996 (PALEX 3) and lasted 8, 10 and 13 days, respectively. Experimental plankton communities were collected in the south-eastern part of the Gulf of Trieste (northern Adriatic) at a depth of 1–2 m at ~09:00 h. A submerged pump was used to collect samples that were pre-screened through 112 μm Nytex mesh to remove zooplankton grazers. Samples of 80–100 l were thoroughly mixed and subsamples were taken for initial measurements.

Plankton communities

Initial plankton communities that were manipulated with nutrient additions were dominated by diatoms (49–88% of total phytoplankton abundance) in PALEX 2 and PALEX 3 experiments (Table I), and were also characterized by rather low heterotrophic bacteria numbers ($1.5–4.8 \times 10^8$ cells l^{-1}). The experimental plankton community used for the PALEX 1 experiment was dominated by microflagellates (>50% of total phytoplankton abundance), high numbers of cyanobacteria ($>2.5 \times 10^7$ cells l^{-1}) and a high heterotrophic bacterial abundance ($>9 \times 10^8$ cells l^{-1}). Initial chlorophyll *a* (Chl *a*) biomass was low in PALEX 1 and PALEX 3 experiments (<1 μg Chl *a* l^{-1}), while it was higher than 3 μg Chl *a* l^{-1} in the PALEX 2 experiment. Some differences were also noted in the 'nutrient history' of communities used in the experiments: plankton sampled in 1995 seemed to be the most severely P-limited [total inorganic nitrogen:phosphate ratios (TIN:P) >100], although low phosphate concentrations were also measured in 1994 and 1996 (0.03–0.1 μM). In addition, in the PALEX 3 experiment environmental concentrations of inorganic N were also rather low (1.01–1.53 μM). Environmental temperatures

ranged between 10.1 and 12.1°C (PALEX 2), 9.1 and 11.0°C (PALEX 3) and between 21.6 and 23.6°C (PALEX 1).

Nutrient additions and experimental set-up

Based on knowledge of the nutrient concentrations in the studied area and results of our previous enrichment experiments (Mozetič *et al.*, 1998a) we supplied experimental communities with single additions of N (nitrate and ammonium corresponding to 7 μM), P (corresponding to 0.6 μM) and silicate (corresponding to 11 μM), a mixture of all three macronutrients at the Redfield ratio, and separate rain and river water additions, both at 15% v/v. No nutrients were added to controls. The third experiment (PALEX 3) was carried out only with additions of all nutrients and phosphate. Nalgene bottles (8 and 20 l) containing natural plankton communities in prescribed nutrient treatments were incubated *in situ* at 2 m depth. Samples were withdrawn daily or every second day between 09:00 and 10:00 h.

Monitored parameters

The following parameters were monitored: primary and bacterial production using radiolabelling techniques, inorganic nutrient concentrations, cell counts [nano- and microphytoplankton, eukaryotic picoplankton, cyanobacteria, heterotrophic bacteria, heterotrophic nanoflagellates (HNAN)], phytoplankton pigments (chlorophylls and carotenoids), DOC, total dissolved nitrogen (TDN), POC, particulate organic nitrogen (PON), particulate carbohydrates (PTCHO) and dissolved carbohydrates (DTCHO).

Samples were preserved with neutralized formaldehyde (1.5% final concentration) and cells counted following standard procedures. Micro- and nanophytoplankton were identified on an inverted microscope using the Utermöhl (Utermöhl, 1958) technique. Cyanobacteria and taxonomically unidentified 2–3 μm eukaryotic cells were counted in green excitation using an epifluorescence microscope (Takahashi *et al.*, 1985). Heterotrophic bacteria were counted after staining with 4′,6-diamidino-2-phenylindole (DAPI; 1 μg ml^{-1} final) according to the

Table I: Relative taxonomic composition of the initial phytoplankton compared with the composition on Day 6 and at the end of the experiments in treatment with addition of nitrogen, phosphorus, silicate (NPSi)

Experiment	Start (% of total no.)		NPSi–day 6 (% of total no.)		NPSi–end (% of total no.)	
PALEX 1	Microflagellates	55%	Microflagellates	97%	Microflagellates	76%
N:P = 60	Diatoms	39%	Diatoms	3%	Diatoms	23%
Chl *a* (μg l^{-1}): 0.68	*Chaetoceros* spp.		*Chaetoceros* spp.		*Chaetoceros* spp.	
			Navicula sp.		*Cylindrotheca closterium*	
					Navicula sp.	
	Dinoflagellates	4%				
	Other	2%	Other	<0.5%	Other	1%
PALEX 2	Microflagellates	11%	Microflagellates	5%	Microflagellates	5%
N:P >100	Diatoms	88%	Diatoms	95%	Diatoms	95%
Chl *a* (μg l^{-1}): 3.39	*Pseudonitzschia pseudodelicatissima*		*P. pseudodelicatissima*		*P. pseudodelicatissima*	
	Skeletonema costatum		*S. costatum*		*S. costatum*	
	Other	1%				
PALEX 3	Microflagellates	42%	Microflagellates	11%	Microflagellates	7%
N:P = 23	Diatoms	49%	Diatoms	86%	Diatoms	90%
Chl *a* (μg l^{-1}): 0.94	*Chaetoceros* spp.		*Chaetoceros* spp.		*Chaetoceros* spp.	
	P. pseudodelicatissima		*P. pseudodelicatissima*		*P. pseudodelicatissima*	
	Bacteriastrum sp.		*Bacteriastrum* sp.		*Bacteriastrum* sp.	
	Cylindrotheca closterium					
	Coccolithophorids	5%	Coccolithophorids	2%	Coccolithophorids	1%
	Emiliania huxleyi		*E. huxleyi*		*E. huxleyi*	
	Other	4%	Other	1%	Other	2%

N:P is total inorganic nitrogen:phosphate ratio. The species with >5% of the abundance within different phytoplankton groups are listed.

protocol of Porter and Feig (Porter and Feig, 1980) and HNAN using primuline (63 μg ml^{-1} final) according to Caron (Caron, 1983). The biovolume of bacteria was converted into carbon biomass using 19.8 fg C $cell^{-1}$ as the conversion factor (Lee and Fuhrman, 1987) and the biomass of HNAN was calculated using mean cell volume 12.8 μm^3 and the conversion factor 0.21 pg C μm^{-3} (Holligan *et al.*, 1984).

Chl *a* was analysed in acetone extracts using a Turner 112 fluorimeter (Strickland and Parsons, 1972). The number and size of different phytoplankton species were counted and measured on an inverted microscope using an ocular micrometer. For biovolume calculation an average size of at least 15 specimens of each species was taken and cell volumes were calculated from geometric formulae based on cell shapes (Edler, 1979). The carbon content was calculated from the cell volume, which was assumed to be equal to the plasma volume for all groups except for diatoms. Here the plasma volume was calculated by subtracting 90% of vacuole volume from the cell volume (Strathmann, 1967). The conversion factor from plasma volume to cell carbon was in all cases 0.11 pg C μm^{-3} (Strathmann, 1967), except for armoured dinoflagellates where a conversion factor of 0.13 pg C μm^{-3} was taken (Smetacek and Pollehne, 1986).

Phytoplankton primary productivity was measured using the ^{14}C technique (Steeman-Nielsen, 1952). A total of 75 ml of seawater were poured into light and dark polycarbonate bottles and a known concentration of ^{14}C was added (6 μCi of $NaH^{14}CO_3$). The bottles were incubated *in situ* at the same depth as the experimental bottles for 3 h. Particulate primary production was measured for cells retained by polycarbonate filters of different pore sizes (10, 2, 0.6 and 0.2 μm). A subsample of 0.2 μm filtrate was also acquired to determine dissolved organic ^{14}C released (extracellular organic carbon release, EOC). The acidified filters were put in vials containing 5 or 10 ml of scintillation cocktail and the activity measured on a Canberra TriCarb 2500 scintillation counter. Assimilation of carbon was calculated as described by Gargas (Gargas, 1975), considering 5% isotope discrimination, activity of added ^{14}C and total CO_2 concentration calculated from temperature, salinity and pH.

For EOC determination the 0.2 μm filtrates were acidified with 100 μl of 1 M HCl and left open in a hood for 24 h; samples were analysed for radioactivity after addition of 10 ml of scintillation cocktail. Data were not corrected for bacterial uptake. Percentage of EOC was calculated as EOC/total primary production × 100, where total primary production equals the sum of the particulate primary production and EOC.

Low- and high-molecular-weight fractions of EOC were separated by gel filtration on PD-10 Sephadex columns (Pharmacia Corp.) using blue dextran, glucose and $CoCl_2$ as standards. Ten millilitres of scintillation cocktail were added and the radioactivity of separated fractions was measured as described for phytoplankton productivity. For the calculation of ratios between high- and low-molecular-weight fractions, d.p.m. values were used.

Phytoplankton pigments (chlorophylls and carotenoids) were determined according to Mantoura and Llewellyn (Mantoura and Llewellyn, 1983), modified by Barlow *et al.* (Barlow *et al.*, 1993). One hundred millilitres of subsamples were filtered on precombusted glass fibre filters (Whatman GF/F). The filters were frozen or immediately extracted in 4 ml of 90% acetone, using sonication, and centrifuged to remove cellular debris and filter fibres. An aliquot of clarified extract was mixed with 300 μl of 1 M ammonium acetate and 100 μl was ejected in a gradient HPLC system (Varian gradient pump Star 9010, spectra-Physics UV/Vis spectrophotometer UV2000-021 and FL2000-030 detector, a column Pecosphere, 35 × 4.5 mm, Perkin Elmer). Chlorophylls and carotenoids were detected by absorbance at 440 nm, while detection of phaeopigments was accomplished with a detector using an excitation wavelength of 420 nm and emission at 620 nm.

The incorporation of ^3H-labelled thymidine was used to estimate bacterial production (Fuhrman and Azam, 1982; Smith and Azam, 1992). Production based on the amount of thymidine incorporated into DNA was calculated according to Fuhrman and Azam (Fuhrman and Azam, 1982) and Lee and Fuhrman (Lee and Fuhrman, 1987).

Nutrients were analysed in filtered (NO_2, NO_3, N-total, PO_4, P-total) and unfiltered (NH_4 and Si) samples using standard colorimetric procedures (Grasshoff *et al.*, 1983). POC and DOC were measured with an integrated system including a Shimadzu TOC 5000 carbon analyser, the corresponding suspended solid module (SSM) in line with the carbon analyser (Cauwet, 1994). The oxidation of organic matter under conditions of the apparatus (high temperature catalytic oxidation at 700°C for DOC and dry combustion at 900°C for POC) produced CO_2 from total combustion. Combustion gases were carried by pure oxygen to the non-disperse infrared detector (NDIR). Filters were first acidified with 5 N phosphoric acid to eliminate carbonates and dried at 200°C before being combusted in a furnace at 900°C. POC was estimated by comparison with the calibration curves of standard solutions of glucose. Precision was 2 μg of C, corresponding in this experiment to ~10 μg C l^{-1}.

DOC was measured after elimination of inorganic carbon by acidification and bubbling by direct injection of 100 μl of the sample in a vertical furnace on a Pt/silica catalyst (Cauwet, 1994). Calibration was carried out with potassium hydrogen phthalate (KHP) solutions (50–300

μM C l^{-1}); the precision was 20 μM C l^{-1}. Precision was limited by the need to have low and stable blanks, and was in the range of 10–15 μM but sometimes increased up to 30 μM. Blanks did not significantly affect precision when they were stabilized, which can require up to 2–3 days when starting with a new set of catalysts.

PTCHO and DTCHO were determined using the MBTH (3-methyl-2-benzothiazolinone hydrazone hydrochloride) method. For PTCHO determination samples (50–100 ml) were filtered onto precombusted Whatman GF/F filters. Dissolved monosaccharides were determined directly by a slightly modified MBTH method (Johnson and Sieburth, 1977), and using glucose as a standard. Concentrations of PTCHO and DTCHO were determined after hydrolysis with 1.7 M HCl (100°C, 3.5 h) (Senior and Chevolot, 1991). The concentration of polysaccharides was calculated subtracting the monosaccharide concentration from the total carbohydrate concentrations.

RESULTS

Nutrient pulse and transition to nutrient-limited conditions had profound effects on the particulate organic matter (POM) and DOM dynamics in all three experiments. Enrichment followed by gradual nutrient limitation affected rates of primary and bacterial production, influenced plankton abundance and community structure, and caused a transient POM increase followed by DOC accumulation.

Nutrient concentrations

Initial nutrient concentrations in different treatments after amendment are given in Table II. Values measured in controls indicate ambient seawater concentrations during the studied periods and reflect seasonal differences in the northern Adriatic system (Degobbis *et al.*, 2000; Cantoni *et al.*, 2003).

Phosphate concentrations levelled off to detection limit by the second or third day in enclosures without addition of P. Phosphate concentration decreased rapidly also in enclosures with phosphate additions reaching values between 0.05 and 0.27 μM on day 4 and stabilized between 0.01–0.02 μM after day 6. Phosphate availability affected the N uptake as well: in NPSi amended enclosures the ammonium concentration quickly decreased to below 0.5 μM, followed by rapid decrease of nitrate concentration. However, total inorganic N concentration remained rather stable around 1.5 μM after day 5. In contrast, nitrate concentration stayed above 2 μM in enclosures amended with ammonium + nitrate, silicate, rain and river water. Concentrations of silicate were above 4 μM in all treatments throughout the first experiment and in enclosures amended with all nutrients in the third experiment. During the second experiment, the situation was different: an already quite low initial silicate concentration (around 1.2 μM) decreased to below 0.5 μM in enclosures amended by phosphate and N alone, and to 1.02–1.05 μM in enclosures with addition of all nutrients.

Production rates, extracellular organic carbon release and biomass accumulation

The added nutrients enhanced both particulate primary and bacterial production (Figure 1). During all three experiments, the greatest enhancement of primary production (Table III) was obtained by the addition of all nutrients (NPSi), reaching maximum hourly rates of 16.85 μg C l^{-1} h^{-1} on day 6 after addition during the first experiment (PALEX 1), 16.80 μg C l^{-1} h^{-1} on day 4 during the second (PALEX 2) and 7.76 μg C l^{-1} h^{-1} on day 6 during the third experiment (PALEX 3).

Quite high production rates were also measured in PALEX 1 and PALEX 2 in treatments with phosphate alone, while during the third experiment this treatment yielded low values. The addition of rain and river water also stimulated primary production, reaching maximum values during the second experiment (4.33 and 6.89 μg C l^{-1} h^{-1} on days 6 and 4, respectively). Primary production in controls without nutrient additions varied between 0.56 and 1.12 μg C l^{-1} h^{-1} during the first experiment, between 0.55 and 2.18 during the second, and between 0.11 and 0.61 μg C l^{-1} h^{-1} during the third experiment. Primary production rates in enclosures amended with N and silicate alone were not significantly different ($P < 0.05$) from controls: in these treatments, they ranged from 67 to 140 expressed as a percentage of the control.

Notwithstanding similar maximum rates of particulate primary production during the first and second experiments, there were marked differences in the partitioning of particulate primary production in different phytoplankton size-fractions. During PALEX 1, the 2–10 μm size fraction contributed on average 77 ± 8% of particulate primary production, the contribution being slightly lower in bottles with an addition of all nutrients (69 ± 8%) and the highest with the addition of P alone (83 ± 6%). The opposite was found during the second experiment when, on average, 79 ± 7% of particulate primary production was associated with the largest size fraction (>10 μm); this fraction contributed even more in treatments with all nutrients (82 ± 7%). Despite lower particulate primary production during PALEX 3, the largest fraction prevailed again contributing, on average, 76 ± 14% (88 ± 9% in treatments with all nutrients). In all three experiments the smallest size fraction (0.6–2 μm)

Table II: Initial nutrient concentrations in the enclosures after addition of different combinations of nutrients, river water (15% v/v) and rain water (15% v/v)

Treatment	PO_4 (μM)	NO_3 (μM)	NH_4 (μM)	SiO_4 (μM)
PALEX 1				
Control	0.10	3.44	2.44	8.11
NPSi	0.45	16.47	3.71	19.77
P	0.50	3.03	2.01	6.99
N	0.16	15.59	3.94	7.55
Si	0.16	3.62	2.40	19.99
Ra	0.18	11.53	6.20	19.47
Ri	0.23	10.89	2.33	11.03
PALEX 2				
Control	0.01	3.58	1.16	1.22
NPSi	0.61	8.68	2.96	11.82
P	0.61	3.58	1.16	1.2
N	0.01	3.58	1.16	1.22
Si	0.01	8.68	2.96	11.21
Ra	0.05	3.58	1.16	5.13
Ri	0.04	4.98	1.62	11.43
PALEX 3				
Control	0.03	1.01	0.07	1.85
NPSi	0.53	6.11	5.77	12.83
P	0.53	1.01	0.97	1.83

NPSi, addition of nitrogen, phosphorus, silicate; P, addition of phosphorus; N, addition of nitrogen; Si, addition of silicate; Ra, addition of rain water; Ri, addition of river water.

Table III: Maximal values of particulate primary production rates and biomass accumulation (as Chl a) reached in different treatments and day when maximum was reached after amendment during three experiments

Treatment	Particulate primary production rate (μg C l^{-1} h^{-1})						Chl *a* (μg Chl *a* l^{-1})					
	PALEX 1		PALEX 2		PALEX 3		PALEX 1		PALEX 2		PALEX 3	
	Max	Day	Max	Day	Max	Day	Max	Day	Max	Day	Max	Day
Control	1.12	6	2.18	6	0.61	1	1.22	8	4.48	8	1.43	11
NPSi	16.85	6	16.80	4	7.76	6	12.61	8	20.38	5	22.00	6
P	12.57	6	12.17	3	1.39	3	3.46	6	8.06	3	2.13	6
N	2.94	6	2.27	6			0.87	6	4.98	10		
Si	1.56	6	2.17	6			0.69	5	4.23	4		
Ra	2.77	6	4.33	6			1.50	8	4.09	10		
Ri	3.22	6	6.89	4			1.24	8	6.29	8		

NPSi, addition of nitrogen, phosphorus, silicate; P, addition of phosphorus; N, addition of nitrogen; Si, addition of silicate; Ra, addition of rain water; Ri, addition of river water.

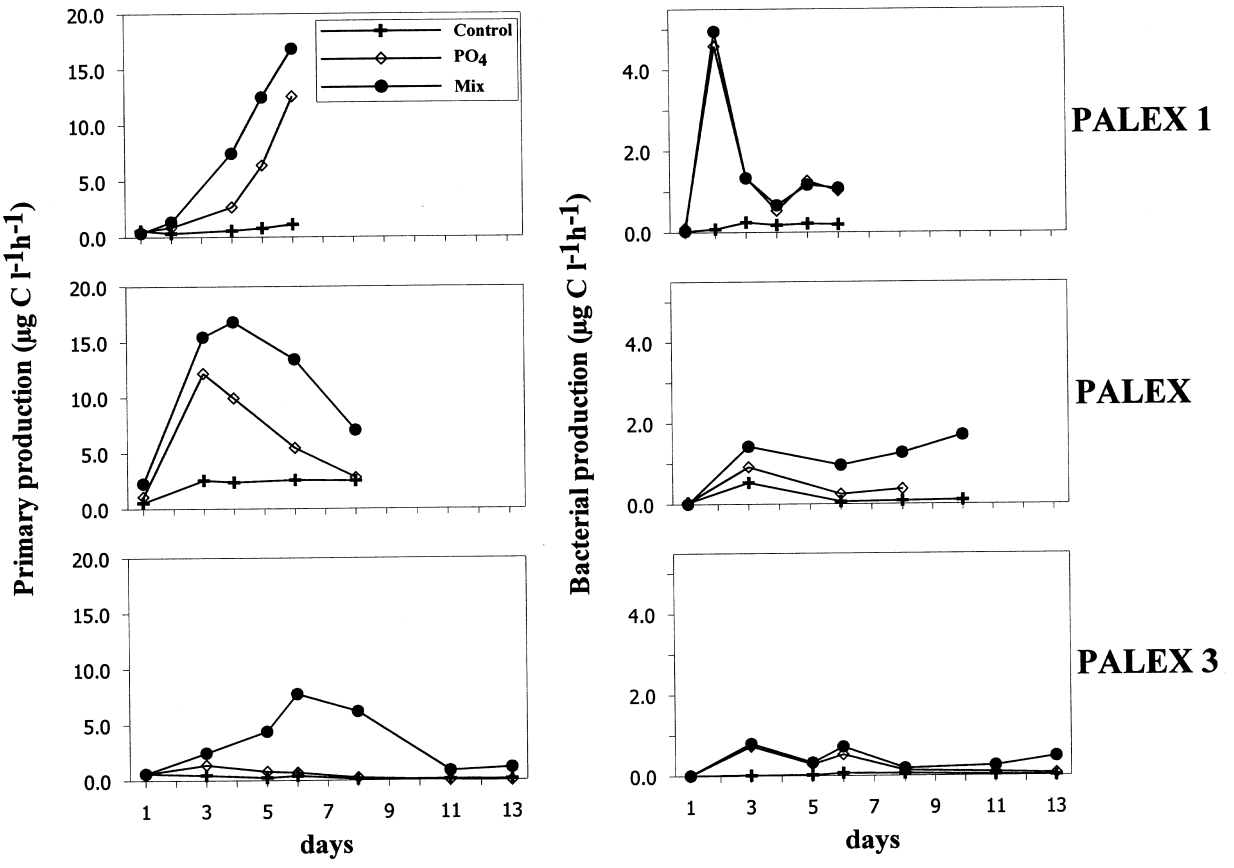

Fig. 1. Time courses of particulate primary production and bacterial production in control (+), phosphorus enriched (◊) and nitrogen, phosphorus, silicate enriched enclosures (•) during three experiments (PALEX 1, June 1994; PALEX 2, April 1995; PALEX 3, March/April 1996).

was least stimulated, contributing, on average, <5% to particulate primary production.

The EOC roughly paralleled particulate primary production with an overall range of <0.1–3.27 µg C l^{-1} h^{-1}. Daily EOC per unit phytobiomass (Chl *a*) was significantly higher during the first experiment (PALEX 1) than in the second and third. The lowest values were determined in treatments with N and silicate alone, while the highest were measured in treatments with all nutrients (NPSi) followed by phosphate amendment and addition of rainwater (Table IV).

At the beginning of the experiments (days 1–3), the EOC was prevailingly composed of low molecular weight fractions as indicated by HMW:LMW ratios of 0.71:0.87, 0.78:0.97 and 0.71:0.95 during PALEX 1, PALEX 2 and PALEX 3, respectively. With the ageing of enclosed communities, high molecular weight fractions prevailed in all treatments (HMW:LMW ratios >1). The highest HMW:LMW ratios were recorded during PALEX 2 in treatments with all nutrients (NPSi) when, excluding values for the first 2 days, ratios varied between 1.77 and 2.73, indicating a very strong prevalence of high molecular weight components in phytoplankton exudates. After an initial increase, the HMW:LMW ratio decreased again below 1 after 10 days during PALEX 3.

While particulate primary production in nutrient-amended enclosures followed a typical pattern with enhanced rates during the exponential growth of autotrophs and a steady decrease after the community had reached the stationary phase, bacterial production showed a different pattern. In all experiments, bacterial production rates typically exhibited a bimodal pattern with two peaks. The initial peak preceded the primary production maximum, while the second peak, attained 6–10 days after the amendment with nutrients, accompanied the autotrophic population decline (Figure 1). Enhancement of bacterial production after the addition of phosphate alone was equally high as for amendments with all nutrients (NPSi) during the first experiment, slightly lower during PALEX 2, but consistently lower during PALEX 3 (Figure 1; Table V). Addition of rain and river water also stimulated bacterial production,

Table IV: Average daily extracellular organic carbon release (EOC) per unit phytobiomass (Chl a) (mean ± SD) in different treatments during three experiments

Treatment	EOC (µg C µg Chl a^{-1} day^{-1})		
	PALEX 1	PALEX 2	PALEX 3
Control	19.4 ± 24.3	5.7 ± 3.0	2.8 ± 3.8
NPSi	50.6 ± 28.0	10.8 ± 4.6	3.4 ± 3.4
P	24.0 ± 34.2	8.9 ± 3.6	3.6 ± 3.8
N	15.8 ± 16.8	3.8 ± 2.9	
Si	5.5 ± 7.2	3.6 ± 1.9	
Ra	16.8 ± 24.0	9.4 ± 3.1	
Ri	12.0 ± 14.4	7.4 ± 5.0	

NPSi, addition of nitrogen, phosphorus, silicate; P, addition of phosphorus; N, addition of nitrogen; Si, addition of silicate; Ra, addition of rain water; Ri, addition of river water.

although less than amendments with all nutrients and P alone. Peak rates of bacterial production measured in treatments with all nutrients (NPSi) reached values up to 4.95 µg C l^{-1} h^{-1} during the first experiment and up to 1.70 and 0.80 µg C l^{-1} h^{-1} during PALEX 2 and 3, while maximum rates in other treatments were always below 0.8 µg C l^{-1} h^{-1}.

Integration of total primary and bacterial production in different treatments over the studied periods in the three experiments revealed that integrated bacterial production comprised, on average (all three experiments), 40.7 ± 29.1% (n = 21) of the integrated total primary production. However, there were great differences noted between the treatments. A flux of primary production through the microbial food web seemed to be greater in treatments with single additions of either P or N: in these amendments, integrated bacterial production comprised, on average, 69 and 55% of integrated total primary production. Comparatively, overall averages of integrated bacterial versus primary production in treatments with all

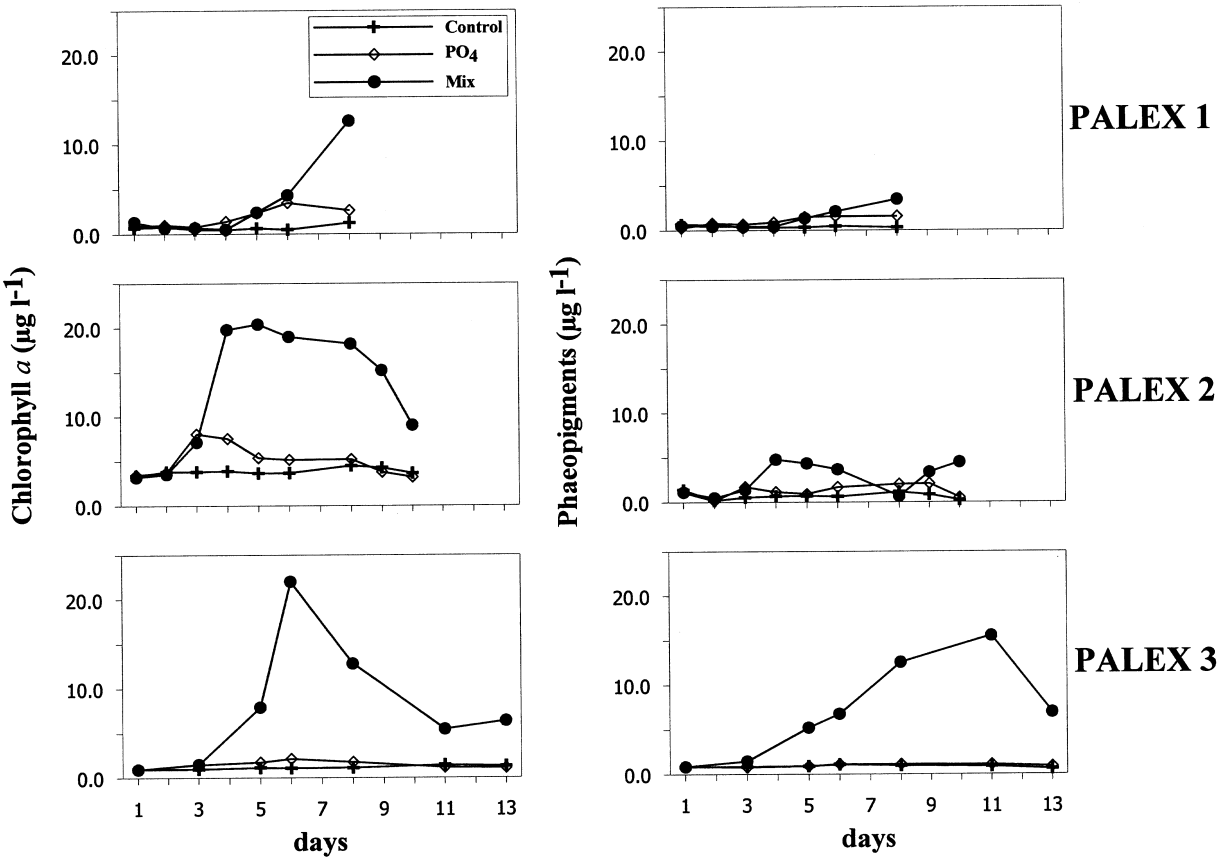

Fig. 2. Development of Chl *a* and phaeopigment concentrations in control (+), phosphorus enriched (◊) and nitrogen, phosphorus, silicate enriched enclosures (•) during three experiments (PALEX 1, June 1994; PALEX 2, April 1995; PALEX 3, March/April 1996).

nutrients (NPSi) and controls were 24 and 38%. There were also some differences between the three experiments (PALEX 1, 2, 3) with the highest ratio of bacterial versus primary production during the first (45.9 ± 19.3%) and the lowest during the second experiment (19.9 ± 4.9%).

Phytoplankton biomass response to added nutrients showed a 3–4 day lag phase (Figures 2 and 3). Generally, phytobiomass attained its highest values on days 4–8 (Table III). Addition of all nutrients (NPSi) provoked the development of blooms reaching a maximum biomass of 12.61 μg Chl *a* l^{-1} in the first experiment, and significantly higher values during PALEX 2 and 3 (20.38 and 22.00 μg Chl *a* l^{-1}, respectively). Biomass accumulation of the autotrophic community was also evident in the treatment with P addition (3.46, 8.06 and 2.13 μg Chl *a* l^{-1} in PALEX 1, 2 and 3, respectively). Chl *a* biomass in other treatments was not significantly different ($P < 0.05$) from controls, the only exception being the enclosure amended with river water, reaching a peak value of 6.29 μg Chl *a* l^{-1} (Table III). Concentrations of Chl degradation products were low at the beginning of the experiments (<0.3 μg l^{-1}), attaining higher values during the second and third experiments (Figure 2).

During PALEX 1, phytoplankton C initially amounted to 46 μg C l^{-1} reaching a maximum value of 270 μg C l^{-1} at the end of the experiment in enclosures amended with all nutrients. Despite their low abundance, the major contribution was initially made by dinoflagellates (82 ± 6% of total phytoplankton C), although dinoflagellate contribution decreased steadily during the course of the experiment. In contrast, microflagellate biomass increased from <10% of autotrophic carbon at the beginning of the experiment to reach over 70% at the end in treatments with P and all nutrients. Initial phytoplankton C during PALEX 2 and 3 was also below 60 μg C l^{-1}. Diatoms dominated the plankton community in PALEX 2, which was reflected in their contribution to total phytoplankton C averaging up to 88 ± 4% of total phytoplankton C. Diatoms also contributed most to total phytoplankton carbon in PALEX 3: >63% in all treatments, and particularly so in the treatment with all nutrients (88 ± 5%). Again, the most significant was the C increase in enclosures amended with all nutrients (NPSi) amounting to an average of 721 ± 123 μg C l^{-1}. In other treatments, phytoplankton C remained lower (<120 μg C l^{-1}).

Phytoplankton accessory pigments, abundance and community structure

Associated with accumulation of the Chl *a* biomass were increases of the main accessory phytoplankton pigments (Figure 3). 19′-hexanoyloxyfucoxanthin and Chl *b* reached peak concentrations of 3.59 and 0.9 μg l^{-1} during PALEX 1 in enclosures amended with all nutrients (NPSi) but remained at quite low levels during the second and third experiments (<0.4 and 0.2 μg l^{-1}). In contrast, fucoxanthin was the dominant accessory pigment during PALEX 2 and 3 reaching the highest values of 12.84 and 14.77 μg l^{-1}, respectively. Concentrations of fucoxanthin remained low in all treatments throughout the course of the first experiment.

Accessory pigment data correlated well with the observed phytoplankton community structure. In the first experiment, the inoculated community was numerically dominated by microflagellates and diatoms contributed <40% of the initial phytoplankton abundance (Table I). After nutrient amendments, the relative proportion of microflagellates to diatoms initially increased, then changed slightly more in favour of diatoms at the end of the experiment. This pattern was paralleled by fucoxanthin concentrations (Figure 3). Nevertheless, diatoms remained below 10% of total phytoplankton (average for all treatments 3.1 ± 2.9%). The only diatom that reached significant numbers in PALEX 1 was *Chaetoceros* (2.2×10^5 cells l^{-1}), while small autotrophic flagellates characterized by 19′-hexanoyloxyfucoxanthin (primnesiophytes) attained very high abundances: up to 1.8×10^7 cells l^{-1}. Absolute abundances of phytoplankton were highest during the second experiment when the diatom *Pseudonitzschia pseudodelicatissima* alone reached 7.61×10^7 cells l^{-1}, followed by *Skeletonema costatum* at 2.8×10^7 cells l^{-1}. Lower total phytoplankton abundances were found during PALEX 1 and 3 (<10^8 cells l^{-1}). *Chaetoceros* was also the most abundant diatom (1.3×10^7 cells l^{-1}) in the third experiment, followed by *S. costatum* (1.2×10^6 cells l^{-1}). Among other phytoplankton organisms, coccolithophorids, specifically *Emiliania huxleyi* (maximum 5.2×10^5 cells l^{-1}), seemed to take up added nutrients quite efficiently. The addition of phosphate alone reflected in an increased abundance of the diatoms *S. costatum* and *Bacteriastrum* sp., while additions of river water and rain enhanced the number of coccolithophorids as well as *Chaetoceros*. Dinoflagellates appeared to be unsuccessful in competing for added nutrients, remaining in low numbers during all three experiments. In addition, cyanobacteria were out-competed completely in nutrient-enriched environments, irrespective of which nutrient combination was used.

Abundance of bacteria and heterotrophic nanoflagellates

Bacterial abundance roughly paralleled bacterial production (Figure 4; Table V). In PALEX 1 and 3, maximum abundances were counted in P-amended enclosures (2.04×10^9 and 1.6×10^9 cells l^{-1}, respectively), while in the second experiment bacteria

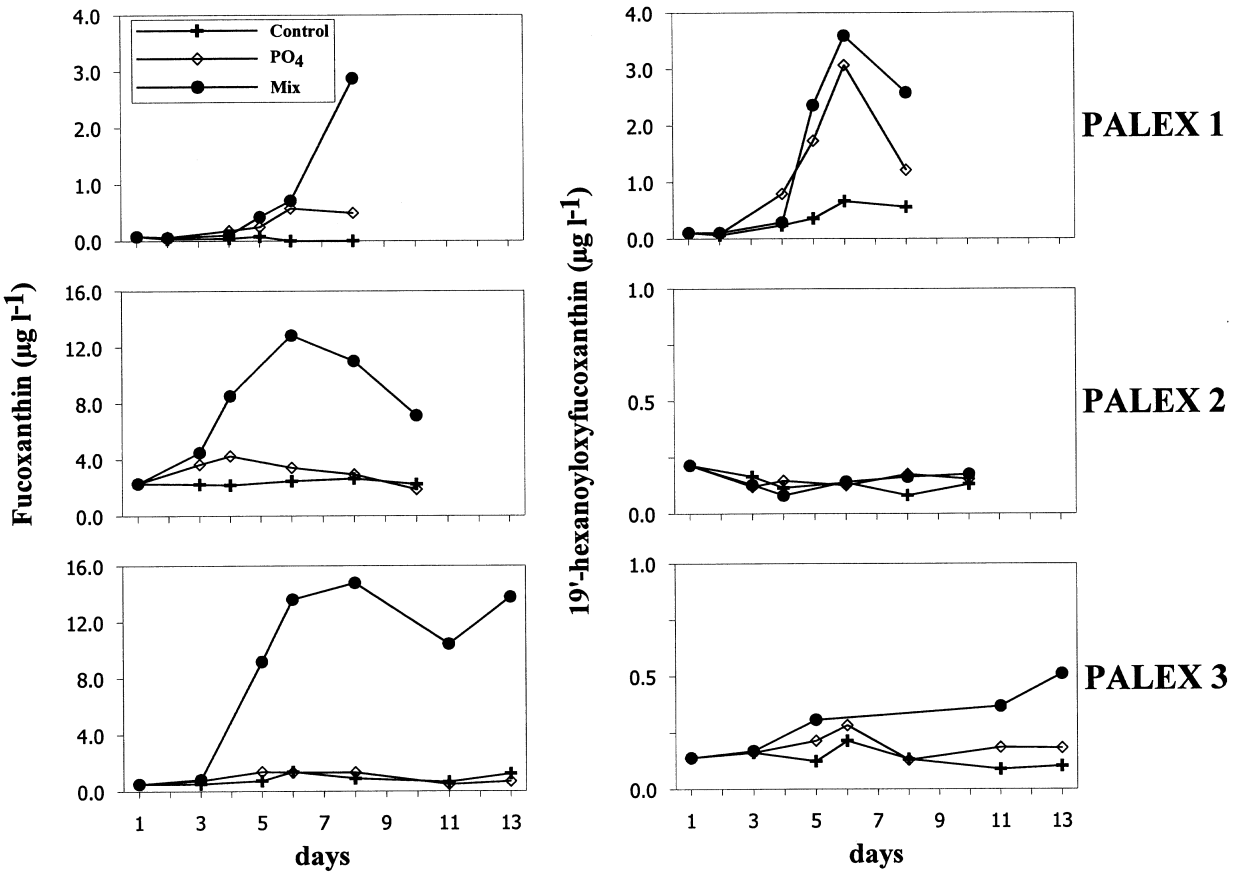

Fig. 3. Fucoxanthin and 19′-hexanoyloxyfucoxanthin concentrations in control (+), phosphorus enriched (◊) and nitrogen, phosphorus, silicate enriched enclosures (•) during three experiments (PALEX 1, June 1994; PALEX 2, April 1995; PALEX 3, March/April 1996).

reached peak abundances in the treatment with all nutrients (NPSi) (1.4×10^9 cells l^{-1}). Initial bacterial abundances varied between 4.8 and 9.3×10^8 cells l^{-1}. In all experiments, initial bacterial C was below 20 µg C l^{-1}, the lowest during PALEX 2. Peak bacterial biomass was measured during PALEX 1 in the phosphate-amended enclosure reaching a value slightly over 40 µg C l^{-1} (Table V). However, in contrast to the peaks of autotrophic biomass, which showed differences of an order of magnitude in different treatments (Table III), differences in peak bacterial biomass were much smaller (Table V). HNAN-associated C increased from initial values below 5 µg C l^{-1} to >10 µg C l^{-1} in PALEX 1 and 6–7 µg C l^{-1} in the third experiment.

Comparison of initial and maximum autotrophic and bacterial biomass in the three experiments revealed that initial phytoplankton C ranged between 40 and 60 µg C l^{-1}, while bacterial C biomass was initially between 9 and 19 µg C l^{-1}, the highest in June (PALEX 1). Maximum bacterial biomass attained roughly double the initial value during PALEX 1; peak values were lower during PALEX 2 and 3 (Table V). In contrast, autotrophic C increased by nearly an order of magnitude reaching a maximum of over 800 µg C l^{-1} during PALEX 3 (enclosure with addition of all nutrients).

POC, DOC, PTCHO and DTCHO

Initial POC was the highest in PALEX 1 (270 µg C l^{-1}) and increased significantly in enclosures amended with phosphate alone (>600 µg C l^{-1}) and with all nutrients (>500 µg C l^{-1}) (Figure 5). Lower initial POC concentrations were found during the second and third experiments (200 and 180 µg C l^{-1}, respectively). During PALEX 2 and 3, the increase of suspended matter corresponded to over 800 µg C l^{-1} in enclosures with the addition of phosphate and/or all nutrients. In all three experiments, the contribution of phytoplankton C to total POC increased from between 17–30% initially to over 50% 5–7 days after the amendment with P and all nutrients. Contribution of bacterial carbon to total POC was less, reaching a

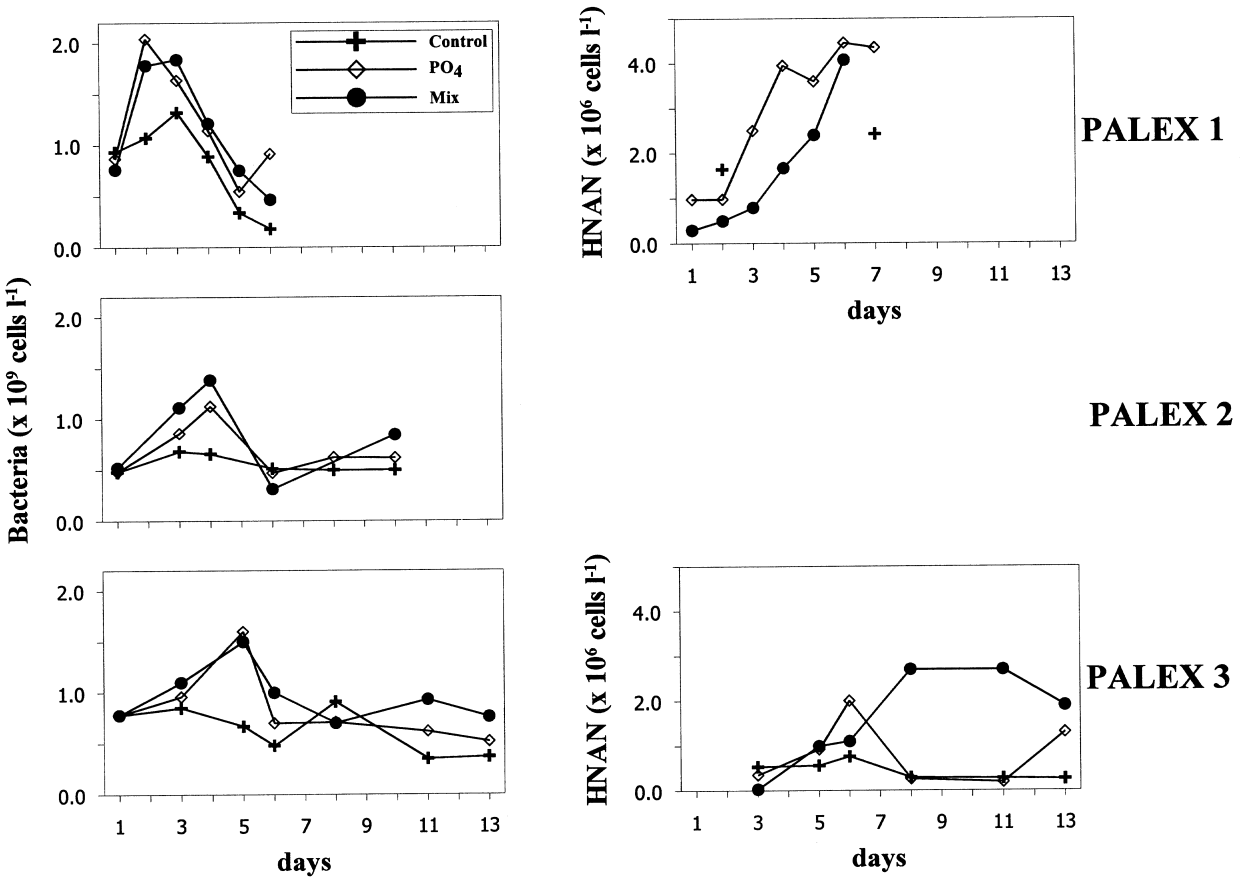

Fig. 4. Heterotrophic bacteria and heterotrophic nanoflagellate (HNAN) abundances in control (+), phosphorus enriched (◊) and nitrogen, phosphorus, silicate enriched enclosures (•) during three experiments (PALEX 1, June 1994; PALEX 2, April 1995; PALEX 3, March/April 1996).

maximum of ~15% in the P-treated enclosure during PALEX 1 but remaining well below 10% in all treatments during PALEX 2 and 3.

As with POC, initial DOC concentrations were also significantly higher in PALEX 1 compared with PALEX 2 and 3. Only a slight change in DOC occurred during PALEX 1 over the course of the experiment. In contrast, DOC concentrations increased in all enclosures during the second experiment, attaining the highest values of 1848, 2364 and 2523 μg C l^{-1} after 11 days in enclosures receiving phosphate, river water and all nutrients. During PALEX 3, DOC concentrations showed a slight increasing trend after the initial small decrease from 1464 to 1224 μg C l^{-1} (Figure 5) to reach the highest value of 2028 μg C l^{-1} in the treatment with all nutrients at the end of the experiment.

The pattern of (PTCHO evolution was slightly different from POC during PALEX 1 (Figure 6). In contrast to a larger POC increase in the phosphate treatment than that of all nutrients, PTCHO increased significantly only in enclosures amended with all nutrients (from an initial 40 to ~140 μg C l^{-1}). DTCHO were approximately two to three times the level of PTCHO and behaved similarly to DOC remaining without significant changes throughout the experiment. In PALEX 2, particulate and dissolved carbohydrates initially increased more slowly than POC and DOC. After the autotrophic community had reached the stationary phase (5–7 days after amendment with nutrients), increases of PTCHO closely matched the increase of POC, indicating a prevalent synthesis of carbohydrates. After the initial small decrease, DTCHO increased steadily, and at the end of the experiment amounted to ~1800 μg C l^{-1} (Figure 6). While DTCHO represented ~25% of DOC at the beginning of the experiment, this contribution increased to nearly 70% at the end of the experiment. A similar pattern was also observed during PALEX 3 with little accumulation of carbohydrates in particulate form during the exponential growth of autotrophs. An increase of PTCHO, when the population reached the stationary

Table V: Maximal values of bacterial production and biomass reached in different treatments and day when maximum was reached after amendment during three experiments

Treatment	Bacterial production (µg C l^{-1} h^{-1})						Bacterial biomass (µg C l^{-1})					
	PALEX 1		PALEX 2		PALEX 3		PALEX 1		PALEX 2		PALEX 3	
	Max	Day	Max	Day	Max	Day	Max	Day	Max	Day	Max	Day
Control	0.24	3	0.53	3	0.07	6	26.12	3	13.50	3	18.02	8
NPSi	4.95	2	1.70	10	0.80	3	36.37	3	27.4	4	29.70	5
P	4.58	2	0.92	3	0.73	3	40.39	2	22.29	4	31.68	5
N	0.52	3	0.29	3			26.35	2	17.19	4		
Si	0.48	2	0.58	3			26.59	3	19.36	3		
Ra	0.2	5	0.73	3			31.03	3	13.78	4		
Ri	0.56	3	0.79	3			32.51	3	17.52	4		

NPSi, addition of nitrogen, phosphorus, silicate; P, addition of phosphorus; N, addition of nitrogen; Si, addition of silicate; Ra, addition of rain water; Ri, addition of river water.

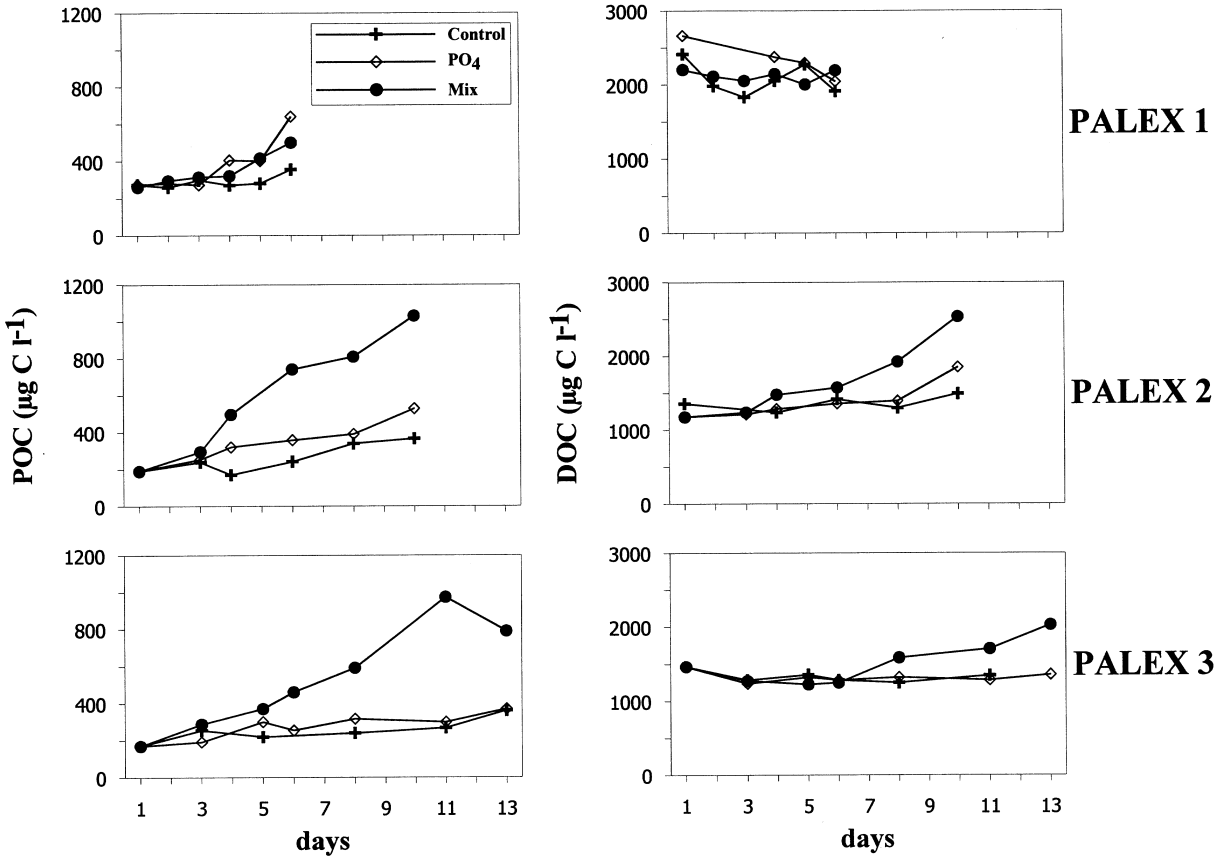

Fig. 5. Particulate organic carbon (POC) and dissolved organic carbon (DOC) in control (+), phosphorus enriched (◊) and nitrogen, phosphorus, silicate enriched enclosures (•) during three experiments (PALEX 1, June 1994; PALEX 2, April 1995; PALEX 3, March/April 1996).

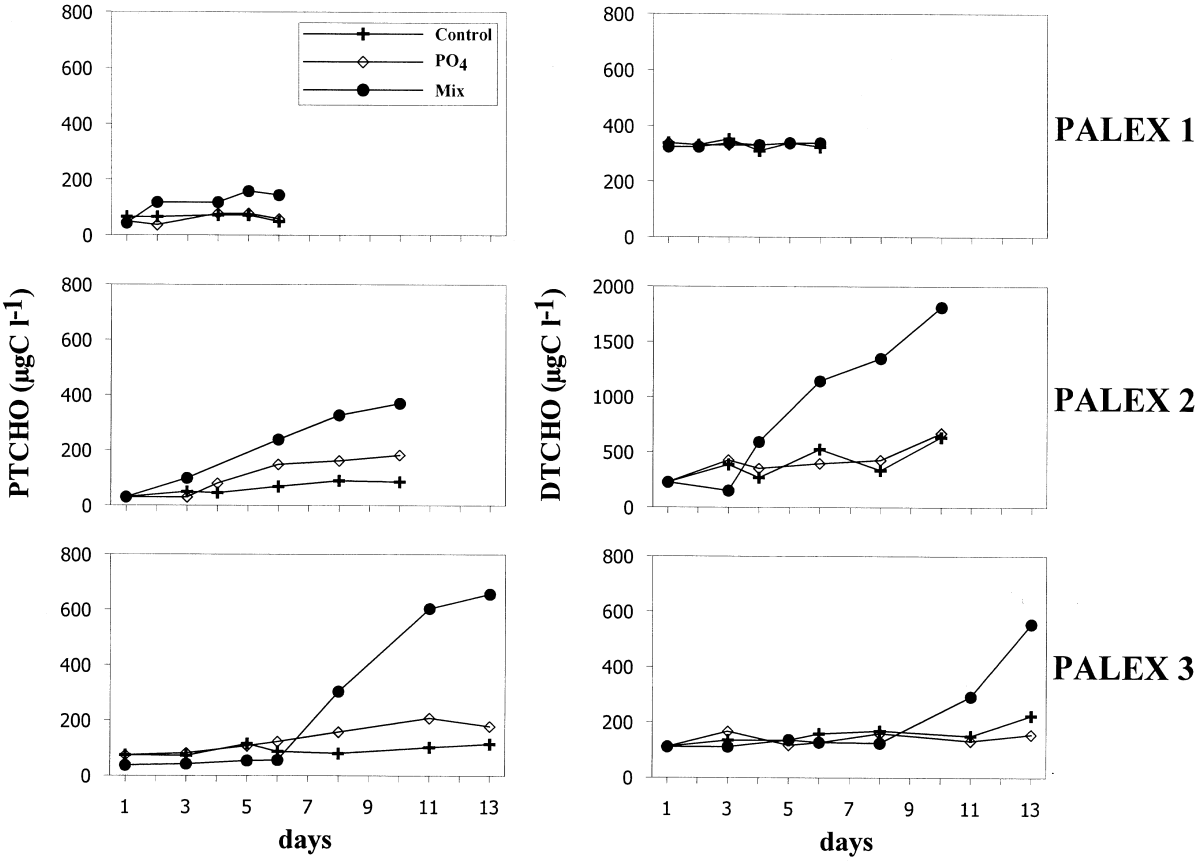

Fig. 6. Particulate total carbohydrates (PTCHO) and dissolved total carbohydrates (DTCHO) in control (+), phosphorus enriched (◊) and nitrogen, phosphorus, silicate enriched enclosures (•) during three experiments (PALEX 1, June 1994; PALEX 2, April 1995; PALEX 3, March/April 1996).

phase, was followed by the DTCHO increment (Figure 6). Again, the highest contribution of DTCHO to DOC was found in the treatment amended by all nutrients.

DISCUSSION

Our experiments were designed to mimic naturally occurring inputs of nutrients in the studied area and to follow the particulate and dissolved organic matter dynamics after introduction of nutrients with the aim of contributing to the understanding of the mechanisms leading to the mucilage phenomenon in the northern Adriatic. Rates of primary and bacterial production measured during our enrichment experiments ranged within values encountered in the natural environment of the studied area and the rest of the northern Adriatic (Karner and Herndl, 1992; Malej *et al.*, 1995; Heilmann and Richardson, 1999; Socal *et al.*, 2002; Cantoni *et al.*, 2003). This shallow marine area exhibits seasonally and spatially different conditions from oligotrophy to eutrophy (Harding *et al.*, 1999). Peak biomass and abundance values of primary and bacterial producers, POC, DOC, PTCHO and DTCHO concentrations, measured during our experiments have also been found seasonally in eutrophic areas of the northern Adriatic (Posedel and Faganeli, 1991; Terzić *et al.*, 1998; Pettine *et al.*, 1999; Turk *et al.*, 2001). Therefore, we may consider that developments during our experiments resembled natural conditions in the northern Adriatic reasonably well.

Our results show that the addition of macronutrients (NPSi) and to a lesser extent phosphate alone induced changes that conformed to classical stages of phytoplankton growth: an initial lag phase of 2–3 days followed by a period of rapid growth (4–6 days). The stationary phase seemed to be shorter in PALEX 3 compared with the second experiment, despite similar environmental conditions and community composition. In both, diatoms prevailed, although with different dominant species. *Pseudonitzschia pseudodelicatissima*, *S. costatum* and *Chaetoceros* sp. prevailed during PALEX 2; four species of *Chaetoceros*

(*C. compressus*, *C. decipiens* and two non-identified species), *Bacteriastrum* sp., *P. pseudodelicatissima* and *Cylindrotheca closterium* dominated in PALEX 3. We did not reach the stationary phase during PALEX 1 in enclosures amended with all nutrients (NPSi), but in P treatment phytobiomass had already begun to decline. Similarly, in PALEX 2 and 3, production rates and Chl *a* biomass began to decline earlier in P-treated enclosures than in amendments with all nutrients indicating that N was co-limiting phytoplankton. Enhancements of the production and biomass accumulation were much smaller in treatments with N and silicate alone and were not statistically different from controls ($P < 0.05$). Common to all treatments was the fast removal of P during the first 3–5 days of the experiments. This pattern, together with results of the enrichment experiments carried out earlier in the study area (Mozetič *et al.*, 1998b) and other parts of the northern Adriatic (Graneli *et al.*, 1999), is consistent with the general notion that P limits primary production and autotrophic biomass most of the time, with N becoming co-limiting at times (Degobbis *et al.*, 2000).

Rain and river water additions also stimulated phytoplankton activity: integrated primary production was, on average, roughly double compared with controls in both experiments (PALEX 1 and 2) in which these treatments were applied. Slightly higher primary production rates were measured in river-treated enclosures, while EOC per unit biomass attained higher values in rain treated enclosures (Table IV).

Despite some differences, the three enrichment experiments indicated a similarity between POM and DOM patterns, particularly in PALEX 2 and 3. Particulate primary production in nutrient-repleted conditions was nearly an order of magnitude higher than bacterial production (Figure 1). In contrast to primary production rates, which decreased after 4–6 days, bacterial production showed a bimodal pattern with an initial peak that preceded the primary production maximum and a secondary peak after 6–10 days. Similar dynamics were observed during microcosm experiments with natural plankton communities from the western Mediterranean (Berdalet *et al.*, 1996) and are probably related to shifts in bacterial community composition (Riemann *et al.*, 2000). Enhanced primary and secondary production led to accumulation of particulate biomass as larger grazers were excluded before inoculating the experimental community. The increase of bacterial biomass was modest compared with the biomass of autotrophs, which was also observed by Duarte *et al.* (Duarte *et al.*, 2000). Autotrophic production rates, especially of microplankton, decreased 4–8 days after nutrient additions and phytoplankton began to decay as indicated by the increase of pigment degradation products (Figures 2 and 3). The decay rate constants for Chl and fucoxanthin in enclosures amended with P and all nutrients (NPSi) were in the range of 0.08–0.2 and 0.07–0.15 during PALEX 2 and 3, respectively. These values are similar to or slightly higher than those found by Bianchi *et al.* (Bianchi *et al.*, 2000), indicating that 7–20% of autotrophic biomass decayed daily after reaching the stationary phase.

Peak particulate production rates and maximum particulate biomass were reached 4–8 days after amendment with nutrients in all three experiments in spite of significant differences in temperature and light conditions. This time scale corresponds to our field observations of the effects of new nutrient input in the studied area (Malej *et al.*, 1997). POC increase reached its maximum during the logarithmic growth of phytoplankton and PTCHO production constituted the major fraction of POC synthesis in the stationary phase. This pattern is consistent with the finding that cells produce more storage carbohydrates under nutrient-depleted conditions (Biersmith and Benner, 1998). The maximum DTCHO concentration occurred with a time lag of ~2–4 days after PTCHO peak, i.e. ~6–8 days after nutrient additions during PALEX 2 and 3. Søndergaard *et al.* (Søndergaard *et al.*, 2000) also observed the lag of ~4 days in the DOC production compared with POC in mesocosms that were manipulated with inorganic nutrients; cessation of N addition created the most dramatic change in favour of DOC production.

Accumulation of DOC, particularly of DTCHO during PALEX 2 and 3 (Figures 5 and 6), indicated decoupling of DOM production and consumption. As assessed by low bacterial glucosidase activity during PALEX 3 (Fajon *et al.*, 1999), the utilization of organic C source may have been inhibited by a lack of P. Heterotrophic bacteria need more P compared with phytoplankton as indicated by cell C:N:P ratio (50:10:1) (Fagerbakke *et al.*, 1996), and thus a P-limited condition may affect bacterial activity more than phytoplankton (Obernosterer and Herndl, 1995; Thingstad *et al.*, 1997). At high organic C supply, a switch from C- to P-limitation occurs accompanied by DOC accumulation. Additionally, the quality of DOC substrate may affect its utilization by bacteria (Norrman *et al.*, 1995). Refractory compounds can be formed during algal blooms, particularly diatom blooms that become nutrient limited. The observed pattern of organic matter dynamics during our second and third experiments when diatoms dominated phytoplankton is consistent with these findings.

The microflagellate-dominated community in PALEX 1 released, on average, significantly higher ($P < 0.01$) EOC per unit biomass (Table III) but DOC did not accumulate during the course of this experiment (Figure 5), indicating bio-availability and uptake of released OM. In spite

of the quite high grazing pressure by HNAN (Figure 4) and low P concentrations after 4 days, bacteria seemed to have consumed newly produced DOC efficiently. This experiment illustrates a situation with a rather high primary production when nutrients are replete, with modest POC increase and little change in DOC due to its balanced production and consumption. Moreover, in contrast to the increased contribution of DTCHO to the total DOC pool over time observed during PALEX 2 and 3, during PALEX 1 the proportion of DTCHO in total DOC remained quite constant. DTCHO contributed from 8 to 19% (average 14 ± 5%) to the DOC at the beginning (days 1–3) of all three experiments. After day 5 the contribution of DTCHO in NPSi enclosures accounted for 16 ± 7%, 71 ± 4% and 22 ± 5% of bulk DOC for PALEX 1, 2 and 3, respectively. Aluwihare and Repeta (Aluwihare and Repeta, 1999) observed interspecific differences in chemical composition of DOM released by different phytoplankton species. The diatom *Thalassiosira weissflogii* exuded polysaccharides that closely resembled naturally occurring high molecular weight acyl heteropolysaccharides (APS), which appeared to be more resistant to microbial degradation than other polysaccharides. In contrast, the analysis of the primnesiophyte *Phaeocystis* sp. exudates indicated the presence of only a small fraction of APS. Consonant with these results 19′-hexanoyloxyfucoxanthin-containing phytoplankton that dominated the autotrophic community during PALEX 1 seemed to produce more labile DOC than diatom-dominating communities during PALEX 2 and 3. Similarly, while phytoplankton bloom dominated by diatoms, which were provoked by nutrient addition during a mesocosm experiment by Meon and Kirchman (Meon and Kirchman, 2001), produced refractory DOM, the unenriched mesocosm did not produce any measurable refractory DOM. Alternatively, bacterial DOC consumption might depend not only on liability of DOC, but also on the structure of the microbial food web. Havskum *et al.* (Havskum *et al.*, 2003) found that bacteria competed successfully with algae <10 μm for mineral nutrients, but not with chain-forming diatoms.

On a time scale of up to 14 days, an increase of DOC and particularly of DTCHO accumulation during PALEX 2 and 3 did not result in the formation of large mucilage aggregates. One reason could be the duration of PALEX experiments (<14 days). During additional enrichment experiments that continued for 22 days (unpublished data) and over 1 month (Baldi *et al.*, 1997), we attained similar total organic C values and observed large (up to few centimetres) flocks of marine snow. Another possibility is that POC and DOC accumulations obtained during our three PALEX experiments were not high enough. The peak concentrations of TOC (total organic C) measured in our experiments were 2.78, 3.47 and 2.85 mg C l^{-1} during PALEX 1, 2 and 3, respectively. Estimates of the volume-specific C mass of mucilage-associated suspended matter that accumulated in the water column during mucilage events in 1997 and 2000 yielded values of 47.6 ± 1.7 mg C l^{-1} for dense water column mucilage clouds and 5.9 ± 4.2 mg C l^{-1} for loose mucilage network (Malej *et al.*, 2001). Considerably higher C content (192.85 ± 71.6 mg C l^{-1}) of dense water column mucilage was reported by Müller-Niklas *et al.* (Müller-Niklas *et al.*, 1994). As the peak concentrations of TOC measured during our enrichment experiments were similar to the lowest values reported for TOC concentrations in mucilage, we either did not reach the critical concentrations or appropriate chemical composition of POC and DOC.

CONCLUSIONS

Our experiments showed that the addition of nutrients in concentrations similar to natural inputs had a significant impact on the production and abundance of autotrophic and heterotrophic plankton as well as on the partitioning of organic C. The initial autotrophic community structure and the ratio of autotrophic to heterotrophic biomass were very important factors that influenced the partitioning of new production into POM and DOM pools. The addition of nutrients stimulated primary production proportionally more than bacterial production. Peak autotrophic biomass in enclosures amended with all nutrients, which reached maximum values 4–7 days after nutrient addition, were about an order of magnitude above the initial values, whereas bacterial biomass that reached maximum values 2–5 days after amendment with nutrients only roughly doubled.

Organic C partitioning was similar during the two experiments in which diatoms prevailed in biotic communities (PALEX 2 and 3): during the exponential growth phase it was dominated by POC with an increasing contribution of PTCHO that paralleled gradual nutrient depletion. Transition to the stationary phase and decay of the autotrophic community were accompanied by a net accumulation of carbohydrate-rich DOC. In contrast, in the first experiment, when microflagellates dominated the autotrophic community, and despite the fact that autotrophs released significantly higher DOC per unit biomass, no such DOC and DTCHO accumulation was observed. No large mucilage aggregate formation was observed during our experiments, which lasted up to 14 days.

ACKNOWLEDGEMENTS

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