

Effects of grazer and substrate amendments on nutrient and plankton dynamics in estuarine enclosures

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ABSTRACT: We compared the effects of substrate C:N ratio and macrozooplankton activity on nutrient and chlorophyll dynamics by amending the substrate C:N ratios in carboys containing natural estuarine microplankton (<200 μm) with additions of glucose (High C:N), arginine (Low C:N), or nothing (Control). Adult copepods (*Acartia tonsa*, 10 ind. l^{-1}) were added to 1 carboy of each substrate treatment. Water and copepods were collected from the Choptank River, a subestuary of the Chesapeake Bay (USA) in August 1993. Ambient concentrations of NH_4^+ , NO_3^- , and o-PO_4^{3-} (orthophosphate) and dissolved primary amines (DPA) were all <2.0 $\mu\text{g-at. l}^{-1}$. Dissolved and particulate nutrients and pigments were monitored over a 2 d period. In all carboys, plankton shifted from being net consumers of nutrients during the first 17 to 23 h (Phase 1) to being net regenerators afterwards (Phase 2). Chlorophyll concentrations declined and phaeopigments increased throughout the experiment. Both substrates stimulated microbial activity, as indicated by decreased accumulation of o-PO_4^{3-} during Phase 2, increased accumulation of particulate carbon (PC) and nitrogen (PN) and increased chlorophyll loss during nighttime in the High C:N and Low C:N carboys relative to the Control carboy. In addition, the low C:N substrate resulted in increased accumulations of NH_4^+ , NO_2^- and NO_3^- ; increased chlorophyll concentration; and a day/night pattern in chlorophyll concentration. Copepod additions resulted in greater PC, PN and chlorophyll losses and day/night patterns in chlorophyll concentration. The additions of copepods and substrates together resulted in several interactive effects, most notably, increased accumulations of NH_4^+ , o-PO_4^{3-} , and, in the High C:N treatment, $\text{NO}_2^- + \text{NO}_3^-$, and greater chlorophyll, PC, and PN losses. Estimated rates of ingestion and excretion by the added copepods could not account for the observed changes in chlorophyll and nutrients, especially in the carboys with copepod and substrate additions, suggesting that the copepods increased nutrient regeneration and phytoplankton removal by microzooplankton.

KEY WORDS: Microbial loop · Substrate C:N ratios · Nutrient regeneration · *Acartia tonsa* · Chlorophyll dynamics

INTRODUCTION

Grazing and nutrient availability are intimately linked in sustaining planktonic productivity when nutrient concentrations are low. Mesozooplankton (200 to 2000 μm) usually excrete a relatively minor proportion of the regenerated nutrients (e.g. Bidigare 1983). Despite this, mesozooplankton and larger animals can

have important direct and indirect impacts on the species composition and metabolism of smaller organisms (Carpenter et al. 1987, Roman et al. 1988, Elser 1992, Glibert et al. 1992, Christaki & Van Wambeke 1995). In particular, copepods, presumably through differential grazing or sloppy feeding, can greatly influence the degree to which bacterioplankton and microzooplankton regenerate NH_4^+ (Roman et al. 1988, Glibert et al. 1992, Miller et al. 1995).

Bacterioplankton and microzooplankton (<200 μm) usually play the quantitatively most important roles in

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regenerating NH_4^+ (Caron & Goldman 1990). The rate at which bacteria and microzooplankton supply NH_4^+ for phytoplankton, however, is itself subject to substrate limitation. Bacteria assimilate labile inorganic and organic dissolved substrates for growth and metabolism. The types and C:N ratio of substrates available to bacterioplankton can control both the timing and magnitude of NH_4^+ regeneration by this group of organisms (Goldman et al. 1987, Goldman & Dennett 1991, Jørgensen et al. 1994). As mentioned above, the rates of nutrient regeneration by bacterioplankton and microzooplankton are also apparently affected by trophic interactions.

Experimentally, the substrate control of phytoplankton is usually studied by adding large amounts of nutrients (e.g. Roman et al. 1988, Paul et al. 1995). When regenerated nutrients sustain productivity, however, nutrient availability to phytoplankton will usually be dominated by the factors which control nutrient regeneration by microheterotrophs, rather than large pulses of nutrients. Factors affecting nutrient regeneration include the quality of dissolved inorganic and organic substrates available to bacteria and the interactions between the microplankton and mesozooplankton. Thus, while there are several studies of plankton responses to nutrients or grazers that directly control phytoplankton biomass or growth, we are just beginning to study the more subtle aspects of how plankton assemblages respond to factors that control microheterotrophic regeneration of inorganic nutrients (e.g. Carlsson et al. 1995).

Temperate estuaries, such as the Chesapeake Bay (USA) and its tributaries, support seasonally high productivity at several trophic levels. Typically, both bacteria- and phytoplankton-based food webs are well developed. High phytoplankton biomass in the springtime is supported by high concentrations of riverborne nutrients. In the late summer, however, external inputs of inorganic nutrients are low and planktonic productivity depends on *in situ* regeneration of nutrients, nitrogen in particular, by both benthic and pelagic heterotrophs (Malone et al. 1988, 1991, Glibert et al. 1995). Summertime productivity in the Chesapeake Bay is intensely grazed by microzooplankton (White & Roman 1992) which can in turn be consumed by mesozooplankton, such as the dominant copepod *Acartia tonsa* (Dolan 1991, White & Roman 1992). This intense grazing fuels the supply of nutrients via regeneration.

Here we investigated the effects of mesozooplankton and of the quality of dissolved substrates, alone and together, on the short-term nutrient and chlorophyll dynamics in a microplankton assemblage from a Chesapeake Bay tributary. We altered the abundance of the copepod, *Acartia tonsa*, predicting that the added copepods would result in increased NH_4^+ con-

centrations and decreased phytoplankton biomass. Simultaneously, we altered the ambient C:N ratios of dissolved substrates. We predicted that an organic substrate which was nitrogen-rich (substrate C:N ratio ≤ 4) would result in increased concentrations of inorganic nitrogen (NH_4^+ , NO_3^- , and NO_2^-) and increased phytoplankton biomass. Alternately, we hypothesized that a nitrogen-poor (substrate C:N ratio ≥ 10) organic substrate would not result in increased nutrient concentrations or chlorophyll biomass. Finally, while we expected the copepods and substrates to impact nutrient concentrations, we were uncertain of the net effect they would have in combination.

METHODS

Water from the Choptank River, a subestuary of the Chesapeake Bay, was filtered through a 200 μm sieve as it was dispensed into six 20 l polyethylene Cubitainers[®] on September 2, 1993. The carboys were filled consecutively. The salinity was ca 8 ppt and the surface temperature was ca 28°C. The carboys were kept in a constant-temperature room at 28°C with the lights turned on at 06:30 h and off at 20:00 h. Indirect red lighting was used during nighttime sampling. Carboys were gently aerated through 1 ml disposable glass pipettes. Copepods used in the experiment were collected from the Choptank River on August 30, 1993, with a 200 μm mesh net which had a 0.5 m diameter and a solid cod-end. Adult and C-5 *Acartia tonsa* were isolated, and maintained in an aerated, 8 l carboy of 10 ppt Instant Ocean[®] in the constant-temperature room. Natural 10 to 64 μm particles sieved from the Choptank River water and cultured ciliates (*Uronema marinum*) were used as food for the copepods.

The carboys received 1 of 2 copepod treatments: with (+Copepods, target addition of 10 copepods l^{-1}) or without copepod additions. Carboys also received 1 of 3 substrate treatments: no addition (Control), an organic C-only addition (High C:N, target labile substrate C:N ≥ 10 via glucose addition), or a low substrate C:N addition (Low C:N, target labile substrate C:N ≤ 4 via arginine addition). Immediately prior to the first sampling time, the appropriate number of copepods were rinsed with filtered seawater transfers, transferred to carboy water and divided into 3 aliquots, which were then added to the appropriate carboys. Finally, glucose and arginine were added to the carboys as necessary to achieve the target substrate C:N ratios (Table 1).

Over the next 45 h, we sampled each carboy 7 times for concentrations of dissolved and particulate materials (Table 2). At each sampling time, carboys were mixed by gentle inversion and 2 l of water was re-

Table 1. Target and final substrate C:N ratios, concentrations of ambient substrates, and substrate additions (in $\mu\text{g-at. C or N l}^{-1}$) in experimental carboys

	Substrate treatment		
	Control	High C:N	Low C:N
Target labile C:N	–	≥ 10	≤ 4
Ambient estimated labile DOC ^a	50	50	50
Ambient labile N ^b	0.6	0.6	0.6
DOC added	0	6 ^c	30 ^d
DON added	0	0	20 ^d
Final estimated labile C:N	83.3	93.3	3.9

^aAssumed ambient DOC 300 $\mu\text{g-at. C l}^{-1}$, 10% considered labile (F. Shiah pers. comm.). To insure that additions reached target ratios, we conservatively estimated labile DOC concentrations as 0 and 50 $\mu\text{g-at. C l}^{-1}$ for High C:N and Low C:N treatments, respectively

^bAs ambient NH_4^+ , measured ca 2 h before experiment. We assumed only NH_4^+ -N might be available to bacteria in significant amounts; DPA concentrations are usually ca 0.5 $\mu\text{g-at. N l}^{-1}$. See text for discussion of other forms of ambient DON

^cAs glucose

^dAs arginine

moved. This mixing and the aeration prevented oxygen depletion and a disproportionate accumulation of detritus over the course of the experiment, as was confirmed by PC:PN (particulate carbon:particulate nitrogen) ratios near Redfield values at the end of the experiment. The water was sampled for concentrations of dissolved and particulate material (Table 2). Samples from carboys containing copepods were pre-filtered through a 183 μm mesh sieve. Particulate material was collected on combusted Whatman GF/F glass fiber filters and the filtrates were used for nutrient and total dissolved nitrogen (TDN) analyses. Samples for dissolved primary amines (DPA) were filtered through 0.22 μm pore sterilized Acrodisc[®] filters. All samples were frozen for later analysis. Here we discuss the treatment effects which were larger than the analytical errors of the measurements (Table 1).

RESULTS

Nutrient dynamics

The first 17 to 24 h in all the carboys was generally a period of net nutrient consumption, while the remainder of the experiment was to a greater extent a period of nutrient regeneration. For convenience, we refer to these 2 periods as Phase 1 and Phase 2, respectively. The timing and magnitude of these changes in individual carboys, though, depended upon the substrate and copepod additions and the interaction of the two.

Table 2. Parameters measured and methods used for measurements. Analytical variability, as 95% C.I.'s for replicate samples at representative concentrations (see figures); inorganic nutrients, $\leq 2\%$; DPA, 2 to 6%; pigments, 4%; PC and PN, 10%

Parameter	Technique
NH_4^+	Manual colorimetry (Parsons et al. 1984)
NO_2^-	Manual colorimetry (Parsons et al. 1984)
NO_3^-	Automated colorimetry for $\text{NO}_3^- + \text{NO}_2^-$, corrected for NO_2^- (Parsons et al. 1984)
o-PO_4^{3-}	Automated colorimetry (Parsons et al. 1984)
DPA ^d	Manual fluorometry, glycine standard (Parsons et al. 1984)
Chlorophyll, phaeopigments	Manual fluorometry (Parsons et al. 1984)
Particulate carbon and nitrogen	Automated combustion and gas analysis, Control Equipment 440 CHN Analyzer

^dDissolved primary amines

During Phase 1, in the Control and High C:N carboys, NH_4^+ declined from an initial concentration of 0.4–1.9 $\mu\text{g-at. l}^{-1}$ to about 0.1 $\mu\text{g-at. l}^{-1}$; DPA decreased from about 0.4–0.7 $\mu\text{g-at. l}^{-1}$ to about 0.3 $\mu\text{g-at. l}^{-1}$ (Figs. 1A, B, D, E & 2A, B); and o-PO_4^{3-} declined from about 0.5 $\mu\text{g-at. l}^{-1}$ to a minimum of about 0.4 $\mu\text{g-at. l}^{-1}$ (Fig. 2C). Variability in nutrient concentrations at 0 h

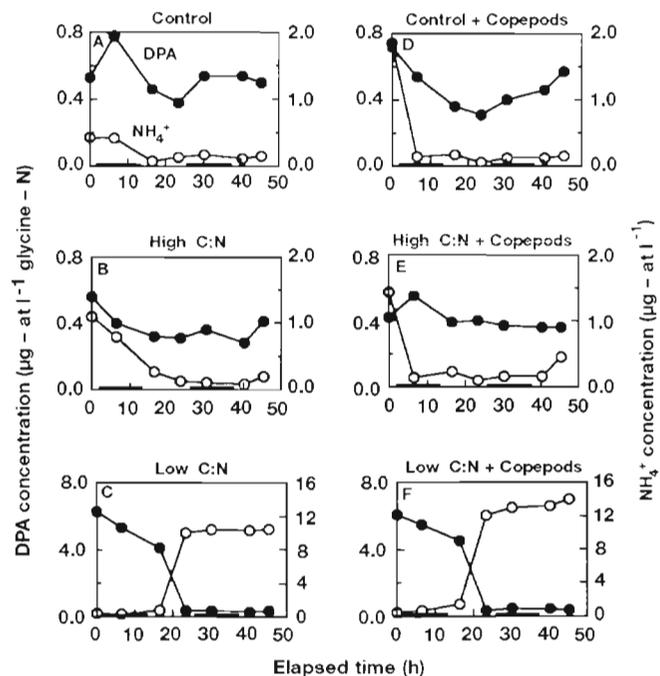


Fig. 1. Concentrations of NH_4^+ and DPA in the carboys during the experiment. Note scale change for Low C:N carboys. Black bars indicate when lights were off

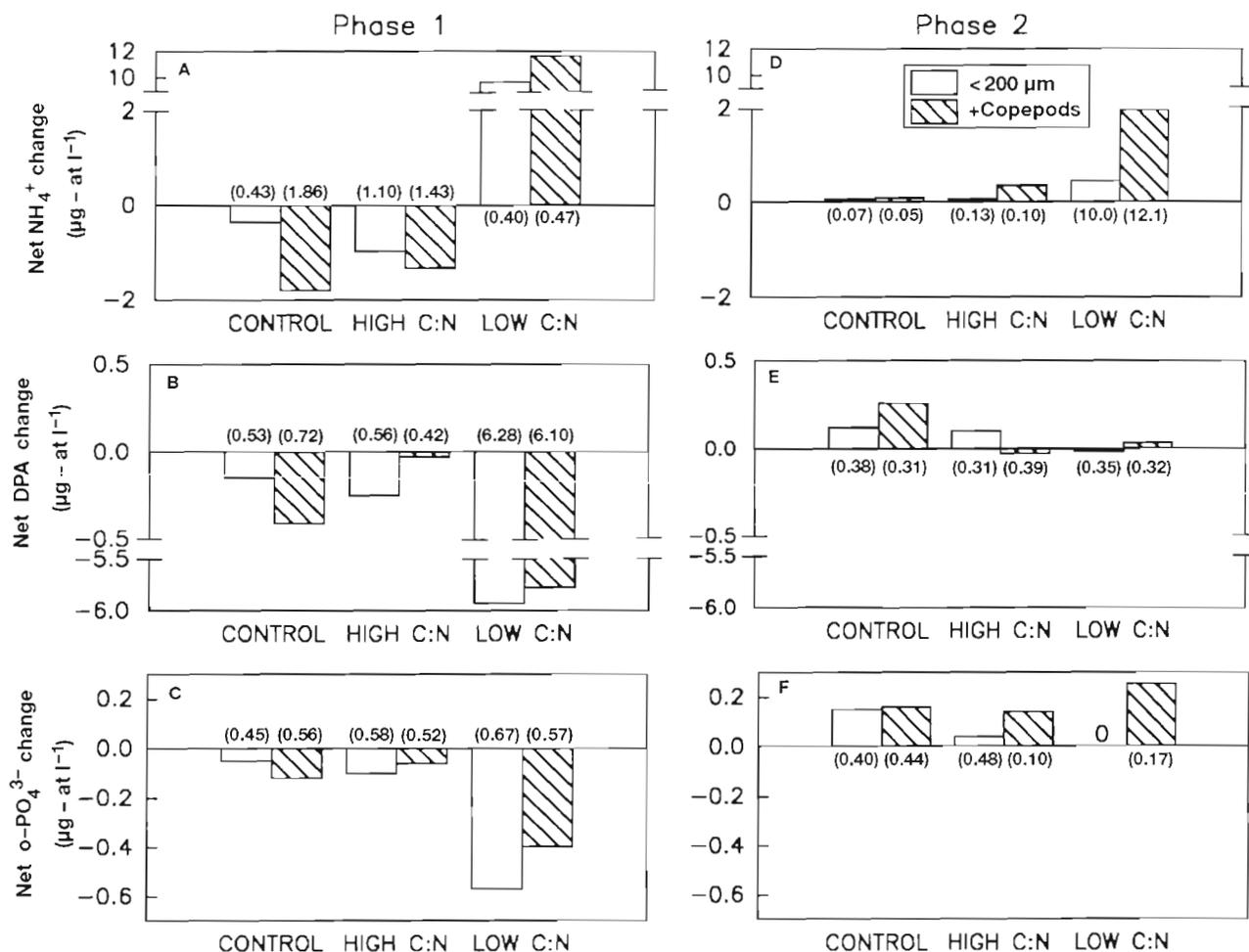


Fig. 2. Net changes in concentrations of NH_4^+ , DPA, and o-PO_4^{3-} during Phase 1 and Phase 2. Phases were separated by the sampling time at which a nutrient reached a minimum (maximum for NH_4^+ in Low C:N treatment) at 18 or 23 h. Numbers in parentheses indicate concentrations at beginning of phase

was likely due to variability in the source water. In the Low C:N carboys, NH_4^+ concentrations increased slightly during the first 17 h as DPA and o-PO_4^{3-} concentrations decreased (Fig. 1C, F). There was then a pronounced transition between 17 and 23 h, when the concentrations of NH_4^+ , DPA, and o-PO_4^{3-} changed most quickly. Concentrations of DPA and o-PO_4^{3-} declined to 0.3 and 0.1 $\mu\text{g-at. l}^{-1}$, respectively, while the concentration of NH_4^+ increased from about 1 to about 11 $\mu\text{g-at. l}^{-1}$ (Figs. 1C, F & 2A, B, C). About 15% more NH_4^+ accumulated in the Low C:N+Copepods carboy than in the Low C:N carboy during this time (Fig. 2A).

The net changes in nutrient concentrations during Phase 2 were particularly sensitive to the substrate and copepod treatments. Often there was a strong interaction between the 2 types of treatments. Net NH_4^+ accumulation after 23 h was highest in the Low C:N+Copepods carboy and decreased in the order Low

C:N+Copepods > Low C:N > High C:N+Copepods carboys (Fig. 2D). The net accumulations of o-PO_4^{3-} and DPA during Phase 2 were influenced in an opposite manner by substrate and copepod additions. In the absence of copepod additions, the net accumulations of both were highest in the Control carboy, followed by the High C:N, then Low C:N carboys (Fig. 2E, F). In the +Copepods carboys, net DPA accumulation was highest in the Control treatment (Fig. 2E). Net o-PO_4^{3-} accumulation in the +Copepods treatment was highest in the Low C:N+Copepods carboy and surpassed that in the carboys without added copepods only in the presence of substrate additions (Fig. 2F).

There was little net change over the 2 d in NO_2^- concentrations in the Control, Control+Copepods, and High C:N carboys (Fig. 3A, C). In contrast, NO_2^- and NO_3^- concentrations increased in the High C:N+Copepods during Phase 2, and in the Low C:N and Low C:N+Copepods carboys throughout the experiment (Fig. 3).

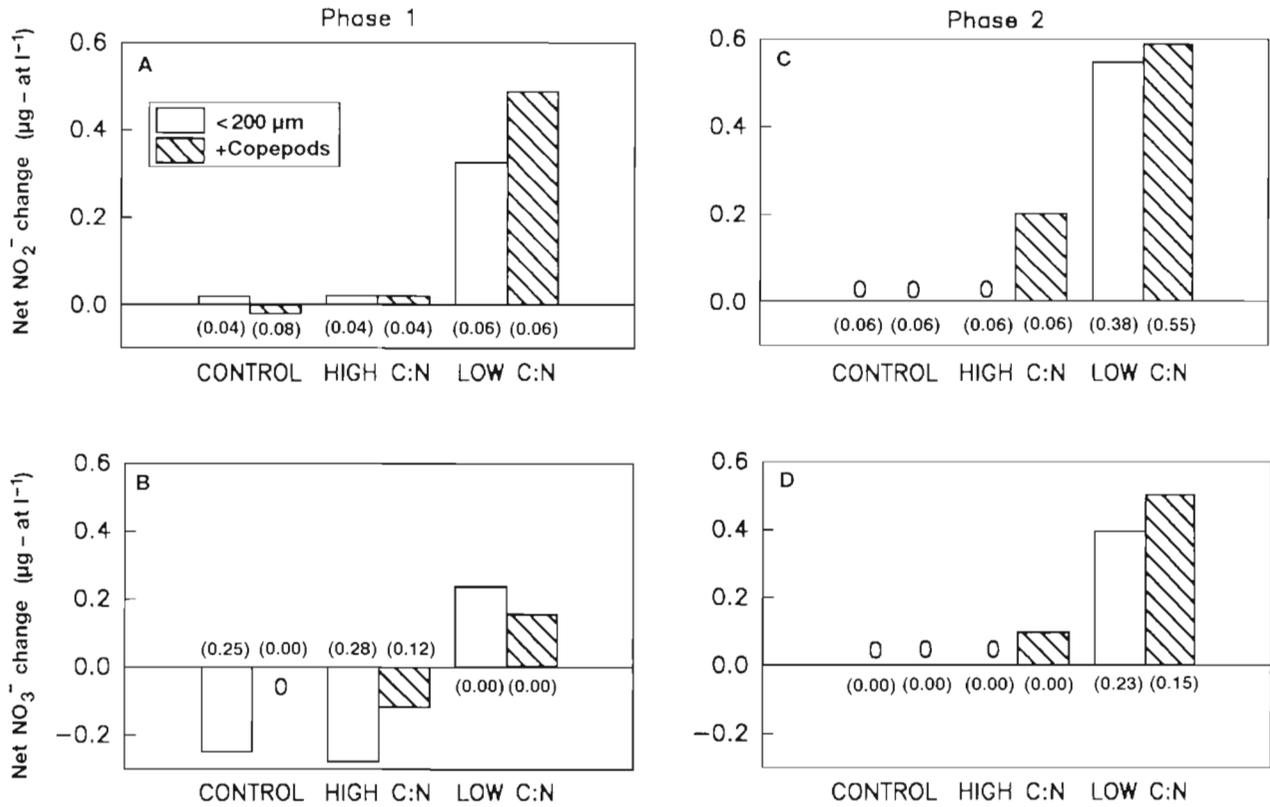


Fig. 3. Net changes in concentrations of NO₂⁻ and NO₃⁻ during Phase 1 and Phase 2. Phases separated at 23 h. Numbers in parentheses indicate concentrations at beginning of phase

The mean DIN (dissolved inorganic nitrogen, NH₄⁺ + NO₂⁻ + NO₃⁻):DIP (dissolved inorganic phosphorus) ratios were about 1 during Phase 1 in all carboys, except in the Low C:N+Copepods carboy, where NH₄⁺ regeneration had already begun and the mean ratio was about 2 (Fig. 4A). During Phase 2 mean ratios decreased to below 1 in the Control, Control+Copepods and High C:N carboys (Fig. 4B)

due to decreased DIN concentrations and increased DIP concentrations. The ratio decreased slightly less in the High C:N+Copepods carboy due to higher NO₂⁻ concentrations in that carboy. The DIN:DIP ratios increased during Phase 2 to about 100 in the Low C:N carboy, and to about 50 in the Low C:N+Copepods carboy, where o-PO₄³⁻ concentrations were higher (Fig. 4B).

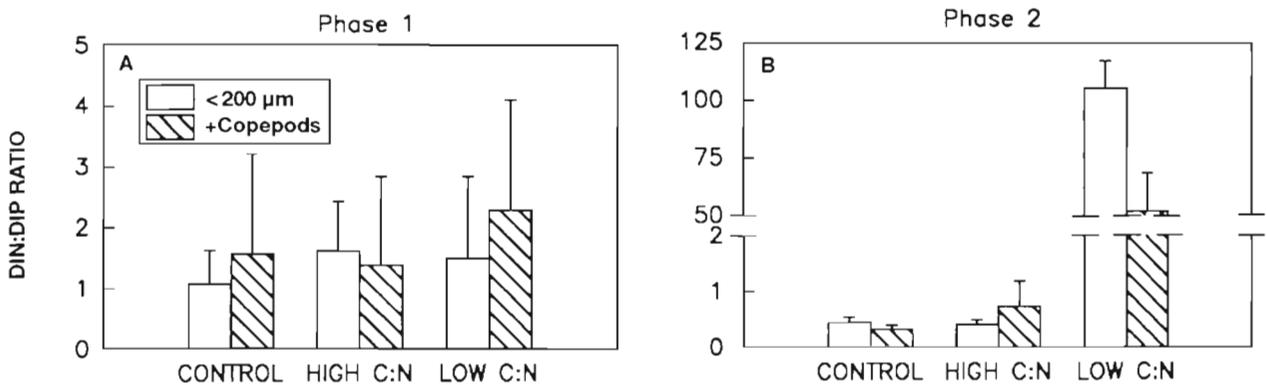


Fig. 4. Mean DIN:DIP ratios (+1 SD) in the carboys during Phase 1 (n = 3) and Phase 2 (n = 4). Phase 1 values for 0 to 17 h; Phase 2 values for 23 to 45 h

Dynamics of PC, PN and pigments

Initial PC and PN concentrations were about $110 \mu\text{g-at. C l}^{-1}$ and $16 \mu\text{g-at. N l}^{-1}$ in all carboys (Fig. 5A, B). Due to lower accumulations of PC and PN during Phase 1, and higher losses during Phase 2, the +Copepods carboys had relatively lower PN and PC concentrations than their counterparts without copepod additions by the end of the experiment (Fig. 5C, D), significantly so in the case of PN ($p < 0.05$, paired *t*-test for the difference in final PN concentrations in carboys with vs without copepod additions within each treatment). A relatively large change also occurred in the Low C:N carboy, where PC increased to nearly $140 \mu\text{g-at. C l}^{-1}$ and PN increased to nearly $25 \mu\text{g-at. N l}^{-1}$ during Phase 1, resulting in a net increase of PN and PC in that carboy. The mean PC:PN ratios varied little; mean atomic PC:PN ratios ranged from 6.3 (Low C:N+Copepods) to 6.8 (High C:N) during Phase 1 and 5.9 (Low C:N) to 6.9 (High C:N) during Phase 2.

Chlorophyll concentrations generally increased or remained constant during the first daylight period, then decreased over the course of the experiment, while phaeopigment concentrations increased throughout the experiment, regardless of treatment (Fig. 6). However, the magnitude of the net chlorophyll change

was related to both the copepod abundance and substrate amendments. The net chlorophyll loss was smallest in the Low C:N carboy (Fig. 7A). Net chlorophyll losses in the High C:N+Copepods and Low C:N+Copepods carboys were higher than in their counterparts without copepods (Fig. 7A). Net phaeopigment accumulation in the carboys without copepods was similar across treatments (Fig. 7B), but additional phaeopigment accumulation in the +Copepods carboys was highest in the Low C:N treatment and decreased in the order Low C:N treatment > Control treatment > High C:N treatment (Fig. 7B). The final chlorophyll concentration in the Low C:N carboy was about 60% higher than in the other 5 carboys ($10.9 \text{ vs } 6.70 \pm 0.86 \mu\text{g l}^{-1}$, mean \pm 95% CI; $p < 0.01$, *t*-test of final chlorophyll concentration in Low C:N carboy vs mean of final chlorophyll concentrations of all other carboys). Conversely, the final phaeopigment concentration in the Low C:N+Copepods carboy was significantly ($p < 0.05$, comparison as above) higher than in the other 5 carboys.

Chlorophyll concentrations fluctuated on a day/night basis in all +Copepods carboys and in the Low C:N carboy without added copepods (Fig. 6). Nighttime chlorophyll losses occurred to some degree in all carboys, but the losses increased with the addition of both substrates

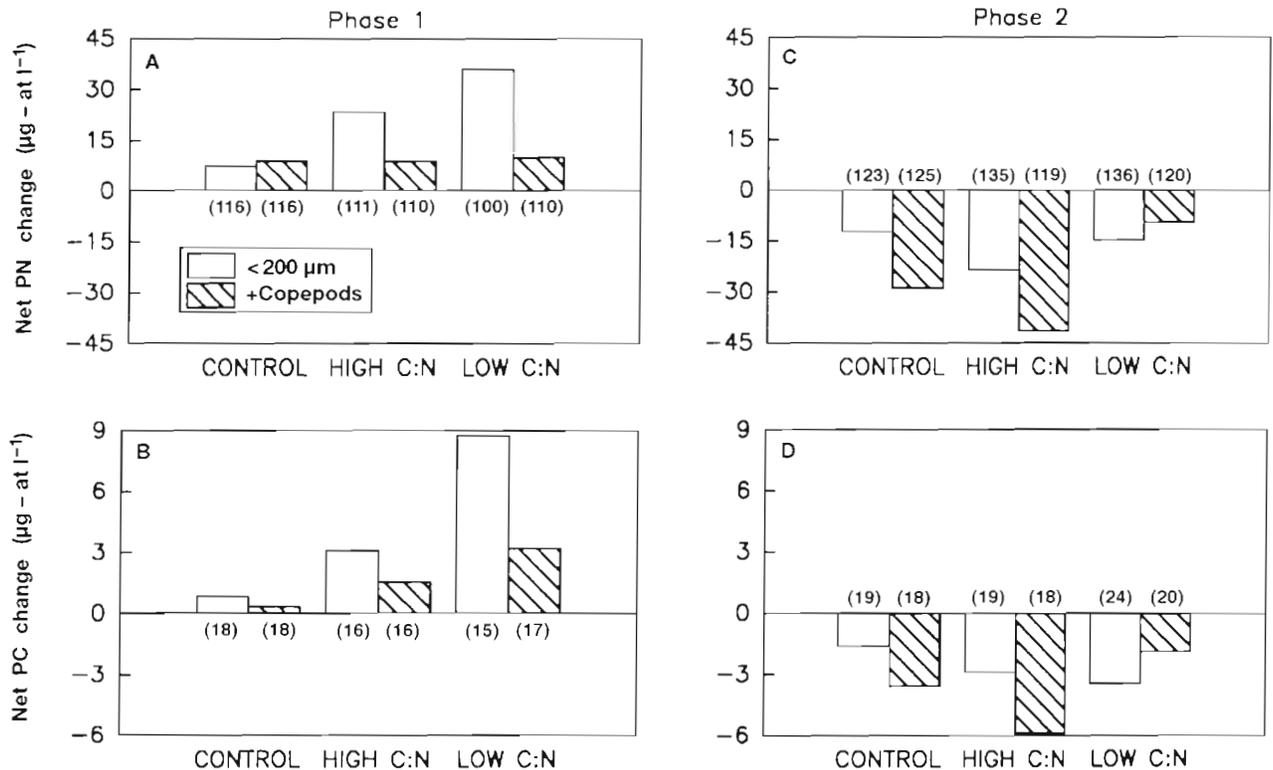


Fig. 5. Net changes in PC and PN concentrations during Phase 1 and Phase 2. Phases separated at 23 h (High C:N at 30 h). Numbers in parentheses indicate concentrations at beginning of phase

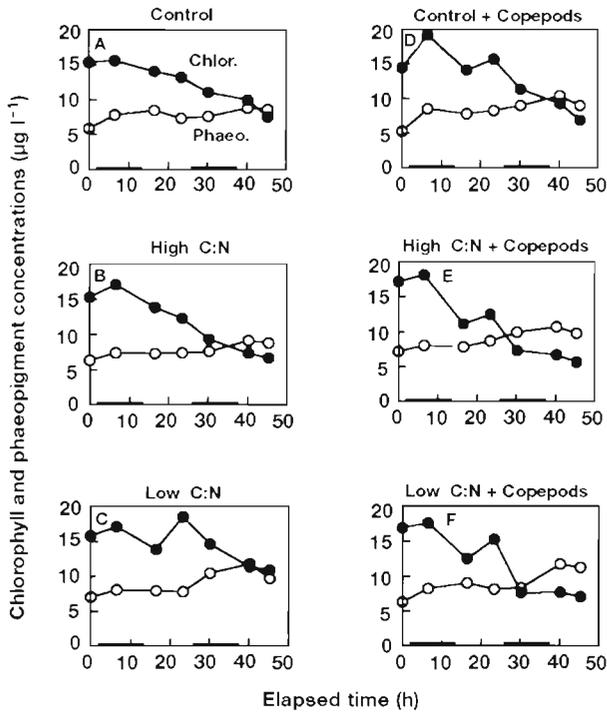


Fig. 6. Concentrations of chlorophyll and phaeopigment in the carboys during the experiment. Black bars indicate when lights were off

(Fig. 7C). Chlorophyll losses during the nighttime hours were about $0.35 \mu\text{g h}^{-1}$ faster in the +Copepods carboys than in the corresponding carboys without copepods, regardless of the substrate treatment (Fig. 7C).

DISCUSSION

In this experiment our measurements focused on bulk changes in nutrient and particulate concentrations. Changes in these parameters in such closed systems are due to changes in either the number of organisms (i.e. biomass), organism-specific metabolic rates, or some combination of the two. While we do not have data on community composition, the biomass and nutrient data that we do have are strongly indicative of certain processes.

The plankton assemblage we sampled appeared to be highly dependent on a tight coupling between nitrogen regeneration and uptake, which is typical of summertime plankton assemblages in Chesapeake Bay. The PC:PN ratios were at the Redfield ratio and both NH_4^+ and o-PO_4^{3-} were measurable, indicating that the phytoplankton were nitrogen-sufficient. The DIN:DIP ratios, on the other hand, were well below the Redfield ratio of 16 required for balanced growth, suggesting that nitrogen availability might limit phytoplankton growth. We could have underestimated the

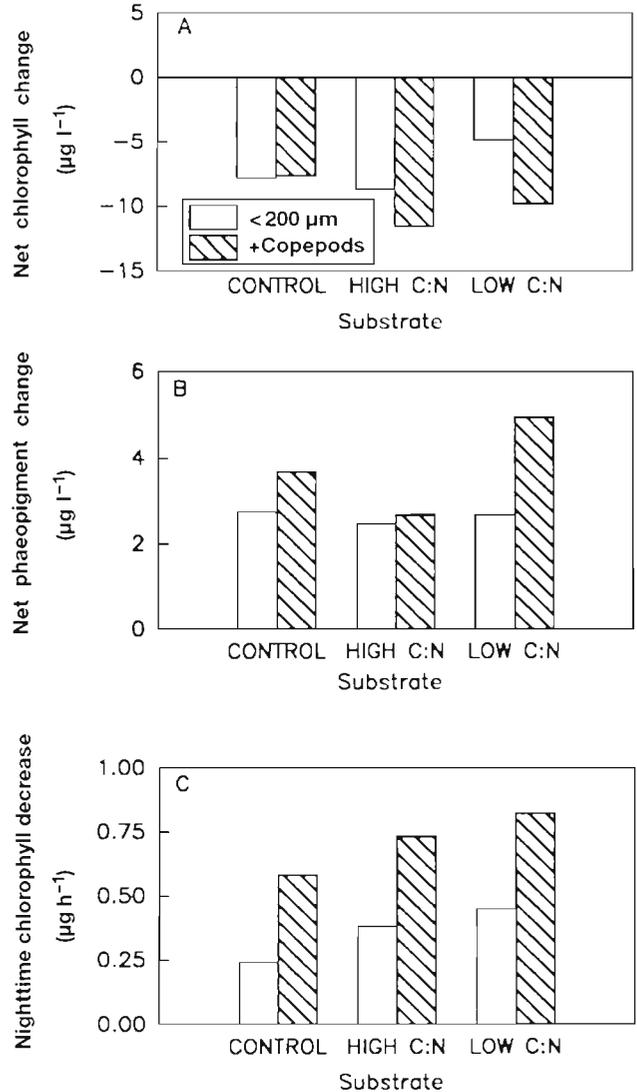


Fig. 7. Net changes in (A) chlorophyll and (B) phaeopigment concentrations over the course of the entire experiment and (C) mean rates of decrease of chlorophyll concentration during 2 nighttime periods (7 to 17 h and 23 to 30 h)

total amount of nitrogen available to phytoplankton, by only including inorganic sources of nitrogen in our estimate of available nitrogen, but it is unlikely that other types of nitrogen available to phytoplankton, such as urea, were plentiful enough (ca $7 \mu\text{g-at. N l}^{-1}$) to increase the ratio to one of balanced growth. Therefore, nitrogen regeneration was likely occurring and phytoplankton were likely to be particularly sensitive to factors affecting nutrient regeneration.

Grazer effects

Copepod additions consistently resulted in increased chlorophyll accumulation during the day and increased

chlorophyll loss at night. In the absence of added substrates (Control treatment) these 2 processes balanced each other and thus there were no effects by copepods alone on the net chlorophyll concentration (Figs. 6A, D & 7A). Copepods often eat more during the night and their presence in enclosures can result in higher phytoplankton biomass than in enclosures with only microplankton (e.g. Roman et al. 1988, Christaki & Van Wambeke 1995), but resultant day/night differences in chlorophyll concentrations are not always observed. Increases in phytoplankton biomass on such a short time scale can be due to reduced grazing or increased availability of nutrients in the presence of copepods. Although both come into play to a certain extent, the importance of increased nutrient availability is supported by previous research showing that *Acartia tonsa* densities of 10 ind. l⁻¹ stimulate NH₄⁺ release on the scale of hours (Glibert et al. 1992), and by the fact that the same day/night pattern occurred in the nutrient-replete Low C:N carboy (Fig. 6C). The 10 adult *A. tonsa* l⁻¹ that we added to the carboys in this experiment is higher than the typical summertime densities of adults in Chesapeake Bay. Such densities have been reported from other estuaries (Ambler et al. 1985), however, and in Chesapeake Bay the densities of adult plus subadult *A. tonsa* together can easily exceed 10 copepods l⁻¹ (Roman et al. 1993).

Substrate effects

In the Low C:N treatment, we manipulated the substrate C:N ratio by adding an amino acid which has been shown to support bacterial nitrogen regeneration (Goldman et al. 1987). Rapid decreases in amino acid concentrations and increases in NH₄⁺ concentrations after a 15 to 20 h lag have been observed in cultures of marine bacteria after experimental manipulations of substrate C:N ratios (Goldman et al. 1987, Goldman & Dennett 1991, Jørgensen et al. 1994). The present results demonstrate that a low C:N addition to intact microplankton assemblages also results in the uptake of DPA and the regeneration of NH₄⁺ after a lag period.

Bacterial regeneration of NH₄⁺ is not only a function of substrate C:N ratio, but also depends on the specific substrates available. When low substrate C:N ratios are achieved with combinations of glucose, NH₄⁺, and amino acids, little or no NH₄⁺ is regenerated (Goldman et al. 1987, Goldman & Dennett 1991, Jørgensen et al. 1994), indicating that bacteria regenerate significant amounts of NH₄⁺ only when labile DON (dissolved organic nitrogen) is the nitrogen source and when DOC (dissolved organic carbon) has become limiting (Goldman & Dennett 1991). The rapid appearance of 50 to 60% of the added substrate as NH₄⁺ (Fig. 1) sug-

gests that this might have been the case in our Low C:N carboys. Alternatively, NH₄⁺ regeneration could have been due to bacterial growth which was subsequently grazed and catabolized by bacterivores. The rapid changes in nutrient concentration consistent with those observed in bacterial cultures, however, suggest that the nutrient dynamics observed between 17 and 23 h were due to bacterial processes. Regardless of the nutritional status of the bacteria, or even the group of organisms responsible for regenerating it, the NH₄⁺ regenerated could and did support increased phytoplankton biomass and bacterial nitrification in the Low C:N treatment.

While dramatic NH₄⁺ regeneration occurred only in the Low C:N carboys, there is evidence that the high C:N substrate might have stimulated microbial processes also. Glucose additions have previously been shown to stimulate bacterial productivity, and, sometimes, bacterial density in estuarine and mesohaline waters (Chin-Leo & Benner 1992, Shiah & Ducklow 1994). Likewise the addition of high C:N (ca 56) humic material to large mesohaline cylinders resulted in increased bacterial density and productivity (Carlsson et al. 1995). In our experiment, the High C:N carboy had higher accumulations of PC and PN during Phase 1 (Fig. 5) and lower accumulations of o-PO₄³⁻ during Phase 2 (Fig. 2F) than the Control carboy did, and other changes suggestive of increased herbivory (discussed below). We hypothesize that the ambient o-PO₄³⁻ was taken up by bacteria along with the labile glucose we added. Nitrogen was available to the bacteria in the High C:N treatment in the form of ambient NH₄⁺ and DPA. In addition, dissolved combined amino acids (DCAA) can occur in relatively high amounts (6 μmol l⁻¹; Coffin 1989) and can be a significant nitrogen source for estuarine bacteria (Coffin 1989, Jørgensen et al. 1994, Kroer et al. 1994). Interestingly, the actual availability of ambient DCAA might have been influenced by our experimental treatment: bacterial uptake of ambient DCAA can be stimulated by the addition of labile substrates with relatively high C:N ratios (10 and 15; Jørgensen et al. 1994).

In addition to influencing nutrient dynamics, the substrate additions might also have influenced herbivory, even in carboys without added adult copepods. The higher losses of PC and PN during Phase 2 (Fig. 5) and higher rates of chlorophyll disappearance during the nights (Fig. 7C) in both the High C:N and Low C:N carboys relative to the Control carboy suggest that the substrate additions resulted in a greater degree of nighttime herbivory by some group or groups of microzooplankton, presumably <200 μm copepodites, which were abundant during this experiment. Microzooplankton densities, along with those of phytoplankton and bacteria, have previously been observed to in-

crease with additions of high C:N humic substances (Carlsson et al. 1995). Results such as these demonstrate how the substrate supply to bacteria can indirectly influence higher order trophic interactions.

Interactive effects

The combined effects produced by the substrate-copepods treatments (Table 3) demonstrate some ways in which grazing and substrate supply interact in intact plankton assemblages. The accumulations of NH_4^+ , o-PO_4^{3-} (Fig. 2D, F), and $\text{NO}_2^- + \text{NO}_3^-$ (Fig. 3C, D) during Phase 2 were higher in the carboys with both copepod and substrate additions than in the corresponding carboys with only substrate additions. There were also larger net changes in PC, PN (Fig. 5), and chlorophyll (Fig. 7A, B) in the +Copepods carboys in the High C:N and Low C:N treatments than in the Control treatment. Thus copepods quickly modify the degree to which nutrients are sequestered into biomass.

Copepods can directly increase nutrient concentrations via the release of metabolic products. The nutrient increases we observed, however, were larger than those expected by copepod nutrient release alone. We estimate that the added *Acartia tonsa* released 0.12 to 0.18 $\mu\text{g-at. NH}_4^+ \text{ l}^{-1}$, 0.03 $\mu\text{g-at. DPA l}^{-1}$, and 0.004 $\mu\text{g-at. o-PO}_4^{3-} \text{ l}^{-1}$ during the experiment [rates of NH_4^+ and DPA release estimated from rates for *A. tonsa* collected from Chesapeake Bay in August (Miller 1992); rates of o-PO_4^{3-} excretion calculated by weight ratio (Ikeda 1985)]. These estimates are lower than the accumulations of NH_4^+ and o-PO_4^{3-} in the +Copepods carboys with substrate additions and the DPA accumulation in the Control+Copepods carboy during Phase 2 (Fig. 2D, E, F). Consistent with our results, NH_4^+ accumulation in another enclosure experiment also exceeded that expected from copepod release rates (Roman et al. 1988). Copepods can indirectly stimulate NH_4^+ regeneration by bacteria or microzooplankton through their release of DON during feeding and metabolism (Roman et al. 1988, Glibert et al. 1992) or trophic interactions with larger microzooplankton (Miller et al. 1995) and we hypothesize that the increased nutrient release observed in this experiment was in part due to these types of interactions. Furthermore, the accumulation of NO_2^- and NO_3^- in the High C:N+Copepods carboy, relative to the High C:N carboy, also points to an indirect role for copepods.

Copepods do not release NO_2^- or NO_3^- themselves, but they do release NH_4^+ and organic compounds, which are the substrates for bacterial nitrification. Nitrification occurred in a previous carboy experiment in carboys without added copepods, but with relatively high NH_4^+ concentrations (Bronk & Glibert 1993). That, together with our observations of NO_3^- and NO_2^- accumulation only in the High C:N carboy with copepods, suggests that the NH_4^+ regeneration enhanced by the copepods is what stimulated nitrification in that carboy. Copepods, then, can modify both the amount and type of regenerated nutrients available to phytoplankton and bacteria. This might explain to some degree why the addition of high C:N humic substances, together with the addition of copepods, to mesohaline cylinders resulted in higher bacterial, phytoplankton, and microzooplankton densities, even in the absence of nutrient accumulation (Carlsson et al. 1995). In that experiment, however, copepods were added to all cylinders, so grazer effects could not be separated from substrate effects.

Likewise, copepods can directly remove particulate material via ingestion. Again, the differences in changes in particulate N and C concentrations between carboys with and without copepods are larger than those estimated based on copepod ingestion rates. In these experiments, the additional net carbon loss in the +Copepods carboys represents ingestion rates of about 9, 20, and 13 $\mu\text{g C copepod}^{-1} \text{ d}^{-1}$ in the Control, High C:N, and Low C:N treatments, respectively. Similarly, the additional net loss of chlorophyll in the +Copepods carboys represents respective rates of 0, 15, and 18 $\mu\text{g C copepod}^{-1} \text{ d}^{-1}$, calculated using C:chlorophyll ratios from our t_0 data. Rates of ingestion of phytoplankton for *Acartia tonsa* in Chesapeake Bay in September are about 5 $\mu\text{g C copepod}^{-1} \text{ d}^{-1}$, although a rate of about 9 $\mu\text{g C d}^{-1}$ has been measured in July (White & Roman 1992). Our chlorophyll-based rates are subject to error in the C:chlorophyll ratio, but together with those cal-

Table 3. Summary of the major substrate, copepod, and interactive effects on nutrient and particulate dynamics in experimental carboys. +, -: increase or decrease relative to the Control carboys

Substrate-only effects	Copepod-only effects
<i>Both substrates</i>	+ Daytime chlorophyll accumulation
- Phase 2 o-PO_4^{3-} accumulation	+ Nighttime chlorophyll loss
+ Net PC and PN accumulation	+ Net phaeopigment accumulation
+ Nighttime chlorophyll loss	- Net PC and PN accumulation
<i>Low C:N addition only</i>	Interactive (substrate + copepods) effects
+ Phase 1 o-PO_4^{3-} loss	+ Phase 2 NH_4^+ and o-PO_4^{3-} accumulation
+ Phase 2 NH_4^+ accumulation	- Phase 2 DPA accumulation
- Phase 2 DPA accumulation	+ $\text{NO}_2^- + \text{NO}_3^-$ concentrations (High C:N)
+ Phase 2 DIN:DIP	- Net chlorophyll, PN/PC accumulation
+ Net $\text{NO}_2^- + \text{NO}_3^-$ accumulation	
+ Net chlorophyll accumulation	
+ Daytime chlorophyll accumulation	

culated from carbon loss, they point to an additional sink for carbon. We suggest that copepod-microzooplankton interactions, such as trophic cascading within multiple trophic levels of microzooplankton or resource competition, resulted in enhanced microzooplankton grazing on phytoplankton, especially in the carboys amended with both copepods and substrates. It is also possible that copepod ingestion increased in response to higher food quality (Houde & Roman 1987, Cowles et al. 1988) in the carboys with substrate additions, but whether the increased chlorophyll removal in the carboys with both substrate and copepod additions was due to direct or indirect activities of the copepods, it supports our hypothesis that both substrates stimulated microplankton processes.

The interactive effects of substrate quality and trophic control on phytoplankton dynamics have often been studied by examining the relative impacts of inorganic nutrients versus those of herbivores. Here we added organic substrates selected to modify bacterial uptake and regeneration of nutrients, and thus indirectly regulate nutrient availability to phytoplankton. Our Low C:N treatment resulted in a large input of NH_4^+ after a time lag. The impacts of copepods on phytoplankton were both positive (increased nutrient regeneration) and negative (increased grazing). The results of our High C:N treatment suggest that through increased microbial activity and trophic interactions between microplankton and copepods, additions of high C:N substrates can also provide regenerated nutrients for primary production. Thus it is essential to know the composition and inputs of dissolved organic matter in estuaries, not only to understand how microbial processes are controlled, but also to understand how estuarine food-webs are controlled.

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