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Variable bottom-up and top-down effects on diversity of different prey assemblages in an estuarine saltmarsh

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ABSTRACT: Bottom-up and top-down effects have been widely studied in the context of food webs. However, there is still inadequate knowledge about how different prey assemblages respond to simultaneous manipulations of consumers and nutrients in benthic meiofaunal and macrofaunal systems, and how these responses affect other trophic levels. Using a factorial experimental design in a salt marsh ecosystem, we investigated the direct influence of nutrients (natural versus enriched) and snail grazing pressure (with versus without *Assiminea latericera*) on 2 different prey communities (microalgae and bacteria). In addition, the indirect influences on nematode communities at other trophic levels were investigated. The abundance of benthic microalgae was regulated mainly by bottom-up forces. Both nutrient supply and grazing pressure significantly affected the diversity of diatoms and bacteria in terms of the Shannon-Wiener diversity index measurement. The amount of nutrient supply significantly reduced diatom and bacteria diversity. The presence of grazers reduced diatom diversity under both ambient and enriched nutrient levels; thus, the algal diversity responses did not support the grazer-reversal hypothesis (species richness of prey decreases with high grazing in nutrient-poor ecosystems, while it increases with high grazing in nutrient-rich ecosystems). The presence of grazers enhanced bacterial diversity mainly under the ambient nutrient level. The feeding selectivity of snail consumers was the likely mechanism influencing the different responses of microalgae and bacteria. Nematodes did not respond to experimental manipulations, indicating the weak indirect effects of bottom-up and top-down forces. We suggest that different prey components within a system may respond to bottom-up and top-down effects in different ways, and other trophic levels in below-ground food webs may not be sensitive to cascade effects.

KEY WORDS: Nutrient enrichment · Snail consumer · Bacteria · Benthic diatom · Diversity cascade · Nematodes

INTRODUCTION

Consumer-induced top-down effects and resource-induced bottom-up effects are primary drivers of biological diversity in food webs (Paine 1966, Menge 1992, Denyer et al. 2010). Early studies focused on the relative importance of top-down and bottom-up forces, but the interaction of these forces has received more attention in recent years (Worm et al. 2002, Hillebrand 2003, Hillebrand et al. 2007, Jaschinski et al. 2010). Worm et al. (2002) used multivariate models to test the simultaneous effects of consumer and resource manipulations in marine ecosystems, and showed that they consistently depend on each

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other, and the direction of their effects shifts as a function of productivity. Other works suggest that the relative strength of resource supplies and consumption pressures can vary with ecosystem type, trophic status, and consumer feeding preferences (Power 1992, Proulx & Mazumder 1998, Hillebrand et al. 2007, Jaschinski et al. 2010). By using a cross-system analysis, Hillebrand et al. (2007) studied the responses of producer diversity following manipulations of resource supply and herbivory, and found that system productivity and producer evenness determine the direction and magnitude of top-down and bottom-up control of diversity. Proulx & Mazumder's (1998) grazer-reversal hypothesis emphasized that plant species richness decreases with high grazing in nutrient-poor ecosystems, while it increases with high grazing in nutrient-rich ecosystems. Predator or grazer composition, feeding preference, and diet breadth also play important roles in modulating the relative strength of top-down and bottom-up forces (Hagerthey et al. 2002, Jiang & Morin 2005, Denyer et al. 2010).

In natural communities, it is common that a grazer can feed on different preys. Different prey communities may respond to alterations of grazing intensity and nutrient supply in different ways (Kneitel & Miller 2002, Burgmer et al. 2010). Therefore, to reveal the responses of different prey components is essential for understanding the diversity alterations under environmental changes in natural ecosystems. However, since different prey groups often have been studied in isolation, the simultaneous effects of top-down and bottom-up controls on different prey communities within a benthic system are still poorly understood. Hillebrand et al. (2002) reported inconsistent nutrient enrichment and grazing effects on the biomass of different components of periphyton communities, but their study did not reveal how prey diversity changed in response to experimental manipulations. Burgmer et al. (2010) reported effects of snail grazers and light on a benthic periphyton community. However, the bottom-up effects (light supply) on autotrophic and heterotrophic components were not consistent in their study.

Multitrophic interactions driven by top-down and bottom-up forces also may exist in food webs (Shurin et al. 2002, Dyer & Letourneau 2003). Dyer & Letourneau (2003) showed that manipulation of resources and predators which cause diversity changes at one trophic level can affect diversity at other trophic levels. Understanding this 'vertical' diversity effects may have important implications for incorporating the network nature of ecological systems into studies of biodiversity–ecosystem functioning (Scherber et al. 2010). In a system containing >1 prey group, it remains unknown if changes in different prey groups under grazer and resource manipulations affect other trophic levels in different ways.

To test the simultaneous effects of grazers and resources on different prey communities in benthic ecosystems, we manipulated snail grazing pressure and nutrient supply amounts within a saltmarsh system in the Yangtze River Estuary, China, and examined the responses of 2 different prey (microalgae and bacteria) communities. *Assiminea latericera*, an abundant snail grazer of microalgae and bacteria in Yangtze River estuarine marshes, was manipulated. To test the indirect effects of top-down and bottom-up forces on other trophic levels, benthic nematode communities, which include algal feeders, bacterial feeders, and carnivores were also investigated.

Previous studies in estuaries and salt marshes have revealed that benthic microalgae can be significantly affected by both resource supplies and consumption forces (Hagerthey et al. 2002, Posey et al. 2002, Armitage & Fong 2004, Armitage et al. 2009). Some studies have shown that these 2 forces may not have strong interactions (Posey et al. 2002, Lever & Valiela 2005, Armitage et al. 2009). However, most of these studies have focused on algal biomass and abundance (Posey et al. 2002, Armitage & Fong 2004, Armitage et al. 2009) rather than species diversity. Bacterial communities also have been reported to be regulated by top-down and bottom-up processes (Jiang & Krumins 2006, Williams et al. 2008). Previous research in lake systems has shown that bacteria are limited primarily by resources, and significant grazing regulation is important only in eutrophic environments (Pace & Cole 1994). In salt marsh sediments, nutrient enrichment has minimal effects on bacterial diversity and production, with the primary controls for bacterial community being local-scale environmental heterogeneity (Bowen et al. 2009). In a laboratory experiment, Jiang & Krumins (2006) found that bacterial diversity decreased with increasing nutrient level, but increased under high predation pressure. Overall, top-down and bottom-up controls would be different for microalgal communities and bacterial communities. Thus, we predicted that microalgal and bacterial communities in our study would respond in different ways to snail grazing pressure and nutrient enrichment.

Nematodes are the most abundant meiofauna in the majority of estuarine sediments, and are sensitive

to resource supplies (Bongers & Ferris 1999). Chen et al. (2007) previously showed that bacterial and algal consumers are 2 dominant nematode trophic groups in Yangtze River salt marshes. Thus, in our system, we assumed that the responses in microalgal and bacterial communities caused by differences in nutrient supply and grazing pressures would affect algal and bacterial nematodes, and then cascade to carnivorous nematodes (Fig. 1).

In factorial design field experiments, we investigated the impacts of consumers and resource supplies on different trophic levels. Our experiment explored the following questions: (1) Do nutrient enrichment and grazing pressure variations differentially influence microalgal and bacterial communities? (2) Do nutrient enrichment and grazing pressure variations have interactive effects? (3) Do the alterations in microalgal and bacterial communities caused by top-down and bottom-up forces in turn affect the abundance and diversity of algal-feeding, bacterial-feeding, and carnivorous nematodes?

METHODS

Study site

The study site was located in the Chongming Dongtan National Nature Reserve, Yangtze River estuary, China (31° 25′ to 31° 38′ N, 121° 50′ to 122° 05′ E). The site is characterized by a subtropical monsoonal climate. The annual mean temperature and precipitation are 15.7°C and 1124 mm, respectively. Tides are semi-diurnal, with an annual average tidal range of 2.67 m. The experiment was conducted at the mid-tidal marsh, which was dominated by the common reed *Phragmites australis.* The salinity at the study site ranges from 1.12 to 5.83‰. The sediment is of a clay-silt type, with the 'natural' ambient total nitrogen level being 0.90 ± 0.19 mg g^{-1}.

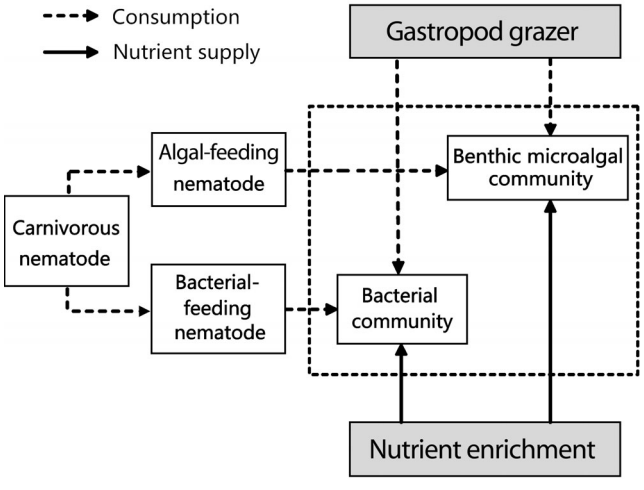

Fig. 1. Conceptual diagram illustrating the grazing and nutrient-enrichment effects on benthic microalgal and bacterial communities, and the relationship between the nematode community and the 2 prey groups. Factors manipulated in our experiment are highlighted in gray, and response variables are presented by rectangular boxes

Experimental design

We used exclusion cages to evaluate the effects of nutrients and grazing on benthic microalgae, bacteria, and nematodes in a factorial design field experiment. A total of 12 plots were randomly distributed at the site. Half of the plots were assigned as nutrient-enrichment treatments, while the others were left at natural nutrient levels (controls). The distance between plots was >6 m. In each plot, 2 cylindrical cages (diameter: 0.62 m, basal area: 0.3 m^2) constructed of aluminum window screening (2 mm mesh) were diagonally placed 0.5 m apart. To prevent animal immigration and emigration, the walling of the cages was 30 cm high and buried 20 cm into the sediment, and the top was covered with 2.5 mm mesh. All plants and epibenthic macrofauna, including snails, were removed from the cages 2 wk before the experiment began. The 2 cages in each plot were assigned to 2 different treatments: snails removed and snails added. Thus, 24 cages were equally divided into 4 treatments, with 6 replicates for each treatment: natural nutrient amounts without snails, natural nutrient amounts with snails, nutrients added without snails, and nutrients added with snails.

We added 1.6 g urea fertilizer (46% N) to the enriched plots during neap tides every 2 wk. Nitrogen loading rate was approximately 0.176 g N d^{-1} m^{-1} (half of the amount suggested by Armitage et al. 2009). After an acclimation period of 2 wk, we added *Assiminea latericera* grazers. The focal herbivore in our study, *A. latericera*, is one of the most common and abundant gastropod species at the site, occurring at mean densities of 192 ind. m^{-2}. Sixty adult snails of 10.2 mm in mean size were added to each grazer treatment. Our previous research on *A. latericera* using fatty acid analysis revealed that this grazer contains both microalgal (20:5n3) and bacterial (15:0 + 17:0 + 18:1n7) biomarker fatty acid signatures (J. H. Wu unpubl. data). Our experiment started in October 2009 and lasted 8 wk.

Sampling and processing

In order to quantify biomass, abundance, and diversity of benthic microalgae at the end of the experiment, we randomly scraped ten 2 × 4 cm areas of surface sediment per cage to a depth of 5 mm. For bacterial and nematode analyses, 8 cores were collected in each cage using a modified O'Connor split corer to a depth of 50 mm (inner diameter: 32 mm). The top 5 mm of sediment of ten 2 × 4 cm areas from each cage was manually homogenized and split into 3 parts. A portion of sediment was preserved in 4% formalin for microalgal species identification and counting. One gram of sediment was frozen at −20°C until chlorophyll *a* (chl *a*) concentration analyses could be performed. The other portion of the sediment was dried to constant weight at 80°C to determine sediment water content. The homogenized top 50 mm of sediment also was divided into 3 portions. For DNA extraction, ~0.5 g was frozen at −4°C. For nematode analyses, 180 g was fixed in 8% formalin. The rest was dried at 80°C to constant weight to determine sediment water content.

Chl *a* was measured using spectrophotometry (Lorenzen 1967). Pigments were extracted with 90% acetone in 10 ml centrifuge tubes. The samples were mixed thoroughly and centrifuged for 15 min at 4000 rpm. The absorbance of the sample extracts was determined at 750 and 665 nm before acidification, and determined at 750 and 665 nm again after acidification with 1 M HCl in a TU-1901 double beam UV-Vis spectrophotometer.

To determine benthic microalgal abundance, formalin-fixed samples were examined and the number of microalgae was counted using a light microscope at 400× magnification. For diatom identification and numeration, Canada balsam mounted slides were prepared. Most diatoms were identified to species level at 1000× magnification following Wang et al. (2008). Small Pennatae and Centricae diatoms (<12 µm) were not identified to species level, and were classed into Pennatae spp. and Centricae spp. We used the species richness and Shannon-Wiener index (H') to calculate diatom diversity. The equation for the Shannon-Wiener diversity index is as follows:

$$H' = -\Sigma p_i \ln(p_i) \quad (1)$$

where $p_i = n_i/N$, n_i is the number of the ith species, and N is the total number of species in a sample. Diatom taxa constituting >8% of the total abundance were defined as dominant taxa.

We used polymerase chain reaction–denaturing gradient gel electrophoresis (PCR-DGGE) to examine benthic bacterial diversity. DNA was extracted and purified using a FastDNA Spin Kit for Soil (MP Biomedicals). Extracted DNA was PCR-amplified with the general bacterial primers 8F and 1492R to obtain a nearly complete 16S rRNA gene fragment (Green et al. 2008). Then, the variable V3 region of 16S rDNA was amplified with the universal bacterial primers 341F (with a 40 bp GC clamp at its 5′ end) and 534R (Muyzer et al. 1993). DGGE of the PCR products was performed using 10% (w/v) acrylamide/bisacrylamide (37.5:1) gels containing a 35 to 65% linear gradient of formamide and urea (100% denaturing solution contained 40% formamide and 7 M urea). The electrophoresis was run at 60°C, using a D-Code system (Bio-Rad Laboratories) for 7 h at 160 V. The gels were stained with EB for 15 min and scanned by a Tanon-2500 Imaging System (Tanon Science & Technology). The number of bands and intensity of each band were measured using Quantity One software (Bio-Rad Laboratories). A band was detected if its relative intensity was >0.5% of the total lane intensity (Crouzet et al. 2010). We used operational taxonomic units (OTU) to define each band (Reche et al. 2005). OTU numbers were named according to their relative intensity; the highest one was OTU 1, and so forth. In calculating the Shannon-Wiener (H') index, n_i was defined as the intensity of the ith OTU, and N was the total intensity of all OTUs in the sample. A dominant bacterial OTU was defined if its band intensity constituted >4% of the total band intensity.

Sediment samples for nematode analyses were filtered through 45 µm mesh sieves and floated with Ludox TM (density 1.17 g cm^{-3}). Total numbers of nematodes were counted under a dissecting microscope. Data were normalized to dry weight of air-dried sediment. Nematodes were dehydrated in anhydrous glycerol, prepared on slides, and identified to species level. For each sample, we randomly selected 100 ind. for identification. Nematodes were classified into 4 trophic groups according to Yeates et al. (1993): plant feeders, algal feeders, bacterial feeders and carnivorous or omnivorous nematodes. The Shannon-Wiener (H') diversity index of nematode communities and each feeding group were calculated.

Statistical analysis

All analyses were executed with Statistica (Version 7.0, StatSoft). We used 2-way factorial ANOVAs to evaluate the effects of nutrient enrichment and grazing pressure on benthic microalgal, bacterial, and nematode variables. Least-square-difference (LSD) tests

were used for multiple comparisons of biomass, abundance, taxon richness, or diversity indices of microalgae, bacterial OTU, and nematodes. Variation of dominant diatom taxa and dominant bacterial OTUs between snail treatments were analyzed with independent *t*-tests. The numeric data were log-transformed or log(x + 1) transformed, and the proportional data were arcsine-square-root transformed to meet the assumptions of ANOVAs or *t*-tests, where necessary.

RESULTS

Microalgal biomass and abundance

Nutrient addition significantly enhanced benthic microalgal biomass in terms of chl *a* concentration (ANOVA: $F_{1,20}$ = 13.55, p = 0.0015), and snails grazing significantly reduced benthic microalgal biomass (ANOVA: $F_{1,20}$ = 5.69, p = 0.0271). There was no significant interaction between nutrient amount and snail presence/absence effects. LSD tests indicated that only in natural-nutrient treatments were chl *a* concentrations significantly different between snail/no-snail treatments (Fig. 2A).

A total of 97 species of benthic microalgae were identified. Among them, there were 88 Bacillariophyta species (Table S1 in the supplement at www.int-res.com/articles/suppl/m472p015_supp.pdf), 5 Cyanophyta species, 2 Chlorophyta species, and 2 Chrysophyta species. Bacillariophyta was the most numerically abundant group (80.11%), followed by Cyanophyta, Chlorophyta, and Chrysophyta (17.88, 1.98, and 0.03%, respectively). Because diatoms

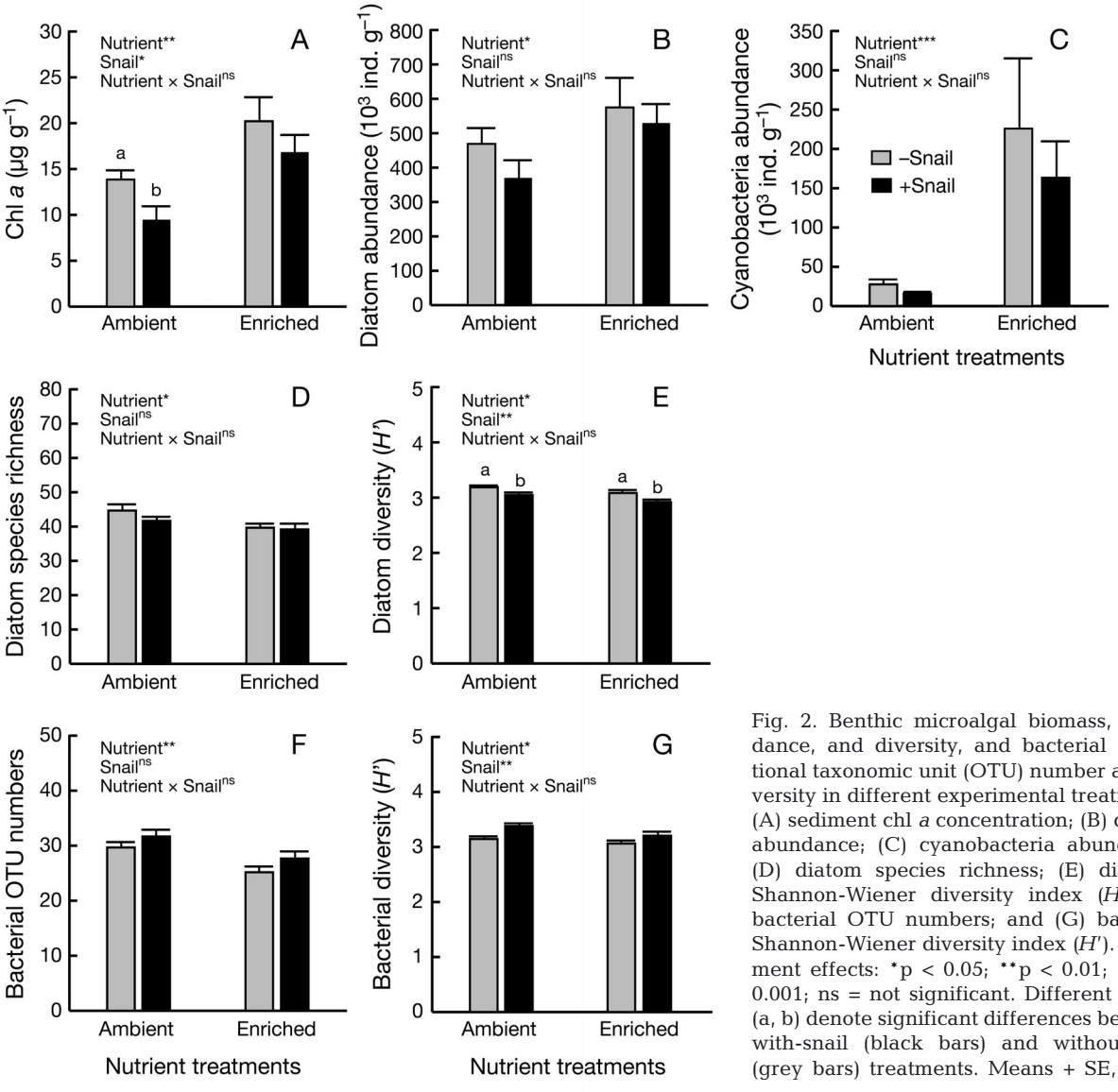

Fig. 2. Benthic microalgal biomass, abundance, and diversity, and bacterial operational taxonomic unit (OTU) number and diversity in different experimental treatments: (A) sediment chl *a* concentration; (B) diatom abundance; (C) cyanobacteria abundance; (D) diatom species richness; (E) diatoms, Shannon-Wiener diversity index (*H'*); (F) bacterial OTU numbers; and (G) bacteria, Shannon-Wiener diversity index (*H'*). Treatment effects: *p < 0.05; **p < 0.01; ***p < 0.001; ns = not significant. Different letters (a, b) denote significant differences between with-snail (black bars) and without-snail (grey bars) treatments. Means + SE, n = 6

(Bacillariophyta) and cyanobacteria (Cyanophyta) comprised >97% of the total microalgal abundance, we analyzed the abundance of these 2 groups only. Both diatom and cyanobacterial abundances in nutrient-enrichment treatments were significantly higher than in natural nutrient level treatments (ANOVA for diatom: $F_{1,20}$ = 5.48, p = 0.0298; ANOVA for cyanobacteria: $F_{1,20}$ = 51.37, p < 0.0001; Fig. 2B,C). Grazing effects on these groups were not significant.

Diatom species richness and diversity

The species richness of diatoms was significantly lowered when nutrients were added, although the changes were small in magnitude (ANOVA: $F_{1,20}$ = 6.54, p = 0.0188), but was not affected by grazing pressure (Fig. 2D). Both nutrient enrichment and grazing negatively affected the Shannon-Wiener diversity index measurement of diatoms (Fig. 2E,F). No significant interaction was found between nutrient and grazing treatments.

We identified 4 dominant diatom taxa (>8% of total diatom abundance), which represented approximately 42% of the total diatom abundance. The relative abundance of each dominant taxon tended to increase due to the presence of snails. (Fig. 3A,B).

Bacterial OTUs number and diversity

The number of bacterial OTUs (DGGE bands) was not significantly affected by grazing. However, nutrient enrichment reduced bacterial OTU numbers (ANOVA: $F_{1,20}$ = 13.39, p = 0.0016; Fig. 2F).

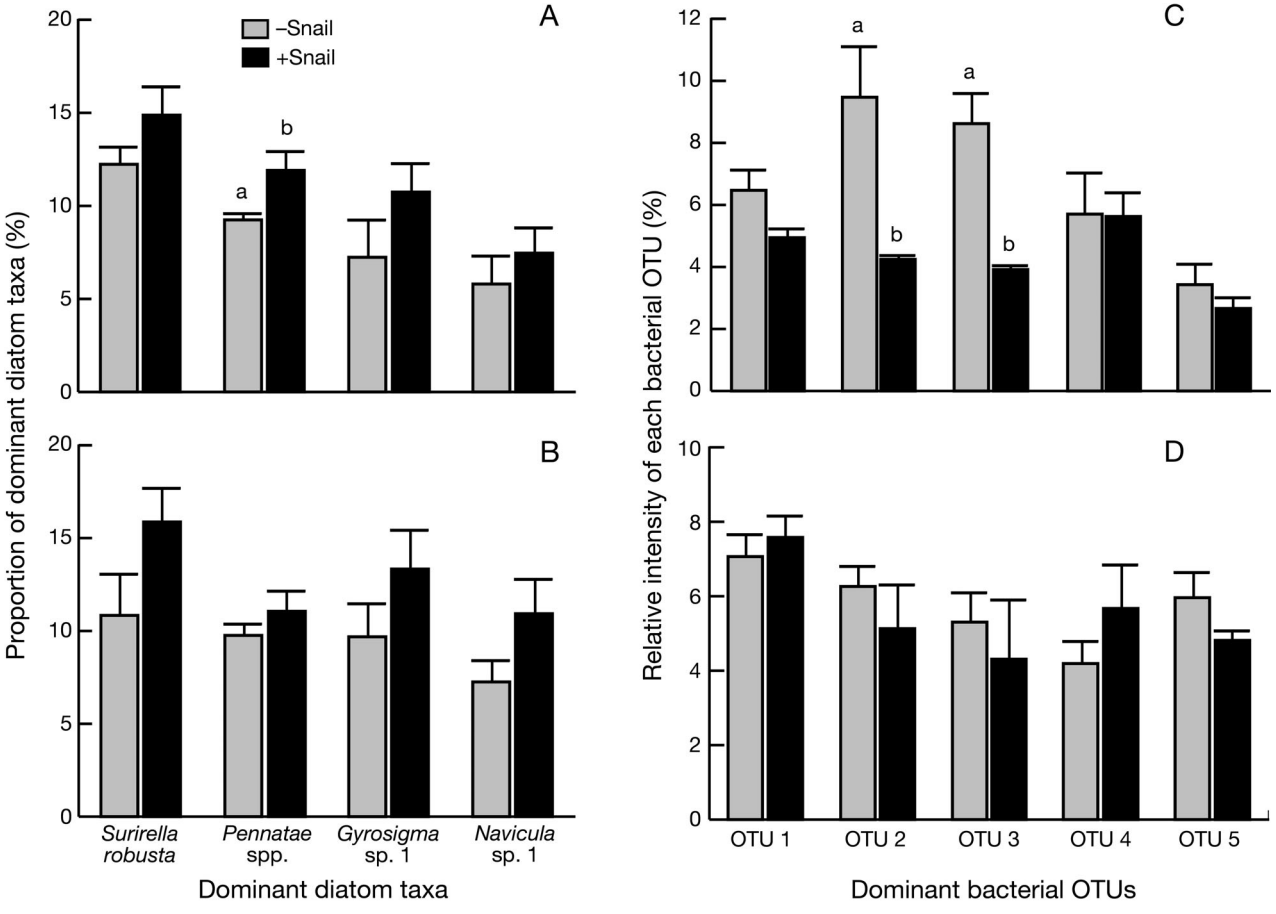

Fig. 3. Variation of dominant diatom taxa in (A) ambient-nutrient treatments and (B) nutrient-added treatments, and variation of dominant bacterial operational taxonomic units (OTUs) in (C) ambient-nutrient treatments and (D) nutrient-added treatments. Different letters (a, b) denote significant differences between with-snail (black bars) and without-snail (grey bars) treatments. Means + SE, n = 6. Pennatae spp.: small-sized (<12 µm) pennate diatom species

Nutrient enrichment had a marginally significant effect on the Shannon-Wiener diversity index measurement of bacteria (ANOVA for H': $F_{1,20}$ = 5.32, p = 0.0319), and bacterial diversity decreased under enriched nutrient conditions (Fig. 2G). The bacterial diversity was higher in the snail-added treatment than in the snail-removed treatment, with significant differences in natural-nutrient treatments (Fig. 2G). No significant interaction between nutrient-enrichment and grazing treatments was detected.

A total of 5 dominant bacterial OTUs (DGGE bands) were identified, with each comprising >4 % of the total band intensity (Table S2 in the supplement at www.int-res.com/articles/suppl/m472p015_supp.pdf). In ambient-nutrient treatments, snail grazing tended to reduce the intensity of the 5 dominant bacterial OTUs (Fig. 3C). In nutrient-enrichment treatments, dominant bacterial OTUs showed inconsistent responses to the presence of snails in terms of intensity (Fig. 3D).

Nematode communities

We found a total of 32 nematode species from 27 genera (Table S3 in the supplement at www.int-res.com/articles/suppl/m472p015_supp.pdf), 19 of which were bacterial feeders, 7 carnivorous or omnivorous nematodes, 4 macrophyte root feeders ('plant feeders' hereafter), and 2 microalgal feeders ('algal feeders' hereafter). Although the algal-feeding group included only 2 species, this group was dominant in terms of density (52 %), followed by bacterial feeders (35 %). Nutrient enrichment significantly reduced total nematode abundance and Shannon-Wiener diversity index value (ANOVA for abundance: $F_{1,20}$ = 4.48, p = 0.0470, for H': $F_{1,20}$ = 4.39, p = 0.0490; Fig. 4). Snails had no effects on nematode density, species richness, or diversity index.

Nutrient enrichment significantly lowered the abundance of bacterial-feeding and carnivorous/omnivorous nematodes, but had no significant effect on algal-feeder abundance (Fig. 4, Table 1). No sig-

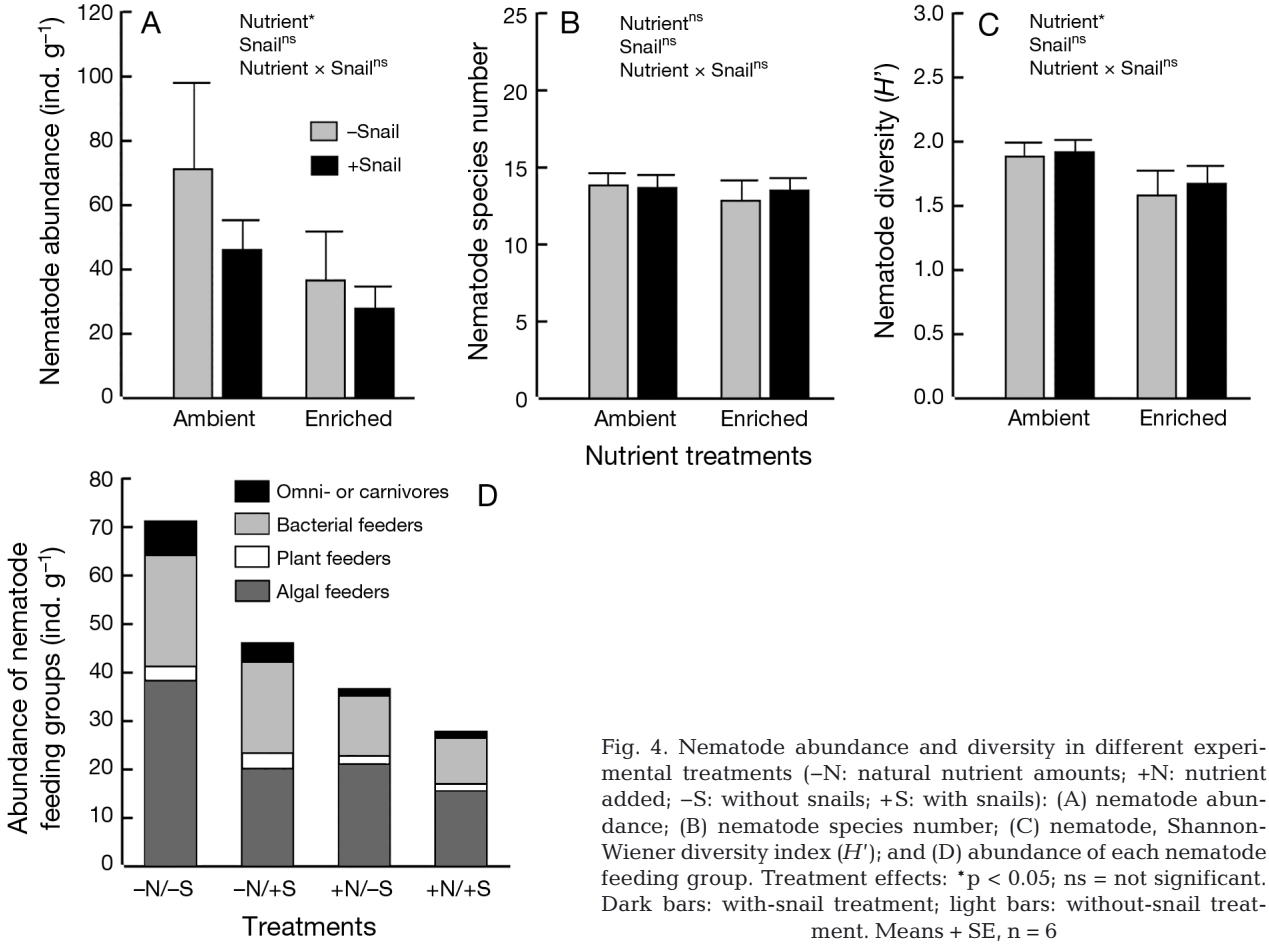

Fig. 4. Nematode abundance and diversity in different experimental treatments (–N: natural nutrient amounts; +N: nutrient added; –S: without snails; +S: with snails): (A) nematode abundance; (B) nematode species number; (C) nematode, Shannon-Wiener diversity index (H'); and (D) abundance of each nematode feeding group. Treatment effects: *p < 0.05; ns = not significant. Dark bars: with-snail treatment; light bars: without-snail treatment. Means + SE, n = 6

Table 1. Two-way ANOVA for the abundance and species diversity (Shannon-Wiener index H') of different nematode feeding groups in response to nutrient-enrichment and snail-addition treatments. Significant p-values in **bold**. Note: abundance data were log(x + 1) transformed

Feeding group	Nutrient			Grazing			Nutrient × Grazing			Residual
	df	F	p	df	F	p	df	F	p	
Algal feeders										
Abundance	1	1.31	0.266	1	0.37	0.552	1	0.12	0.734	20
H'	1	0.21	0.653	1	0.17	0.683	1	0.03	0.857	20
Bacterial feeders										
Abundance	1	**5.46**	**0.030**	1	0.01	0.929	1	0.06	0.807	20
H'	1	0.01	0.917	1	0.16	0.689	1	0.99	0.332	20
Carni- or omnivores										
Abundance	1	**36.93**	**<0.001**	1	2.61	0.122	1	2.06	0.166	20
H'	1	0.66	0.428	1	0.01	0.910	1	0.02	0.902	20
Plant feeders										
Abundance	1	2.31	0.144	1	0.61	0.443	1	0.82	0.376	20
H'	1	1.31	0.266	1	0.04	0.845	1	1.00	0.329	20

nificant changes were caused by experimental manipulations on the species diversity of nematode feeding groups (Table 1).

DISCUSSION

The diversity of diatoms and bacteria (in terms of the Shannon-Wiener diversity index measurement) was significantly affected by both nutrient supply and grazing pressure. The presence of grazers reduced diatom diversity under both ambient and enriched nutrient levels; thus, the algal diversity responses did not support the grazer-reversal hypothesis. The presence of grazers enhanced bacterial diversity mainly under the ambient nutrient level. This confirmed our hypothesis that microalgal and bacterial communities respond in different ways to alterations in grazing and nutrient supply.

Prey abundance was primarily controlled by bottom-up processes

Our experiment revealed a significant effect of nutrient enrichment on the abundance of diatoms and cyanobacteria. Since diatoms and cyanobacteria were the main components of the microalgal community, we conclude that bottom-up processes exerted a strong positive control on benthic microalgae, while the influence of top-down control was relatively weak.

Enhanced benthic microalgal abundance due to nutrient enrichment has been reported previously (Pinckney et al. 1995, Armitage et al. 2009). Microalgal biomass generally has been found to respond to nutrient enrichment in a consistent way, especially in non-eutrophic sites (Posey et al. 2002, Armitage & Fong 2004, Lever & Valiela 2005). In line with these reports, our study indicates that nitrogen addition significantly enhances benthic microalgal abundance and biomass. Nutrient enrichment resulted in a greater increase in the abundance of cyanobacteria (up to 8 times) than diatoms (+30%). This suggests that cyanobacteria are better competitors for nitrogen than other algal groups (Fong et al. 1993). Grazing had a lesser impact on benthic microalgal abundance compared to nutrient effects, likely because the influence of herbivory was masked by high microalgal growth rates, or because snail grazing may influence microalgal growth by providing nutrients via their feces or via the turn-over of sediment (Sommer 1997, Armitage & Fong 2004).

Prey diversity was controlled by both top-down and bottom-up processes

Both nutrient enrichment and grazing influenced diatom diversity significantly. Similarly, bacterial diversity also strongly responded to the 2 manipulated factors. Thus, we conclude that top-down and bottom-up forces both have strong effects on the diversity of 2 prey (microalgae and bacteria) groups.

Hillebrand et al. (2000) reported that grazing reduced microalgal diversity at low nutrient supply, but enhanced it at high nutrient supply. Experiments in marine ecosystems have also shown the 2 effects on macroalgal diversity consistently depend on each other (Worm et al. 2002). However, no significant

interaction between grazing and nutrient enrichment was detected in our study. Furthermore, we did not find a significant interaction of nutrient amount and grazing on bacterial communities either. Thus, our results suggest that the links between top-down and bottom-up effects were weak, and did not follow the pattern expected from conceptual models suggested by Worm et al. (2002). Similar results have also been reported in other studies for periphyton, benthic diatom, and microphytobenthic communities (Hillebrand 2003, Lever & Valiela 2005, Armitage et al. 2009). For example, Hillebrand (2003) showed no significant interaction between nutrient-enrichment and grazing effects on periphyton diversity in lake and coastal habitats. Considering the relatively independent effects of top-down and bottom-up processes reported by various researchers (Posey et al. 2002, Lever & Valiela 2005, Armitage et al. 2009), the interaction between enrichment and grazing may not be general. In addition, the differences in study duration and the ranges of experimental treatment are likely to provide different results (Posey et al. 2002, Worm et al. 2002, Guerry 2008).

Different responses of diatom and bacterial diversity to top-down and bottom-up processes

The direction of top-down and bottom-up controls on 2 different prey (microalgae and bacteria) assemblages in our study were not the same. Nutrient enrichment and grazing pressure both significantly reduced diatom diversity. However, snail and nutrient effects on bacterial diversity differed (i.e. the nutrient effect on bacterial diversity was negative, while the grazing effect was positive).

Similar to Hillebrand's (2003) study, we found decreased taxon number and diversity of diatoms and bacteria in nutrient-addition treatments. Hillebrand (2003) suggested that competition for nutrients enhanced the growth of a few dominant species and decreased the evenness of periphyton communities. However, our experiment did not show significant changes in abundance of the dominant diatom species between low- and high-nutrient treatments ($p > 0.05$). For bacterial OTU intensity, the nutrient-addition effects were inconsistent among 5 dominant bacterial OTUs, thus suggesting that, in this study, the decreased diversity under enriched nutrient conditions was not because of the overgrowth of dominant species.

Our findings suggest that the grazing decreased diatom diversity both under natural nutrient conditions and under enriched conditions, reaching a result inconsistent with the grazer-reversal hypothesis. Snail grazing greatly reduced benthic diatom diversity, which may be related to its selective feeding habits (Pape et al. 2008). Our experiment showed that the proportions of dominant diatom taxa tended to increase when snails were present. This may be related to changes in microalgal community composition caused by gastropod selective grazing (Liess & Kahlert 2007). We suspect that *Assiminea latericera* disproportionately affected just a few diatom species and therefore changed diatom diversity.

In contrast, grazers were observed to affect bacterial diversity positively. Snail grazing tended to reduce the relative intensity of 5 dominant bacterial OTUs in natural-nutrient treatments. We suspect that *Assiminea latericera* selectively graze on common bacterial species, therefore increasing the relative abundance of rare species and increasing bacterial diversity. An alternative mechanism is that the snail might reduce the competitive dominance of the major bacterial taxa, thereby allowing less-dominant taxa to persist. Other non-trophic factors may also have played a role or interacted to regulate bacterial communities (Hillebrand et al. 2002). For example, the mucus and feces of snail or starfish grazers may have stimulated bacterial regeneration (Pillay et al. 2009, Dawson & Pillay 2011).

The highest diversity of diatoms was found under natural-nutrient conditions without consumers, while the highest diversity of bacteria was found under natural-nutrient levels when consumers were present. This also confirmed the different responses of the autotrophic and heterotrophic components of the prey communities to top-down and bottom-up processes.

Cascading effects on nematode communities

Previous studies revealed that meiofaunal abundance and diversity can be regulated by their food sources (Danovaro & Gambi 2002). However, our study showed that the abundance of nematodes did not indicate a strong link with changes in prey groups. A certain decrease in algal-feeding nematode abundance was found in the nutrient-enrichment treatment, which was the opposite of the increase in microalgal abundance caused by nutrient addition. Between the snail/no-snail treatments, no significant difference was observed in the abundance of all nematode feeding groups. Despite changes in microalgal and bacterial diversity caused

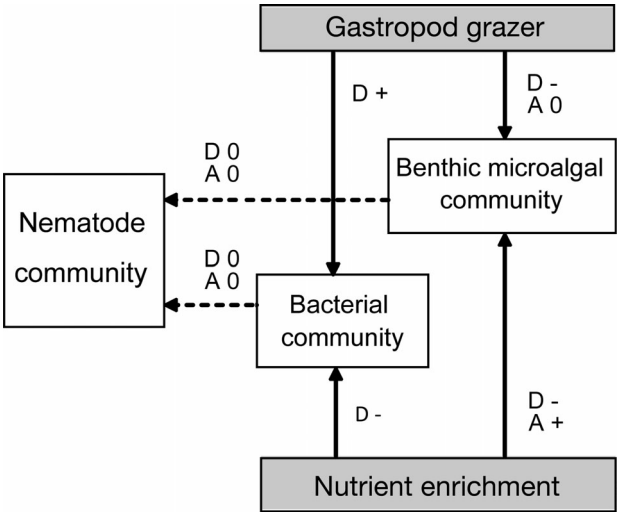

Fig. 5. Summary of the effects of gastropod grazers or nutrient supply on different prey components (microalgal and bacterial assemblages). Dotted lines: indirect effects on the other consumer assemblage (nematode community). D: diversity; A: abundance; +: positive effect; −: negative effect; 0: non-significant effect

by top-down or bottom-up influences, the diversity of algal-feeding, bacterial-feeding, and carnivorous nematodes showed no responses to all manipulated treatments (Table 1). These results reveal that the multitrophic effects in our study system were not as prominent as we hypothesized. This result may be related to the experimental gradient of nutrient and grazing intensity. At intermediate levels of productivity, grazing may influence taxonomic richness in a unimodal way (hump-shaped response). For example, Pillay et al. (2010) found that benthic fauna at intermediate densities were more diverse than those at high or low starfish densities. Scherber et al. (2010) indicated that the bottom-up effects of plant diversity on multitrophic interactions were weaker on below-ground than on above-ground biodiversity. Our study agrees with their results that diversity cascade effects may not be prominent in below-ground food webs (Fig. 5).

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