Phytoplankton and bacterial uptake of inorganic and organic nitrogen during an induced bloom of *Phaeocystis pouchetii*

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ABSTRACT: A mesocosm experiment was conducted during the spring of 2003 in Raunefjord, west Norway. Inorganic nutrients (16 µmol l^{-1} nitrate, 1 µmol l^{-1} phosphate) were added to 2 enclosures with 10 % of those concentrations (1.6 µmol l^{-1} nitrate, 0.1 µmol l^{-1} phosphate) added daily thereafter; a third unamended mesocosm was used as a control. Nitrogen (N) (ammonium, nitrate, urea and amino acid) uptake rates for >0.8 µm (largely composed of phytoplankton) and 0.2 to 0.8 µm (largely composed of bacteria) size classes were measured, as well as nutrient, chlorophyll, phytoplankton and microzooplankton concentrations. The nutrient additions initiated a process resulting in a large bloom of *Phaeocystis pouchetii* colonies. There was a 2.5 wk lag, during which a smaller bloom of phototrophic flagellates, followed by diatoms, formed in all mesocosms; diatoms increased until silicic acid was depleted. After the flagellate and diatom bloom dissipated, the mesocosms were depleted of inorganic N. Dissolved organic N (DON) remained constant in all mesocosms during the flagellate and diatom bloom but increased with the onset of the large *P. pouchetii* bloom, largely due to release of urea and amino acids, which made up >93 % of the DON pool at the end of the experiment. Uptake rates of urea often accounted for the largest percentage of total N uptake in all mesocosms for both the >0.8 µm and 0.2 to 0.8 µm size classes, generally providing the largest percentage (up to 88 %) of bacterial N demand of any substrate. This was particularly true during the *Phaeocystis* bloom when urea uptake rates were up to 65 times greater than the other N substrates. We observed a switch from new production, when nitrate uptake dominated during the diatom bloom, to regenerated production during the *Phaeocystis* bloom. The present study adds to the growing body of evidence that organic N is a substantial source of N for both phytoplankton and bacteria.

KEY WORDS: Nitrogen uptake · Mesocosm experiments · *Phaeocystis* · Diatoms · Bacteria

INTRODUCTION

Liebig's Law of the Minimum (Liebig 1840) states that the overall production in an area will be determined by the availability of the nutrient that is needed for growth and is present in the lowest concentration relative to its demand. In many marine ecosystems the macronutrient nitrogen (N) limits biological production. Dissolved inorganic N (DIN) compounds, ammo-

nium (NH_4^+), nitrate (NO_3^-) and nitrite (NO_2^-), were originally considered the main bioavailable N compounds; however, it has been shown that dissolved organic N (DON) forms, such as urea (e.g. Lomas et al. 2002), amino acids (e.g. Fuhrman 1990) and even humics (See et al. 2006), can also contribute to planktonic N nutrition (reviewed in Berman & Bronk 2003).

The importance of DIN for the nutrition and growth of marine phytoplankton has long been recognized

(e.g. Dugdale & Goering 1967), whereas DIN utilization by bacteria has received less attention. In contrast, DON has been considered to be more important for the nutrition and growth of heterotrophic organisms than for phytoplankton. Competition between phytoplankton and bacteria for available N has been an active area of research (e.g. Lancelot & Billen 1984, Lancelot & Mathot 1987, Veuger et al. 2004). In general, the small size and resulting high surface to volume ratio of bacteria make them superior competitors.

In the fjords of Norway, phytoplankton blooms occur in early spring as light increases and the nutrient-rich water from winter mixing stratifies within the euphotic zone (Erga & Heimdal 1984). In northern latitudes, blooms of the colony-forming prymnesiophyte *Phaeocystis pouchetii* have been reported simultaneously with or immediately following spring blooms of chain-forming diatoms between February and May, but may also occur in high numbers during the fall (e.g. Heimdal 1974). *P. pouchetii* can be found as single cells or cells embedded in a gelatinous colony. As blooms progress, the number of small individual cells typically decreases coincident with the appearance of more colonies. Finally, as a bloom declines and colonies senesce, bacterial communities are likely involved in the decomposition and remineralization of colony materials. It has been widely reported that bacterial biomass and activity increase following *Phaeocystis* spp. blooms (e.g. Fernández et al. 1992, Nejstgaard et al. 2006).

Large blooms of *Phaeocystis pouchetii* colonies are significant because they can increase export of material to the deep ocean via the biological pump (Verity & Medlin 2003) and can produce large quantities of dimethyl sulfide, which act as cloud condensation nuclei resulting in a climate-cooling effect due to cloud formation (Charlson et al. 1987). They are also classified as a harmful algal bloom (HAB) species because they are known to produce toxins which are lethal to fish larvae (Eilertsen & Raa 1995). These dense blooms may also affect higher trophic levels, leading to negative influences on human activities such as fisheries (net clogging and fish mortality), fish farming (altering fish taste) and tourism (odorous foams of a declining/decaying bloom on coastal beaches, Schoemann et al. 2005 and references therein).

Phaeocystis pouchetii often dominates over diatoms in waters with low silicic acid (H_2SiO_3) concentrations (<2 µmol l^{-1}) and surplus NO_3^- (>5 µmol l^{-1}) and phosphate (PO_4^{3-}, >0.2 µmol l^{-1}) (e.g. Egge & Jacobsen 1997). However, H_2SiO_3 availability is not a unique predictor of *P. pouchetii* versus diatom dominance (e.g. Larsen et al. 2004). Blooms of *P. pouchetii* typically comprise the colonial form, although there are a few reports of blooms of solitary *P. pouchetii* cells (Wassmann et al. 2005). Colony formation could be a means

to escape predation (Fernández et al. 1992), to control buoyancy in order to keep cells in the upper layers of the euphotic zone (Skreslet 1988) or to avoid viral infection (e.g. Bratbak et al. 1998). A fundamental mechanistic understanding of all the factors that contribute to the formation of *P. pouchetii* blooms and colony formation, however, remains elusive (Larsen et al. 2004, Schoemann et al. 2005, Nejstgaard et al. 2006). Nevertheless, many mesocosm studies have shown that a bloom of *P. pouchetii* can be initiated by the addition of both N and phosphorus (P) during the early spring (Egge & Heimdal 1994 and references therein).

The present study was conducted as part of a larger mesocosm project designed to investigate the onset of colony formation in *Phaeocystis pouchetii* while mimicking the formation of a typical bloom in Raunefjord, Norway (Nejstgaard et al. 2006, Verity et al. 2007b). The use of mesocosm enclosures allows for an experiment of larger spatial and temporal scales without complicating issues typically seen in smaller scale experiments or *in situ* field studies. Mesocosm enclosures contain large volumes of water and therefore allow multiple investigators to conduct experiments simultaneously without sacrificing experimental integrity due to insufficient sample volumes. Furthermore, bottle effects are essentially eliminated due to the large sample size, and the experiments are more representative of processes that occur in nature due to maintenance of realistic temperature and irradiance levels and the inclusion of higher trophic levels of natural plankton assemblages, including grazers that are often excluded from bottle experiments. An added benefit of mesocosm studies is that one does not have the difficulty of following a given water mass (i.e. drogue studies), and the experiment can continue for a longer period of time without complications arising from advection of surrounding water masses.

In the present study, experiments were conducted during the early spring of 2003 in Raunefjord, Norway, in mesocosms amended with NO_3^- and PO_4^{3-} and monitored for 34 d. The present study focuses on the utilization of nutrients, succession of phytoplankton species and the uptake of both inorganic and organic N compounds in phytoplankton (>0.8 µm) and bacterial (0.2 to 0.8 µm) size classes during the onset and decline of a *Phaeocystis pouchetii* bloom. We hypothesized that inorganic N would be relatively more important early in the experiment and that the reliance on organic N would increase as the *P. pouchetii* bloom developed and declined. We also hypothesized that rates of bacterial uptake would be lower than phytoplankton uptake and would increase with the decline of the *P. pouchetii* bloom. Experimental results indicate that both hypotheses were true.

MATERIALS AND METHODS

Experimental design. Mesocosm studies were conducted at The University of Bergen's Marine Biological Field Station in Espegrend, Norway (www.bio.uib.no/pages/mbs.php). Experiments were conducted over a 34 d period from 28 February to 2 April 2003. Three mesocosm enclosures were attached to a floating dock 200 m offshore in Raunefjord. The enclosures were 2 m in diameter, 4.5 m deep and contained 11 m^3 of water. The bags, made of a transparent polyethylene material, were open at the top and designed to allow ca. 90% penetration of photosynthetically active radiation. The water within each enclosure was kept well mixed using an airlift system that pumped ca. 40 l water min^{-1} (Nejstgaard et al. 2006). All enclosures had 10% of their water renewed daily with adjacent fjord water to allow for the introduction of new species, to avoid substantial pH changes due to primary production and to replace water removed for analyses (see Williams & Egge 1998 for more information on water renewal).

All enclosures were filled with unfiltered fjord water from a depth of 5 m on 28 February 2003. One of the enclosures (M1) was treated as a control and the other two were amended with nutrients (M2 and M3). The 2 nutrient-amended mesocosms had 16 µmol l^{-1} NO$_3^-$ and 1 µmol l^{-1} PO$_4^{3-}$ added initially in the forms of NaNO$_3$ and KH$_2$PO$_4$, respectively, and then 10% (or 1.6 µmol l^{-1} NO$_3^-$ and 0.1 µmol l^{-1} PO$_4^{3-}$) of the nutrients added daily thereafter at noon to replace nutrients lost by the daily 10% water exchange. Amendments to M2 were stopped on Day 19 to initiate decline of the bloom in that enclosure; additions to M3 continued daily throughout the experiment. Mesocosms were sampled between 08:00 and 09:00 h daily for chlorophyll *a* (chl *a*) and approximately every 3 d for nutrient, phytoplankton and microzooplankton concentrations, as well as N uptake rates; for further details of the experimental design see Nejstgaard et al. (2006).

Biomass and nutrient measurements. Concentrations of chl *a* and particulate N (PN) were measured as proxies for biomass. Concentrations of chl *a* were measured fluorometrically on a Turner Design Model 10-AU fluorometer; samples were filtered onto Sartorius filters (25 mm diameter, 0.45 µm pore size), extracted in 90% acetone at 4°C overnight and analyzed according to Parsons et al. (1984). Concentrations of PN were measured at the end of incubation during the analysis of mass spectrometer samples as described below.

Phytoplankton and microzooplankton cells were identified and enumerated by epifluorescence microscopy. Plankton <10 µm were analyzed in triplicate samples (15 ml) that were prepared by gently passing water through a 10 µm nylon mesh. Each sample was fixed with HPLC-grade glutaraldehyde (0.5% final

concentration), stained with proflavin (100 ng ml^{-1} final concentration) and DAPI (25 ng ml^{-1} final concentration) as described by Porter & Feig (1980), with the exception that higher concentrations of stain were required to effectively stain *Phaeocystis pouchetii* cells. Epifluorescent slides were made by gently filtering the fixed and stained samples onto black 0.2 µm 25 mm diameter polycarbonate filters using a hand pump with <0.5 mm Hg vacuum pressure. Filters were mounted onto glass slides with a cover slip and stored at –20°C until analyzed. Cells were visualized by epifluorescence microscopy using an Olympus BX-60 fluorescence microscope equipped with a 60× Planapo NA 1.40 oil objective. For each slide a minimum of 20 fields or 300 cells were examined. Plankton >10 µm were enumerated in 60 ml of whole water that was treated with EDTA (10 mmol l^{-1} final concentration) before fixing, staining and storing as above. The EDTA was added to disperse *P. pouchetii* colonies without affecting cell morphology or total cell abundance (Nejstgaard et al. 2006). Flagellated *P. pouchetii* solitary cells and other small cells were enumerated in the <10 µm samples. Microflagellates (autotrophic, heterotrophic and mixotrophic), diatoms and total *P. pouchetii* cells (solitary and colonial) were identified and enumerated in the EDTA-treated samples. Ciliates and large dinoflagellates (<50 µm diameter) were enumerated in the EDTA-treated samples by screening and counting all cells present on the whole slide (15 ml).

Nutrients were monitored in all mesocosms throughout the experiment and analyses were performed in duplicate (urea) or triplicate (all others). Unless otherwise noted all nutrient samples were filtered upon collection and frozen immediately in acid-cleaned bottles until analysis; urea and NH$_4^+$ samples were stored in polypropylene bottles, and all other nutrients were stored in high-density polyethylene (HDPE) bottles. Concentrations of NH$_4^+$ were determined colormetrically using the manual phenol hypochlorite technique (detection limit 0.05 µmol N l^{-1}, Koroleff 1983). Concentrations of NO$_3^-$ and NO$_2^-$ were measured on an O.I. Analytical Alpkem Flow Solution IV autoanalyzer using the Parsons et al. (1984) colorimetric technique (detection limit 0.003 and 0.002 µmol N l^{-1}, respectively). Urea concentrations were measured manually using the monoxime method (detection limit 0.025 µmol N l^{-1}, Price & Harrison 1987). Amino acid (AA) concentrations were measured as dissolved primary amines (DPA) using a fluorometric OPA (*o*-phthaldialdehyde) technique (detection limit 0.04 µmol N l^{-1}; Parsons et al. 1984). In waters with low concentrations of NH$_4^+$, measurements of free AA and DPA are approximately equal (Kirchman et al. 1989); therefore we will refer to them as AA. Total dissolved N (TDN)

was measured using the Valderrama (1981) persulfate oxidation technique as described in Bronk et al. (2000); the oxidized samples were treated like NO_3^- samples as described above. The concentration of DON was calculated as the difference between TDN and the sum of NH_4^+, NO_3^- and NO_2^-; the SD for DON concentration was calculated using a propagation of error analysis (Bronk et al. 2000). Samples (20 ml) for analysis of PO_4^{3-} and H_2SiO_3 were fixed with 0.2 ml chloroform and stored at 4°C until analysis as described in Nejstgaard et al. (2006). Phosphate data were lost for Days 24 through 28 due to an analytical error associated with the instrument.

Measurements of uptake rates. Nitrogen uptake rates were measured approximately every 3 d using standard ^{15}N uptake techniques as described in Bronk et al. (1998). Rates were determined for NH_4^+, NO_3^-, urea and a mixture of AA for 2 size classes: the >0.8 µm fraction and the 0.2 to 0.8 µm fraction.

For each rate measurement, 1 l polyethylene terephthalate copolyester (PETG) bottles were filled with mesocosm water and amended with tracer levels, ≤10% of the ambient substrate concentration, of an ^{15}N-labeled substrate (ammonium chloride, 98%+ ^{15}N; potassium nitrate, 98%+ ^{15}N; urea, 99% ^{13}C, 98%+ ^{15}N; or an algal AA mixture, lot # P-7934, 98%+ ^{13}C, 96 to 99% ^{15}N, all obtained from Cambridge Isotope Laboratories). Bottles were incubated at *in situ* temperatures and light intensities in the fjord by placing them in mesh bags suspended near the mesocosm enclosures for ca. 3 h. Incubations were performed in duplicate and were ended by filtering (<150 mm Hg vacuum) an aliquot over a 0.8 µm silver filter to collect the >0.8 µm size fraction. The filtrate was then passed through a 0.2 µm silver filter to obtain the 0.2 to 0.8 µm size fraction, representing the bacterial size class. All silver filters were frozen initially and then thawed and dried at 40°C in a drying oven overnight prior to simultaneous analysis of PN and particulate carbon concentrations and ^{15}N and ^{13}C isotopic enrichments. All isotope samples were analyzed on a Europa Automated Nitrogen Carbon Analyzer for Solids and Liquids (ANCA-SL) attached to a GEO 20/20 or 20/20 isotope ratio mass spectrometer. Unfortunately, the replicate samples for the >0.8 µm size fraction for urea and AA uptake were irretrievably lost during an errant run on the mass spectrometer. Uptake rates were calculated using the equations originally described in Dugdale & Goering (1967). Rates of NH_4^+ uptake were corrected for isotope dilution, and NH_4^+ regenerations rates were determined using the isotope dilution method (Glibert et al. 1982). Ammonium was isolated using solid phase extraction (Dudek et al. 1986). Uptake rates of NO_3^- were not corrected for isotope dilution. The elevated NO_2^- concentrations observed early in the experiment

(ca. the first 18 d) suggest that nitrification or NO_2^- excretion (Lomas & Lipschultz 2006) may have been occurring such that isotope dilution of the NO_3^- tracer could have taken place. However, with the very high concentrations of NO_3^- present during this time (>14 µmol N l^{-1}), any dilution of isotope due to nitrification or phytoplankton excretion would have had a negligible impact on measured NO_3^- uptake rates. Urea and AA uptake rates were also not corrected for isotope dilution. There were increases in urea and AA concentrations later in the experiment, indicating urea and AA regeneration; this suggests that uptake rates of these substrates may have been underestimated due to isotope dilution. We note that correcting for isotope dilution in urea and AA incubations would enhance the patterns we observed. No correction was done for loss of $DO^{15}N$ during the incubations; as such, the uptake rates presented are all net rates (Bronk et al. 1994).

Bacterial productivity in whole seawater samples was estimated based on incorporation rates of ^3H-leucine determined essentially following the protocol of Smith & Azam (1992), except that to achieve saturated maximal uptake rates, ^3H-leucine was added to yield 60 nmol l^{-1} final concentrations. Initial studies using water samples from mesocosm enclosures were conducted to determine optimal ^3H-leucine concentrations (data not shown). Bacterial productivity was estimated from leucine incorporation rates using conversion factors as described by Kirchman & Ducklow (1993).

RESULTS

Biomass and species composition

Concentrations of chl *a* increased from 0.5 to 5 µg l^{-1} over the first 2 wk in all mesocosms (Fig. 1a); this moderate increase in chl *a* will be referred to as the flagellate and diatom bloom. In the NO_3^- and PO_4^{3-} amended mesocosms (M2 and M3), the chl *a* concentrations continued to increase from 5 up to 30 µg l^{-1} due to a bloom of *Phaeocystis pouchetii.* The highest chl *a* concentrations were observed in M3, the mesocosm that received continuous NO_3^- and PO_4^{3-} nutrient additions throughout the experiment. Concentrations of chl *a* peaked around Day 25 and then began to decline in M2 and M3, although by Day 34 the concentrations were still higher than those observed in the control mesocosm.

Concentrations of total PN (combined >0.8 µm and 0.2 to 0.8 µm fractions) behaved similarly in all 3 mesocosms over the first 2 wk and increased from 1.9 ± 0.3 to ca. 6.0 µmol N l^{-1} (Fig. 1b). In the control mesocosm,
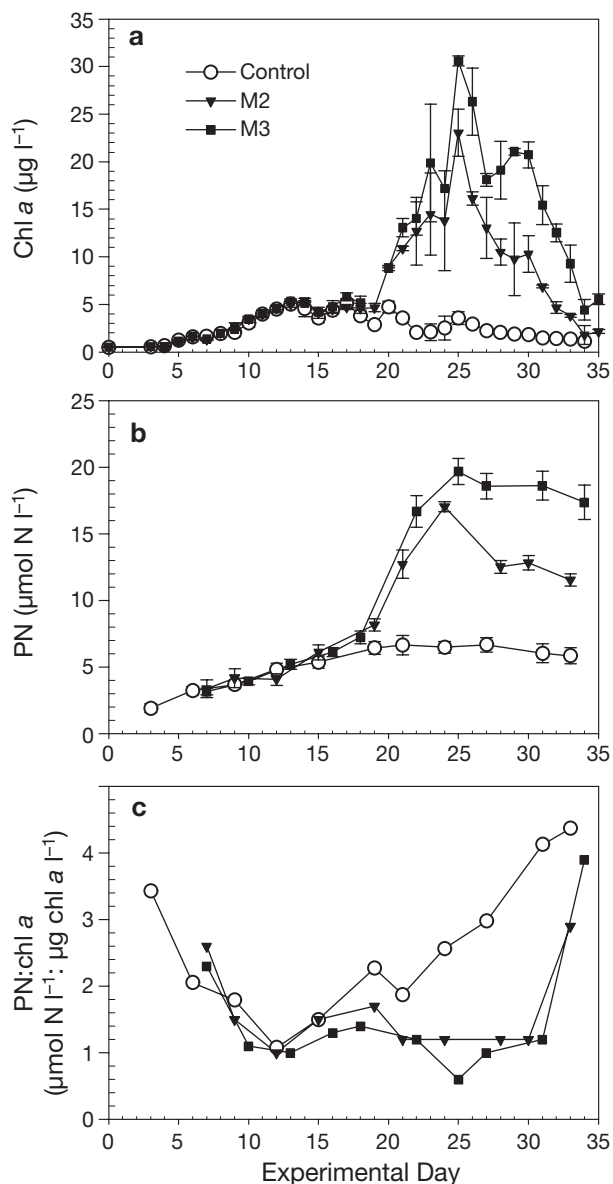
Fig. 1. Concentrations of (a) chlorophyll *a* (chl *a*), (b) total particulate nitrogen (PN) and (c) PN:chl *a*, including both the >0.8 µm and 0.2 to 0.8 µm size fractions, versus experimental day for the control and the 2 nutrient-amended mesocosms, M2 and M3. Error bars in (a) and (b) represent SD; when they do not appear they are smaller than the symbols

PN concentrations leveled off between 6.0 and 6.7 µmol N l^{-1} for the remainder of the experiment, whereas in M2 and M3 PN increased to 17.1 ± 0.4 and 19.7 ± 1.0 µmol N l^{-1} by Days 24 and 25, respectively, before declining. The increase in PN observed in all mesocosms was largely due to the >0.8 µm size fraction. PN in the 0.2 to 0.8 µm size fraction increased slightly in all mesocosms over the entire experiment, starting out at 0.62 ± 0.29 µmol N l^{-1} and increasing to 1.62 ± 0.23 µmol N l^{-1} in the control and to 2.0 ± 0.29

and 2.0 ± 0.46 µmol N l^{-1} in M2 and M3, respectively (data not shown).

At the start of the experiment, ratios of PN:chl *a* (µmol N l^{-1}: µg chl *a* l^{-1}) were ca. 3.4 and decreased as the flagellate and diatom bloom formed to approach 1:1 in all mesocosms within the first 10 d or so (Fig. 1c). This ratio started to increase in the control as the flagellate and diatom bloom declined and continued increasing throughout the remainder of the experiment, reaching 4.4 by Day 33. In M2 and M3 the ratio increased slightly, but remained between 1.0 and 1.5 during the end of the flagellate and diatom bloom and into the start of the *Phaeocystis* bloom. However, by the peak of the *Phaeocystis pouchetii* colony bloom it had decreased to 1.2 and 0.6 for M2 and M3, respectively. These ratios remained close to 1 until the very end of the experiment when the ratio increased to 2.9 in M2 on Day 33 and to 3.9 in M3 on Day 34.

Phytoplankton species composition in all mesocosms was initially dominated by small (2 to 5 µm) phototrophic flagellates, other than *Phaeocystis pouchetii* cells (Fig. 2). There was a small bloom and subsequent decline of diatoms (mainly *Chaetoceros socialis* and *Skeletonema costatum*) during the first 2 wk in all mesocosms. The small phototrophic algae also increased in cell numbers initially before decreasing; however, they remained present at a low level throughout the remainder of the experiment. Heterotrophic flagellates and ciliates were present in low numbers at the start of the experiment and increased slightly during the last third of the experiment. The largest change in species numbers occurred in M2 and M3 where large blooms of colonial *P. pouchetii* developed (Fig. 2b,c). Prior to the colonial bloom (Days 1 to 16), *P. pouchetii* was present largely as solitary cells (see Nejstgaard et al. [2006] for additional community composition data).

Nutrients

Initial concentrations of TDN were ca. 11 µmol N l^{-1} (before nutrient addition) and decreased over the first 21 d of the experiment before leveling off around 5 µmol N l^{-1} (data not shown); the initial decrease was due to the rapid draw-down of inorganic N. At the beginning of the experiment, roughly 65% of the TDN pool was DIN. During the last 1.5 wk of the experiment, however, <5% of the TDN pool was DIN as a result of the increase in DON and substantial DIN uptake.

Initial NO$_3^-$ concentrations were ca. 6.6 µmol N l^{-1}. This dropped in the control mesocosm to 0.06 ± 0.01 µmol N l^{-1} by Day 19 (Fig. 3a). In the amended mesocosms, with the addition of 16 µmol l^{-1} NO$_3^-$ ini-
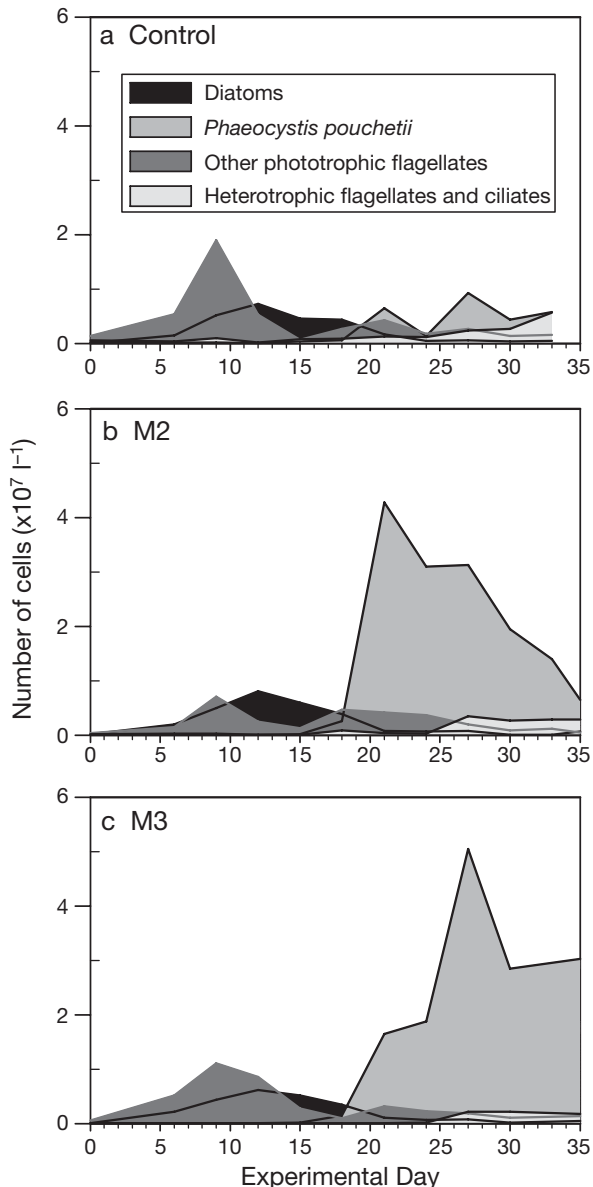
Fig. 2. Species composition in number of cells versus experimental day for (a) control, (b) M2 and (c) M3 mesocosms

tially and 1.6 µmol l^{-1} NO$_3^-$ daily thereafter (except in M2 where NO$_3^-$ additions stopped on Day 19), the concentration of NO$_3^-$ had been drawn down to between 0.01 and 0.02 µmol N l^{-1} by Day 22. Concentrations of NH$_4^+$ in all 3 mesocosms were rapidly drawn down during the first 5 d from initial concentrations of ca. 0.4 µmol N l^{-1} and then remained ca. 0.15 µmol N l^{-1} for the remainder of the experiment (Fig. 3b), likely reflecting a balance between production and consumption. Concentrations of NO$_2^-$ remained fairly constant during the first 15 d, then were drawn down from initial concentrations of ca. 0.10 µmol N l^{-1} to below detection by Day 19 (control) and by Day 24 (M2 and M3, Fig. 3c).

Concentrations of DON in all 3 mesocosms were stable during the first 10 d before decreasing slightly around Day 15 and then increasing for the remainder of the experiment (Fig. 3d). The greatest increase in DON concentration was observed in the nutrient-amended mesocosms, and the increase was mainly due to increases in urea concentrations (Fig. 3e). At the start of the experiment >55% of the DON was undefined, 35% was urea, and AA comprised the remainder (Fig. 3d–f). As the *Phaeocystis pouchetii* bloom developed, the percentage of DON due to urea and AA increased to >80 and 13%, respectively.

Concentrations of PO$_4^{3-}$ declined slowly in the control throughout the experiment (Fig. 4a). In M2 and M3, which had 1.0 µmol l^{-1} PO$_4^{3-}$ added initially and 0.1 µmol l^{-1} added daily thereafter (M2 additions stopped on Day 19), a steady decline in PO$_4^{3-}$ concentration was observed during the flagellate and diatom bloom. This decline continued during the onset and peak of the bloom of *Phaeocystis pouchetii* before increasing slightly during the last week of the experiment (Fig. 4a). Concentrations of H$_2$SiO$_3$ were initially high but decreased rapidly to <0.5 µmol Si l^{-1} by Day 9 in all mesocosms and remained low throughout the rest of the experiment (Fig. 4b).

Generating a mass balance for this experiment was not straightforward. For reasons discussed in the 'Materials and methods', we replaced 10% of the mesocosm volume with water from the fjord each day. Unfortunately, we do not have ambient concentrations for the fjord water over the entire course of the experiment. However, assuming the ambient concentration of the fjord did not change from the initial concentrations at the beginning of the experiment, and taking into account the 10% addition of nutrients and fjord water, we were able to calculate mass balances of N consumption and production. A mass balance was achieved in M1 (control) and M3 over the entire course of the experiment. A mass balance was not achieved in M2; our speculation is that because the bloom in M2 crashed earlier than in M3 there was possibly more export or sedimentation of *Phaeocystis pouchetii* colonies in M2 that would not be sampled in the water column. This is supported by the fact that we saw more consumption of N than we measured in production of N. Along similar lines, mass balances were also not achieved for M2 and M3 during the diatom and flagellate bloom period (the increase in biomass and DON did not equal the disappearance of DIN). We believe this is due to a significant amount of export or sedimentation onto the bottom of the mesocosm bags because large diatoms tend to sink out of the system quickly due to their heavy siliceous tests. Unfortunately, we do not have any data on sedimentation rates in the mesocosms.
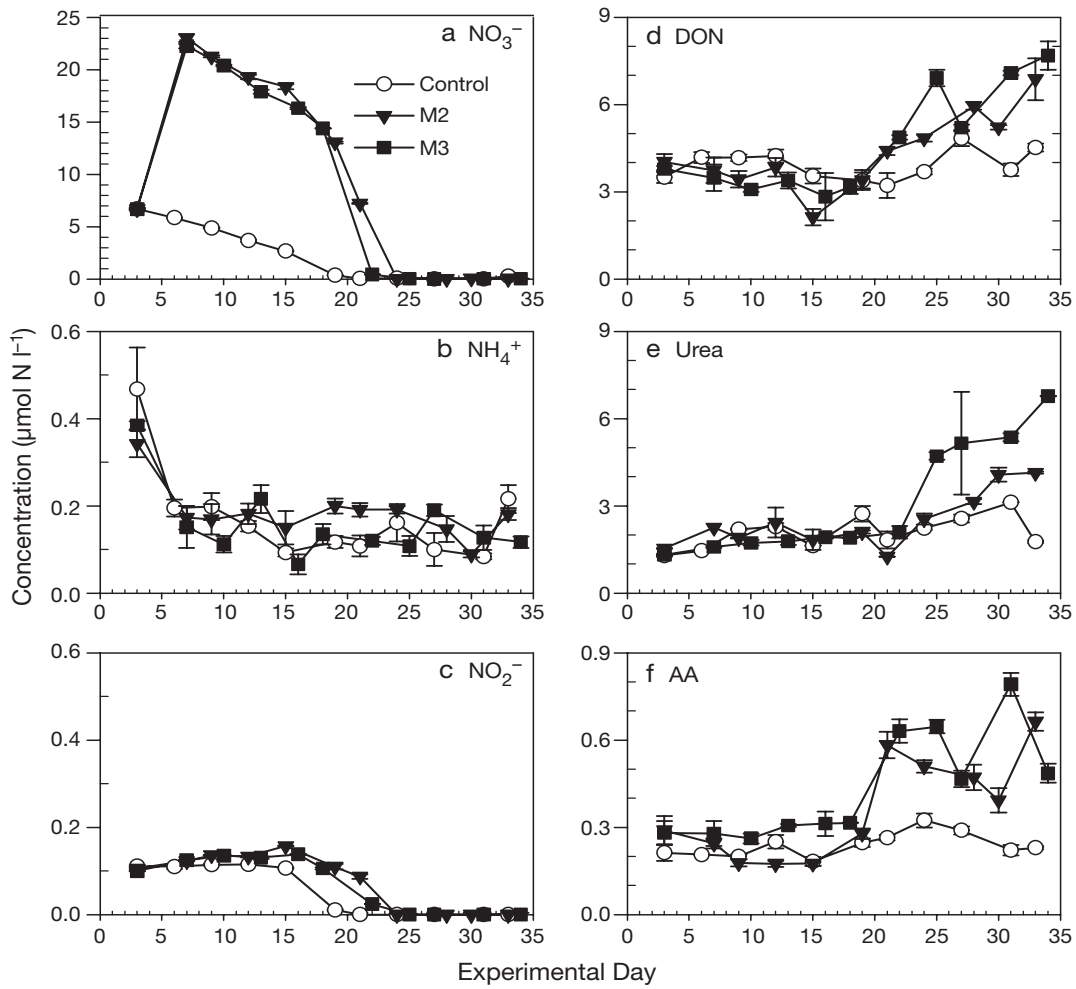
Fig. 3. Concentration of nitrogenous nutrients versus experimental day for inorganic nitrogen forms (a) NO_3^-, (b) NH_4^+ and (c) NO_2^-, and for organic forms (d) dissolved organic nitrogen (DON), (e) urea and (f) amino acids (AA). Error bars represent $\pm$SD; when they do not appear they are smaller than the symbols. There are no ambient nitrogen data for Day 4. Note the differences in scale on the *y*-axes
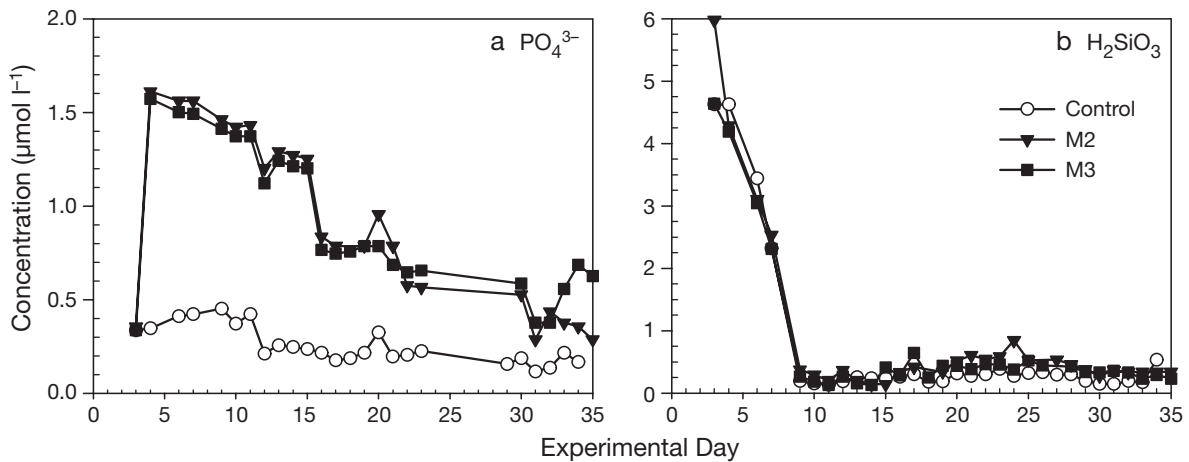

Fig. 4. Concentration of (a) PO_4^{3-} and (b) H_2SiO_3 versus experimental day for the control and the 2 nutrient-amended mesocosms, M2 and M3. Note the differences in scale on the *y*-axes
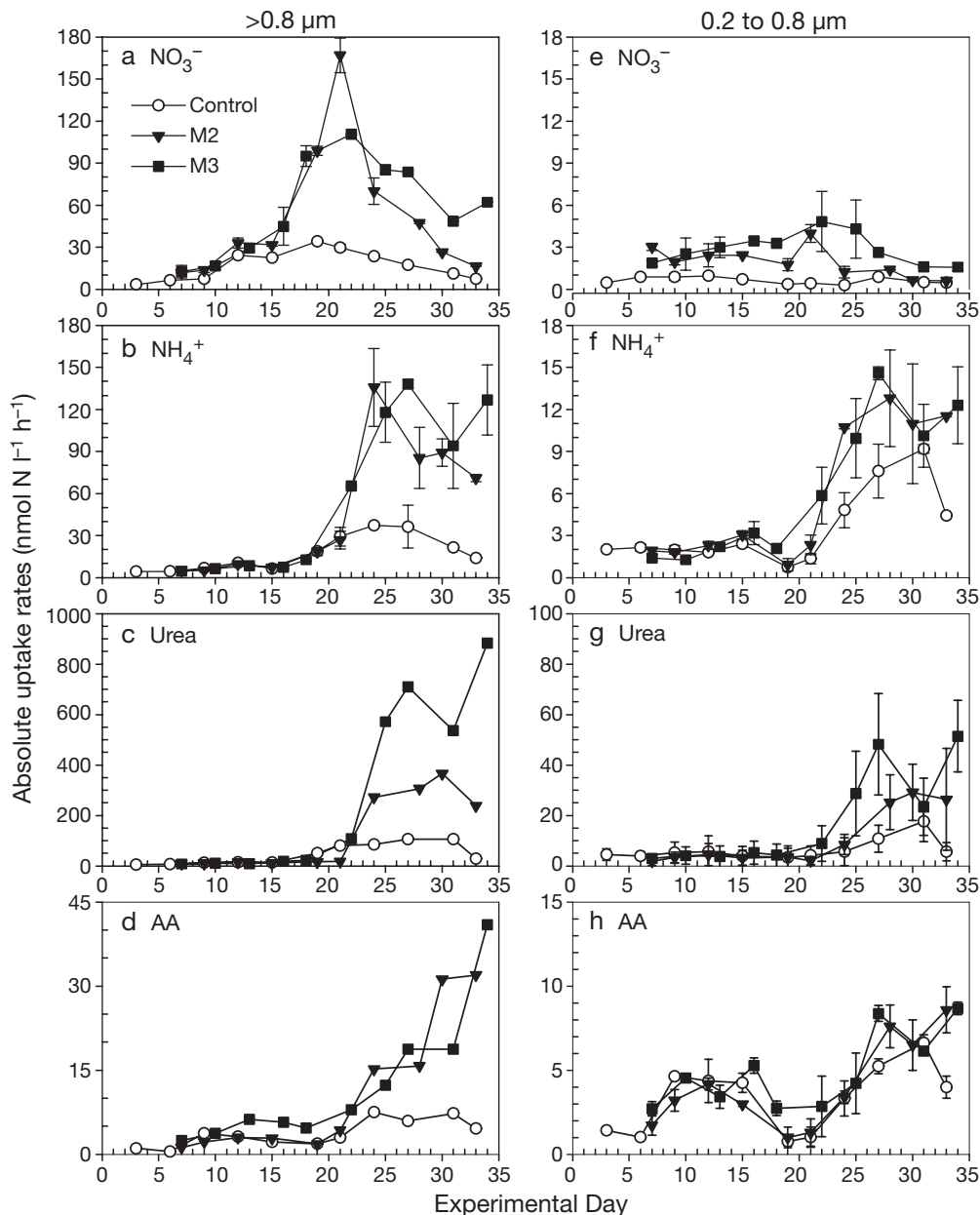
Fig. 5. Absolute uptake rates for (a–d) the >0.8 µm and (e–h) the 0.2 to 0.8 µm size classes for (a,e) NO_3^-, (b,f) NH_4^+, (c,g) urea and (d,h) AA. Error bars represent ±SD; when they do not appear they are smaller than the symbols (except in panels c and d where there are no error bars as noted in 'Materials and methods'). Note the differences in scale on the *y*-axes

Uptake and regeneration rates

In general, absolute uptake rates of all substrates were similar in all 3 mesocosms over the first 15 d of the experiment in both the >0.8 µm and 0.2 to 0.8 µm size fractions (Fig. 5), with the exception of NO_3^- uptake in the bacterial size class (Fig. 5e). In the second half of the present study, uptake rates into the >0.8 µm fraction increased dramatically. Uptake rates of NO_3^- peaked around Day 23, NH_4^+ uptake rates

peaked around Day 25, urea uptake rates were maximal at Days 25 to 30, and AA uptake increased near the end of the present study (Fig. 5a–d). Though the control treatment followed a similar pattern, uptake rates were substantially higher in M2 and M3 during the *Phaeocystis pouchetii* bloom. The highest absolute uptake rates measured were for urea, followed by NO_3^- and NH_4^+, and then AA. In the 0.2 to 0.8 µm fraction, uptake rates of NH_4^+, urea and AA increased towards the end of the *P. pouchetii* bloom, while NO_3^-

uptake remained relatively constant or decreased (Fig. 5e–h).

In general, temporal changes in absolute uptake rates (Fig. 5) were closely correlated to changes in specific uptake rates (Fig. 6), suggesting that the cells in all 3 mesocosms were physiologically similar to each other throughout the experiment. As a result, the large differences in absolute uptake rates observed among the control and M2 and M3 mesocosms were due largely to differences in biomass within the mesocosms. Within a given mesocosm, the changes in specific uptake rates over time indicated that the cells

were changing physiologically in response to changes in nutrients. The magnitude of specific uptake rates were similar in the >0.8 µm and 0.2 to 0.8 µm fractions, in contrast to the large differences observed in the absolute uptake rates between the 2 size fractions. Specific NO_3^- uptake rates in the >0.8 µm size class increased initially, peaking on Day 12 in the control and on Days 18 and 21 in M2 and M3, respectively (Fig. 6a). In the 0.2 to 0.8 µm fraction, specific NO_3^- uptake was highest during the flagellate and diatom bloom in all 3 mesocosms (Fig. 6e). In the >0.8 µm and 0.2 to 0.8 µm fractions, specific uptake rates of NH_4^+

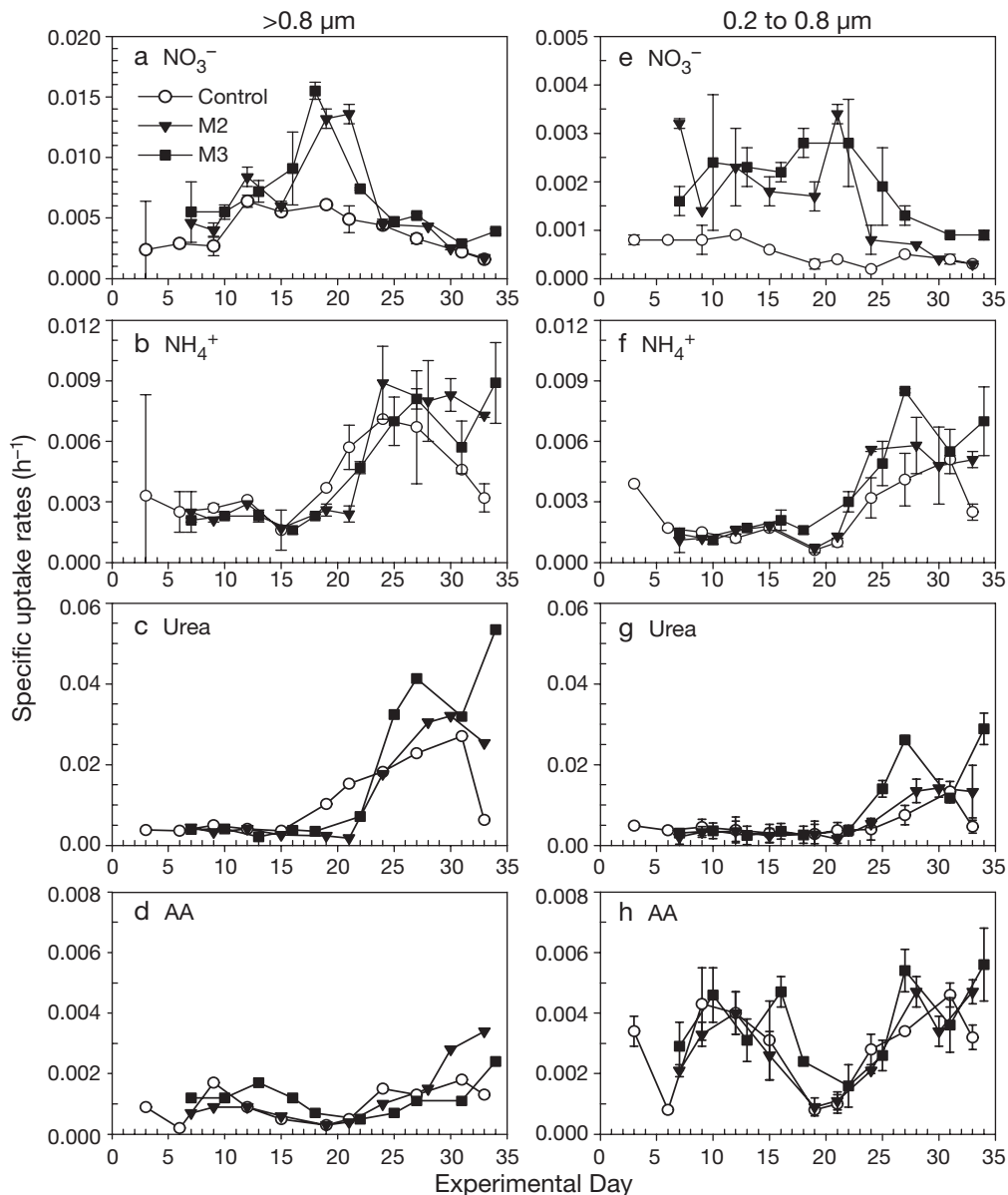Fig. 6. Specific uptake rates (h^{-1}) for (a–d) the >0.8 µm and (e–h) the 0.2 to 0.8 µm size classes for (a,e) NO_3^- (note the difference in scale used for the 2 size classes), (b,f) NH_4^+, (c,g) urea and (d,h) AA. Error bars represent ±SD; when they do not appear they are smaller than the symbols (except in panels c and d where there are no error bars as noted in 'Materials and methods'). Note the differences in scale on the y-axes

were low initially, but increased substantially in all mesocosms (Fig. 6b,f). Regeneration rates of NH_4^+ were relatively low and of similar magnitude during the first half of the experiment in all 3 mesocosms (Fig. 7). Rates of NH_4^+ regeneration increased during the increase in *Phaeocystis pouchetii* and were extremely variable during the final third of the experiment. The high variability in regeneration rates is likely due to patchiness in grazers. Though we normally screen out large grazers (i.e. >100 to 200 µm) which can have a large effect on a given incubation, we were not able to do so in the present study due to the large size of the *P. pouchetii* colonies.

In the case of specific urea uptake, rates were low during the flagellate and diatom bloom but increased dramatically during the *Phaeocystis pouchetii* bloom in both size fractions (Fig. 6c,g). Although rates of specific urea uptake were highest in M2 and M3, they also increased in the control before declining in the final days of the experiment. Rates of specific AA uptake in both size fractions peaked during the flagellate and diatom bloom, similar to NO_3^- uptake, but then increased again during the *Phaeocystis* bloom (Fig. 6d,h). Specific AA uptake rates were also unique in that rates were higher in the 0.2 to 0.8 µm fraction relative to the >0.8 µm fraction.

The >0.8 µm size fraction relied mostly on inorganic N during the diatom and flagellate bloom in all 3 mesocosms, with NO_3^- averaging 36% of uptake in the control and 50% in M2 and M3 (Fig. 8a–c). During this same time period, the 0.2 to 0.8 µm size fraction relied mostly on organic N, with urea averaging 45% of uptake in the control and 29 and 32% in M2 and M3, respectively (Fig. 8d–f). AA uptake was also high during the diatom and flagellate bloom in the bacterial
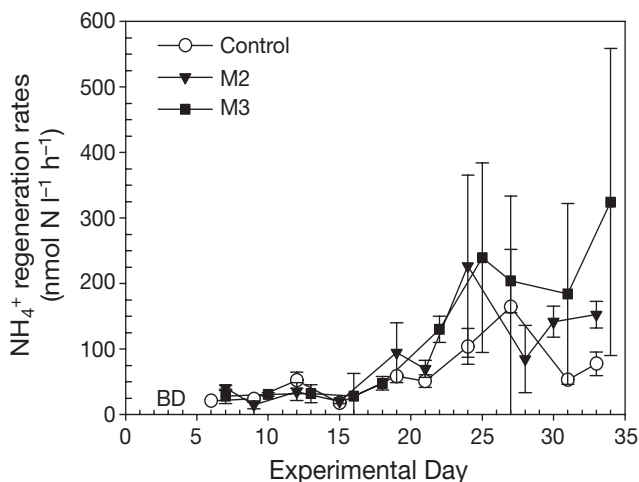

Fig. 7. Ammonium regeneration rates versus experimental day for the control and the 2 nutrient-amended mesocosms, M2 and M3. Error bars represent SD; when they do not appear they are smaller than the symbols

size fraction, averaging 27% of uptake in the control and M2 and 31% in M3. During the *Phaeocystis pouchetii* bloom, the >0.8 µm size fraction relied mostly on organic N, particularly urea, which represented an average of 47 to 59% of uptake in all 3 mesocosms. The bacterial size fraction continued to rely on organic N during the *P. pouchetii* bloom with urea representing an average of 46 to 54% of uptake in all 3 mesocosms. Ammonium uptake represented 21 to 26% of uptake in this size fraction during the *P. pouchetii* bloom, which was slightly more than AA uptake (14 to 20%).

DISCUSSION

As observed in similar mesocosm experiments, the addition of NO_3^- and PO_4^{3-} ultimately resulted in a large phytoplankton bloom dominated by the prymnesiophyte, *Phaeocystis* sp. (e.g. Fernández et al. 1992, Egge & Heimdal 1994). In the present mesocosm study, a small bloom of flagellates and diatoms developed during the first 15 d before *Phaeocystis pouchetii* colonies started to accumulate and any major differences between amended mesocosms and the control were apparent. Our results are similar to naturally occurring *P. pouchetii* blooms, where spring blooms of colonial *P. pouchetii* form yearly in many northern latitudes (Barrents Sea, fjords of Norway, North Sea; Heimdal 1974, Erga & Heimdal 1984, Wassmann et al. 2005). Typically, *P. pouchetii* blooms follow a diatom bloom that forms in nutrient-rich waters. It is hypothesized that the input of nutrients via winter mixing or river runoff (or the addition of NO_3^- and PO_4^{3-} in a mesocosm study) fuels a bloom of diatoms, which are known to rapidly take advantage of new inputs of nutrients (especially NO_3^-) in the presence of H_2SiO_3. As the H_2SiO_3 is depleted and PO_4^{3-} is drawn down, *P. pouchetii* are poised to start blooming. Blooms of phytoplankton supported by inorganic nutrients followed by blooms of an HAB species that is poorly grazed and supported by regenerated organic nutrients are seen in many HAB studies (e.g. Laroche et al. 1997, Sunda et al. 2006 and references therein). Since *P. pouchetii* are also considered detrimental to ecosystems (Schoemann et al. 2005, Verity et al. 2007a) they can be considered HABs or ecosystem disrupting algal blooms (EDABs, Sunda et al. 2006). Many HAB species occur in nutrient-enriched areas after a previous bloom of a non-HAB species has utilized inorganic nutrients and started to decline. The prebloom provides recycled nutrients that are often in forms not immediately available to HAB species and therefore cause a lag between the introduction of nutrients and an HAB event; this was seen in the present mesocosm study,
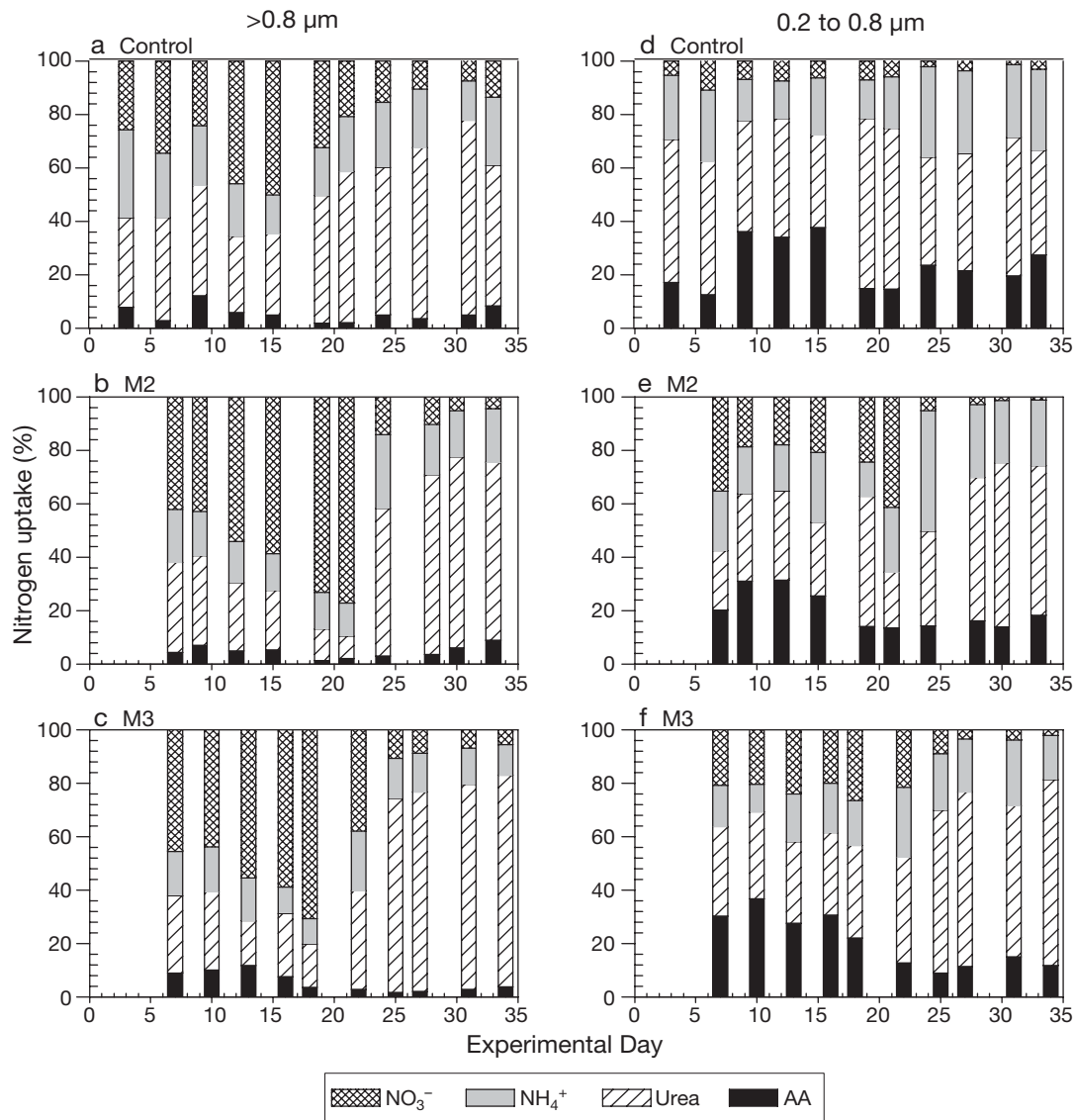
Fig. 8. Percent of nitrogen uptake for (a–c) the >0.8 µm and (d–f) the 0.2 to 0.8 µm size fractions due to NO_3^-, NH_4^+, urea and AA over the course of the experiment for (a,d) control, (b,e) M2 and (c,f) M3 mesocosms

with a few days lag time before the *P. pouchetii* bloom, as well as in studies of *Aureococcus* blooms (Laroche et al. 1997).

Studies of N uptake dynamics during the onset of a *Phaeocystis pouchetii* bloom are sparse, especially those that include both inorganic and organic N forms (NO_3^- and NH_4^+, Muggli & Smith 1993; NO_3^-, NH_4^+ and urea, Tungaraza et al. 2003) and those that include competition between phytoplankton and bacteria for these nutrients (Lancelot & Billen 1984, Lancelot & Mathot 1987). Below we highlight the development and decline of the flagellate and diatom and *Phaeocystis pouchetii* blooms with respect to critical nutrient pools and competition between phytoplankton and bacteria.

Flagellate and diatom bloom

During the first few days of the experiment all 3 enclosures behaved similarly. Particulate N and chl *a* concentrations increased at similar rates, due primarily to an increase in diatoms and small phototrophic algae other than *Phaeocystis pouchetii*. During the flagellate and diatom bloom, *P. pouchetii* was present only as solitary cells; the number of colonial forms began to increase around Day 12 in M2 and M3, but always remained low in the control (Nejstgaard et al. 2006). At first, the PN pool included some detrital N, as ratios of PN:chl *a* were initially high; however, as the blooms progressed the PN pool was increasingly dominated by

healthy growing phytoplankton populations, demonstrated by the PN:chl *a* ratio approaching 1:1 (McCarthy et al. 1977).

Levels of NO_3^- and PO_4^{3-} were initially high enough for *Phaeocystis pouchetii* blooms to occur in the present study; however, they were likely out-competed by small phototrophic algae and by diatoms during the first 15 d. Small phototrophic flagellates started to increase in numbers first, but as diatoms started to bloom, the flagellate bloom decreased (Fig. 2). Diatoms, however, were only able to out-compete *P. pouchetii* when there was sufficient H_2SiO_3 present to support their growth (Fig. 4). Egge & Aksnes (1992) observed diatom blooms in mesocosm studies when H_2SiO_3 concentrations were >2 µmol Si l^{-1}, suggesting that a minimum concentration of 2 µmol Si l^{-1} is required before H_2SiO_3 is utilized. Paasche (1973) found that H_2SiO_3 uptake does not necessarily follow typical Michaelis-Menten kinetics and argued that a threshold concentration of 0.3 to 1.3 µmol Si l^{-1} is required for H_2SiO_3 uptake. In the present study H_2SiO_3 concentrations started out above this threshold, but were drawn down to below 1.2 µmol Si l^{-1} between Days 7 and 9, and the concentration was well below 0.5 µmol Si l^{-1} after Day 9. This utilization of H_2SiO_3 corresponded with an increase in the number of diatoms in each mesocosm; as the H_2SiO_3 was depleted, the small bloom of diatoms dissipated.

As the flagellate and diatom bloom developed, the concentration of TDN decreased to ca. 5 µmol N l^{-1}, due to the near depletion of NO_3^- and NO_2^-, as well as the drawn-down NH_4^+. Concentrations of DON were very low at the start of the experiment and comparable to levels found in the open ocean. There was little change in the organic N forms during the formation of the flagellate and diatom bloom, suggesting that regeneration processes were adequate to balance the measured uptake of urea and AA (Fig. 5). Data suggest that the rise of the diatom bloom in all 3 mesocosms was most likely due to diatoms out-competing the small phototrophic algae for available inorganic N sources and that the decline was due to the depletion of H_2SiO_3. An alternative hypothesis is that a rapid response in grazing pressure from zooplankton contributed to the decline of the flagellate and diatom bloom. However, the biomass of both micrograzers (Fig. 2) and copepods was very low compared to the total phytoplankton during the flagellate and diatom bloom in all mesocosms, as well as during the *Phaeocystis pouchetii* bloom in M2 and M3 (Nejstgaard et al. 2006). Thus, it is unlikely that grazers could significantly affect the development of the major phytoplankton groups. At this stage the mesocosms appeared to be mimicking an area dominated by new production that was uncoupled with respect to phytoplankton and their grazers.

To directly study the affect of grazing during our 34 d experiment, mesozooplankton and microzooplankton experiments were conducted (J. C. Nejstgaard et al. unpubl.) with water from M2 during 3 periods of 24 h (March 12-13, 15-16 and 24-25) using a standard bottle incubation dilution technique as described in Nejstgaard et al. (2001b). The dilution studies clearly show that the microzooplankton community did not have any statistically significant grazing impact on the smaller-sized phytoplankton (caught on 0.45 to 8 µm filters) during any of the 24 h periods. For the >8 µm phytoplankton community, grazing was also negligible during the first 2 periods (grazing removed only 11 to 18 % of the phytoplankton standing stock per day). In contrast, during the third period (March 24-25) the microzooplankton community removed ca. 63 % of the standing stock per day and thus may have contributed to the decline of the larger phytoplankton bloom only at that stage. In general, however, as expected from the low microzooplankton abundances, the grazing impact by the microzooplankton on the phytoplankton appeared to be minor (J. C. Nejstgaard et al. 2006). Further, preliminary experimental data (Nejstgaard et al. unpubl.) also suggest that direct grazing by the mesozooplankton had only a minor effect on the phytoplankton directly. The mesozooplankton, however, may have kept the microzooplankton at a relatively low abundance, as seen in previous mesocosm experiments at this site (e.g. Nejstgaard et al. 2001a) and so affected the phytoplankton community indirectly.

We observed no major difference between the control and the nutrient-amended mesocosms in either absolute or specific uptake rates for NH_4^+, urea and AA in either size class during the flagellate and diatom bloom. During the flagellate and diatom bloom, the largest differences between mesocosms were observed for NO_3^- uptake (both absolute and specific) in the 0.2 to 0.8 µm size classes, where the NO_3^- and PO_4^{3-}-amended mesocosms had rates 2 to 3 times higher than in the control. Several investigators have reported high NO_3^- utilization rates by bacteria (Horrigan et al. 1988, in cultures; Kirchman et al. 1991, in the North Atlantic). Recent work in the Barents Sea also demonstrated high rates of NO_3^- utilization by the bacterial size class (Allen et al. 2002). Allen et al. (2002) suggest that the high rates observed are a function of high ambient NO_3^- concentrations, as in the case of our nutrient-amended mesocosms. Although bacterial abundance and production increased during the flagellate and diatom bloom period, the increase was observed in all 3 mesocosms. This suggests that the higher rates of NO_3^- uptake observed in the 2 nutrient-amended mesocosms for the bacterial fraction were not due

solely to the elevated NO_3^- concentrations (M. E. Frischer et al. unpubl. data). Although outside the scope of the present study, the composition of bacteria communities in each mesocosm was investigated by DGGE analysis of amplified 16S rDNA genes to assess whether nutrient additions resulted in the development of distinct bacterial communities. The results of these investigations suggest that bacterial communities were similar in the fertilized and unfertilized mesocosms, and so it seems unlikely that differences in bacterial community composition would explain higher NO_3^- uptake rates in the fertilized treatments (M. E. Frischer et al. unpubl.).

During the flagellate and diatom bloom, the >0.8 µm size fraction in the control mesocosm utilized both new (NO_3^-) and regenerated (NH_4^+ and urea) forms of N (Fig. 8a). The 0.2 to 0.8 µm size class in the control treatment, however, relied primarily on regenerated forms of N, with urea uptake rates being the highest (Figs. 5 & 8d). The nutrient-amended mesocosms (M2 and M3) were characteristic of an area dominated by new production, relying primarily on NO_3^- in the >0.8 µm fraction (Fig. 8b,c).

Bacterial N demand (BND, %) was estimated based on bacterial productivity using a stoichiometric conversion factor of 5.2 (C:N) determined for exponentially growing bacterioplankton in Raunefjord (Vrede et al. 2002). Bacterial production in terms of N was then compared to absolute uptake rates of the 0.2 to 0.8 µm fraction to determine BND. We found that NH_4^+, NO_3^-, urea and AA could account for 33 to 96% of BND during the flagellate and diatom bloom (Fig. 9). The organic forms accounted for the majority of the BND (urea 10 to 44%, AA 9 to 29%), as was also demonstrated by N uptake in the 0.2 to 0.8 µm size class. Some studies of BND have shown that AA and NH_4^+ are the major sources of bacterial N (Fuhrman 1990, Keil & Kirchman 1991); however, several more recent studies have demonstrated that urea can be a major source of N to bacteria (Middelboe et al. 1998, Jørgensen et al. 1999, Jørgensen 2006), as we have observed in the present study. These data further suggest that additional N (NO_2^- and/or other organics) were likely used by the bacterial fraction during the experiment.

Phaeocystis pouchetii bloom

The flagellate and diatom bloom was followed by a large increase in the abundance of *Phaeocystis pouchetii* colonies present in the mesocosms. It has been hypothesized that the transition from solitary to colonial forms may provide a refuge for *P. pouchetii* from predation (Fernández et al. 1992) and/or from
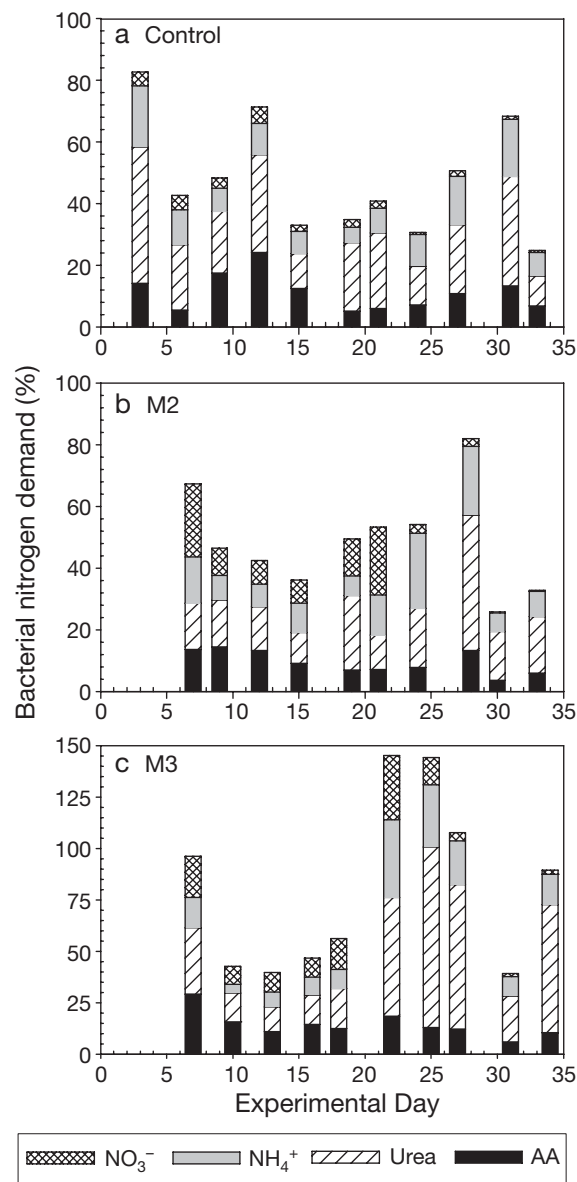

Fig. 9. Percent of bacterial nitrogen demand (0.2 to 0.8 µm size fraction) due to NO_3^-, NH_4^+, urea and AA over the course of the experiment for (a) control, (b) M2 and (c) M3 mesocosms

viral infection, as solitary forms are susceptible to viral attack while colonial forms are not (e.g. Bratbak et al. 1998, Jacobsen et al. 2007). It is also hypothesized that colony formation may assist in buoyancy control, keeping cells in the upper layers of the euphotic zone (Skreslet 1988). During the colonial period of the bloom, large differences between the control and nutrient-amended mesocosms became apparent. The bloom of *P. pouchetii* was only observed in the 2 mesocosms amended with NO_3^- and PO_4^{3-}. Concentrations of chl *a* reached a maximum on

Day 25 and declined more rapidly in M2 than in M3, most likely because nutrient amendment of M2 was stopped on Day 19. Concentrations of PN reached a maximum at the same time as chl *a* concentrations peaked. The concentration of PN is a measurement of all PN including decaying phytoplankton and detritus. As a result, PN remained elevated throughout the decline of the *P. pouchetii* bloom, while PN:chl *a*, which had been close to 1:1 during the peak of the *P. pouchetii* bloom, increased as detrital N accumulated in the PN pool.

During the *Phaeocystis pouchetii* bloom (Days 18 to 34), concentrations of DON increased, likely due to the decline of the flagellate and diatom bloom. The decline of phytoplankton blooms is often a time of enhanced organic matter release due to processes such as physiological stress or high grazing pressure (e.g. Carlson 2002). Increases in organic carbon release have been observed with blooms of *P. pouchetii* (e.g. Lancelot 1983); therefore, increases in DON may also be associated with these blooms. Heterotrophic flagellates and ciliates also increased in all 3 mesocosms during the *P. pouchetii* bloom. Their increase, as well as a lack of inorganic nutrients, appears to have been sufficient to keep any large phytoplankton blooms from forming in the control mesocosm and suggests a tightly coupled system between phytoplankton and grazers. In the nutrient-amended mesocosms, however, the rapid growth of *P. pouchetii* (especially colonies, Nejstgaard et al. 2006, Fig. 6) and the slower growth of grazers allowed the system to become uncoupled. A number of factors likely contributed to the initiation of the *P. pouchetii* bloom including H_2SiO_3 depletion, high NO_3^- concentrations and reduced PO_4^{3-} availability. H_2SiO_3 was depleted by Day 9, at which time diatom numbers declined, reducing competition with *P. pouchetii*. The decline in H_2SiO_3 and resulting decrease in diatom numbers is unlikely to have triggered the *P. pouchetii* bloom, however, because *P. pouchetii* did not begin to increase in numbers until Day 16. Nitrogen availability is also unlikely as the key to initiation because NO_3^- was present at high concentrations (>15 µmol l^{-1}) during the time prior to the increase in *P. pouchetii*. Early in the experiment, PO_4^{3-} concentrations were also high. Veldhuis & Admiraal (1987) found that *P. pouchetii* colonies bloom in culture only when PO_4^{3-} concentrations are below 1 µmol l^{-1}. This is consistent with our observations: phosphorus fell to below 1 µmol l^{-1} on Day 16, the day on which *P. pouchetii* colonies first appeared, and thereafter *P. pouchetii* numbers increased dramatically.

Initial concentrations of urea during the present study were somewhat high (1.2 µmol N l^{-1}) for coastal areas; however, Tungaraza et al. (2003) have reported even higher urea concentrations of up to 6 µmol N l^{-1} in a coastal area of the North Sea, although their site is subject to anthropogenic discharges. Large increases in urea concentration during the present study were observed in the nutrient-amended mesocosms where *Phaeocystis pouchetii* bloomed and not in the control mesocosm, suggesting that urea was coming from regeneration processes and not anthropogenic sources when water was added to the mesocosms. Similarly, regeneration rates of NH_4^+ were roughly 2 times higher in the nutrient-amended mesocosms than in the control. This observation, along with uptake rate data, suggests that the nutrient-amended mesocosms were relying on regenerated forms of N during the *P. pouchetii* bloom (Fig. 8b,c,e,f). A reliance on organics was also seen in the 0.2 to 0.8 µm size class, for which BND was met mostly by urea (11 to 44% in M2, 22 to 88% in M3), followed by NH_4^+ and AA, with NO_3^- contributing the least. These 4 N substrates could account for 26 to 145% of the total BND during the *P. pouchetii* bloom. Jørgensen (2006) reported similar observations associated with a spring bloom in Roskilde Fjord (Denmark) and in bacterial culture studies of isolates from Knebel Vig and Limfjorden (Denmark), where urea accounted for >30% of the BND, which exceeded the BND met by AA. In the present study, urea met the majority of the BND during both the flagellate and diatom and *Phaeocystis* blooms in all mesocosms, once again suggesting that urea, when available, can be a major source of N to the bacterial community.

During the *Phaeocystis pouchetii* bloom, the DON pool comprised largely labile urea and AA (>93%), and organic forms of N were highly utilized. The crash of the *P. pouchetii* blooms was likely due to PO_4^{3-} depletion and perhaps a moderate increase in grazing pressure from the microzooplankton. Supporting the idea of P limitation, Riegman et al. (1992) found that *Phaeocystis* spp. are poor competitors under low P conditions. Viral abundance in the present study increased in all 3 mesocosms, with slightly higher numbers in the 2 nutrient-amended mesocosms, suggesting that viral infection is another factor that may have contributed to the decline of the *P. pouchetii* bloom (Ruardij et al. 2005, Jacobsen et al. 2007).

CONCLUSIONS

In summary, addition of NO_3^- and PO_4^{3-} to our mesocosms eventually resulted in a large bloom of *Phaeocystis pouchetii*. Urea was a highly utilized form of N in both the phytoplankton and bacterial size classes. Overall, in the bacterial size class, organic N (urea and AA) accounted for the largest percentages of total uptake in all 3 mesocosms. In contrast to urea, AA did not appear to be a major source of N for the >0.8 µm

size class. In the bacterial size class, however, specific uptake rates of AA were often equal to or greater than those measured for NH_4^+, suggesting that organic N, especially urea, may be playing a more important role in the nitrogenous nutrition of both phytoplankton and bacteria in these high-latitude waters than previously thought. As hypothesized, inorganic N was relatively more important early in the experiment when the mesocosms were representative of an area dominated by new production. Also as hypothesized, we observed an increase in bacterial utilization of N, especially organic N, towards the end of the experiment. The succession we observed was from a system dominated by the classic food web, with high amounts of new production, to a system dominated by the microbial loop, with high amounts of regenerated production.

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