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Role of microzooplankton grazing in regulating phytoplankton biomass and community structure in response to atmospheric aerosol input

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ABSTRACT: The effect of aerosol deposition on the growth rate (μ) and grazing mortality (*m*) of different-sized phytoplankton was investigated in microcosm experiments using the dilution technique. Experiments were conducted on 3 August 2011 (Expt 1), 14 November 2012 (Expt 2) and 1 February 2013 (Expt 3) at a subtropical coastal site adjacent to the South China Sea. Significant enhancement in both phytoplankton growth and microzooplankton grazing were obtained after aerosol addition, but the degree of impact was different in each size fraction. Aerosol input induced significant phytoplankton biomass accumulation as well as community structure shift, with 2.0-, 2.7- and 2.5-fold biomass increases of micro-sized cells and 1.3-, 1.5- and 1.9-fold biomass increases of pico- and nano-sized cells in the 3 experiments, respectively. A large increase in growth rate was obtained for micro-phytoplankton in 2 of the 3 experiments, while pico- and nano-sized cells suffered increased grazing pressure in the aerosol treatment. The different responses resulted in a reduced proportion of protist consumption to phytoplankton daily production (i.e. *m*/μ ratio) and increased net growth rate (μ – *m*) in micro-phytoplankton but the opposite in nano- and pico-phytoplankon, which explains the clear shift of phytoplankton size structure in response to aerosol addition. The picoplankton populations did not exhibit apparent biomass accumulation due to efficient cropping by heterotrophic nanoflagellates. Our study is the first direct assessment of the impact of aerosol amendment on microzooplankton grazing activity, and our results suggest that it is important to consider the role of protist grazing when evaluating the effects of atmospheric deposition on marine planktonic ecosystems under the scenarios of increasing human activity, atmospheric input and natural perturbations.

KEY WORDS: Aerosol · Microzooplankton grazing mortality · Phytoplankton growth rate · Phytoplankton community structure

INTRODUCTION

Atmospheric input is well documented as an important source of external nutrients for seas and oceans (Jickells et al. 2005, Duce et al. 2008). It can alleviate nutrient limitation of marine phytoplankton, enhance their primary production, change the phytoplankton and bacterial community composition, and ultimately affect biogeochemical cycles (Bonnet et al. 2005, Herut et al. 2005, Pulido-Villena et al. 2008, Lekunberri et al. 2010, Romero et al. 2011, Guo et al. 2012, 2013). In East Asia, aerosols containing large amounts of anthropogenic particles with a high content of NH_3, NO_x and trace metal species, as well as mineral particles from the broad continent (especially the northern desert), are transported over China's marginal seas and the Northwest Pacific region by the prevailing seasonal monsoons and westerlies, attracting the attention of many researchers (Duce & Tindale 1991, Gao et al. 1997, Cohen et al. 2004, Lin et al. 2007, Geng et al. 2009, Kim et al. 2011).

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Although the biogeochemical significance of Asian aerosol deposition in the northwest Pacific region has been well recognized, its impact on biological activities in the subtropical marginal sea has not been well studied. Guo et al. (2012, 2013) conducted aerosol enrichment experiments to investigate the influence of East Asian aerosol on the microbial community in the South China Sea. A clear change in both phytoplankton and bacterial community structure was observed, with a significant increase in microphytoplankton biomass (especially of diatoms), but imperceptible biomass accumulation of small picoplankton, including autotrophic picocyanobacteria and heterotrophic bacteria, in response to aerosol loadings. The increased abundance of protist grazers lead to the hypothesis that the stimulation effects on small plankton might be counterbalanced by enhanced mortality due to grazing, causing a shift in phytoplankton community structure.

Similar observations and speculation of enhanced protist grazing have been previously reported for dust-amended waters in other regions, assuming an increase of bioavailable nutrients as the major effect of aerosol input. Both Lekunberri et al. (2010) and Romero et al. (2011) observed a bacterial decay associated with a peak in heterotrophic flagellate abundance on the second and third day of dust enrichment. Herut et al. (2005) considered a slight increase of ciliates as a possible reason for the lack of increase in picoeukaryote abundance. Other nutrient addition experiments revealed similar results, which are believed to be a consequence of enhanced microzooplankton grazing. Blain et al. (2004) speculated that the decline of pico-phytoplankton on the third day after iron addition was likely due to grazing. Psarra et al. (2005) also recorded a decrease in phytoplankton biomass, and Pitta et al. (2005) reported stable abundance of heterotrophic nanoflagellates and a rapid increase in ciliate abundance after P-addition in the Eastern Mediterranean. A rapid response of the grazing community to the addition of P, in addition to a positive response in copepod gut fullness and egg abundance was also reported by Pasternak et al. (2005). Bonnet et al. (2005) and Marañón et al. (2010) demonstrated that the standing stocks of planktonic microbes tended to show much smaller responses to dust addition than metabolic rates such as primary production and bacterial production. This pattern has also been observed in other nutrient addition experiments (Mills et al. 2004), suggesting a strong trophic control exerted by protist grazers on both bacteria and phytoplankton in the ocean in response to atmospheric input. Although Pulido-Villena et al. (2008) demonstrated that dust-induced increases in bacterial abundance in the Mediterranean resulted in significant carbon mineralization, they also highlighted the importance of elucidating protistan grazing activities in response to atmospheric deposition, which may counteract the dissolved organic carbon (DOC) mineralization and even increase the amount of DOC susceptible to be exported to depth by winter mixing.

Despite speculation in a number of studies, however, no study has provided direct evidence that aerosol input enhances the grazing activity of micrograzers. Thus, it is of major importance to investigate how protistan grazers respond to atmospheric deposition, and whether it is an important mechanism driving plankton community composition change and biomass accumulation in response to atmospheric aerosol input. We conducted 3 aerosol enrichment microcosm experiments using the dilution technique (Landry & Hassett 1982) in a first attempt to evaluate the role of microzooplankton grazing in controlling or influencing the size structure of the phytoplankton community in response to aerosol input.

MATERIALS AND METHODS

Aerosol enrichment experiment

Microcosm experimental design. Three aerosol enrichment experiments were conducted in August 2011 (Expt 1), November 2012 (Expt 2) and February 2013 (Expt 3) using seawater samples collected at Stn PM7 (22° 20.453′ N, 114° 17.703′ E), a fixed sampling station with bottom depth of ~17 m located in Port Shelter, a semi-closed bay in the eastern part of Hong Kong and the south coast of China adjacent to the northern part of the South China Sea (Fig. 1). It is a relatively stable water system without direct influence from the Pearl River. The water in Port Shelter originates from the oceanic South China Sea, presenting strong stratification in summer and intrusion of the China coastal current in winter when northeast monsoonal winds prevail. Aerosol of PM2.5 (which includes particles ≤2.5 µm) was collected by a high-volume sampler at a flow rate of 1130 l min^{-1} on the rooftop of the Academic Building at the campus of the Hong Kong University of Science and Technology (HKUST) (Fig. 1). This location is an area of relatively low pollution, more than 5 km away from the nearest commercial area and 100 m above sea level, facing Port Shelter and the South China Sea (Fig. 1). The aerosol was collected in the same season as each experiment.

Surface seawater (0 to 3 m) was collected using 4 acid-cleaned 20 l carboys, and immediately (within 30 min) transported to the Coastal Marine Lab in HKUST near the coast of Port Shelter. The collected seawater was gently transferred into another 4 acid-cleaned 20 l polycarbonate carboys after being pre-screened over 200 µm mesh to exclude mesozooplankton. Aerosol was added to 2 microcosms at an approximate final concentration of 1 mg l^{-1} to serve as the aerosol treatment; the other 2 microcosms without any amendment served as the controls. The aerosol concentration used in the experiment (~1 mg l^{-1}) was based on the assumption of a high deposition event of approximately 10 g m^{-2} into the mixing layer of 10 m in the sea. All carboys were tightly capped and incubated outside the Coastal Marine Lab in flow-through tanks with running seawater from Port Shelter. The light level was simulated by covering the carboys with 2 layers of neutral density screen, which attenuated the sunlight intensity by 40%, approximately the light condition at the surface layer. Duplicate samples for inorganic nutrients, 20 µm size-fractioned chlorophyll *a* (chl *a*), and picoplankton were collected from each carboy every day during incubation. At time point D0, aerosol was added to the microcosms; dilution experiments were conducted after incubations of 1 d (D1) in Expts 1 and 3, and after 3 h (D0′) and 2 d (D2), respectively, in Expt 2.

Dilution experiment setup and rate estimation. Growth rate and grazing mortality of phytoplankton with different size-fraction and picoplankton populations were measured by the dilution technique (Landry & Hassett 1982) following protocols of Landry et al. (2003). The dilution series were prepared using unfiltered seawater at 15, 50, 73 and 100%. A measured amount of particle-free seawater obtained by gravity filtration using 0.2 µm filter capsule (Pall Corporation) was added to 1.2 l polycarbonate bottles; the bottles were then gently filled (1.2 l) with the unfiltered seawater. The bottles were incubated for 24 h under the same conditions as the carboys. Before the experiments, all bottles, tubes, and capsules used in the experiment were thoroughly washed with 10% HCl and deionized water, and rinsed with the incubated or ambient seawater. Samples for measuring chl *a* concentration and picoplankton abundances were taken from the initial microcosms and each dilution bottle after the incubation.

Based on the exponential model of phytoplankton growth, the net growth rate (K_i) of phytoplankton (chl *a* or specific populations) from each dilution treatment was calculated according to the formula $K_i = \ln(C_i / C_0 \times D_i)$, where C_i is the chl *a* concentration or specific population abundance in the i^{th} dilution treatment after 24 h incubation, C_0 is initial chl *a* concentration or specific population abundance, and D_i is the dilution factor. The calculated K_i from each dilution treatment and D_i were used to derive a linear regression, in order to obtain the instantaneous growth rate of phytoplankton (µ, *y*-axis intercept) and grazing mortality (*m*, regression slope) (Landry & Hassett 1982, Landry et al. 2003). The proportion of production of each picoplankton population that was consumed by microzooplankton was calculated as *m*/µ.

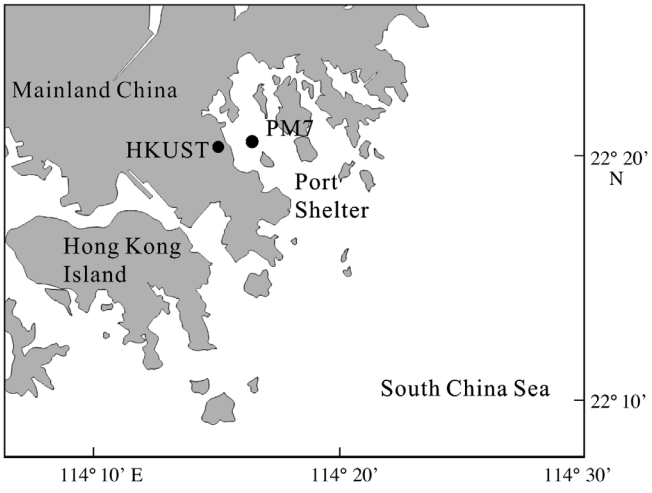

Fig. 1. Location of sampling station PM7 in Port Shelter, adjacent to the South China Sea. Aerosol was collected at the Hong Kong University of Science and Technology (HKUST)

Parameter measurements

Chl a. Duplicate aliquots of 250 ml subsamples were sequentially filtered through 20 µm polycarbonate membranes and Whatman GF/F glass fiber filters, to obtain chl *a* content in plankton greater and smaller than 20 µm. The samples were then extracted in 90% acetone at −20°C in the dark overnight, and determined fluorometrically using a Turner Designs Trilogy fluorometer.

Picoplankton populations. Collected seawater (1.8 ml) was fixed with 0.5% (final conc.) seawater-buffered paraformaldehyde immediately after collection and stored at −80°C until analysis. Abundances of autotrophic picoplankton, *Synechococcus* (Syn) and picoeukaryotes (Peuk) were enumerated using a Becton-Dickson FACSCalibur flow cytometer under the high flow rate (~60 µl min^{-1}), according to the side scattering (SSC) and red/orange auto-fluorescence emitted by chlorophyll/phycoerythrin. Yellowish green fluoresence beads (1 µm, Polysciences) were added

Table 1. Initial hydrographic conditions for the 3 aerosol enrichment microcosm experiments. Number in parenthesis is standard deviation. DO: dissolved oxygen; Syn: *Synechococcus*; Peuk: picoeukaryotes; HNF: heterotrophic nanoflagellates

	Expt 1	Expt 2	Expt 3
Date	3 Aug 2011	14 Nov 2012	1 Feb 2013
Temperature (°C)	31.30	24.59	16.6
Salinity (ppt)	33.15	32.98	32.24
DO (mg l^{-1})	4.35	6.39	9.03
$NO_3 + NO_2$ (µM)	1.3	2.8	<0.1
PO_4 (µM)	0.8	0.9	0.1
>20 µm chl *a* (µg l^{-1})	2.03 (0.29)	0.68 (0.12)	8.71 (1.16)
<20 µm chl *a* (µg l^{-1})	1.80 (0.04)	0.30 (0.13)	3.47 (0.66)
Total chl *a* (µg l^{-1})	3.83 (0.25)	0.98 (0.10)	12.19 (1.82)
Syn (×10^3 cells ml^{-1})	414.38 (0.14)	25.55 (1.19)	0.93 (0.18)
Peuk (×10^3 cells ml^{-1})	20.63 (0.62)	17.92 (2.32)	9.26 (0.64)
HNF (×10^3 cells ml^{-1})	2.67 (0.24)	1.55 (0.19)	1.63 (0.02)

as an internal standard to calibrate and normalize the fluoresence and light scattering signals.

Inorganic nutrients. Samples for measurement of inorganic nutrients $NO_3^- + NO_2^-$, PO_4^{3-} (P) and SiO_4^{4-} (Si) were filtered through GF/F glass fiber filters, and the filtrates immediately frozen at −20°C until analysis. The concentrations of inorganic nutrients were determined colorimetrically using a Skalar autoanalyzer (San Plus) following JGOFS protocols (Knap et al. 1996).

Ion composition and concentrations. Analysis of ionic species was carried out using an ion chromatography (IC) system (DX500, Dionex). The anions (i.e. Cl^-, NO_3^-, and SO_4^{2-}) were determined using an AS-11 column and a gradient elution solution of NaOH. The cations (i.e. Na^+, NH_4^+ and K^+) were determined using a CS-12 column and an isocratic elution solution of methanesulfonic acid. The details of IC analysis are described in Yang et al. (2005).

Table 2. Concentration (µg l^{-1}) of ions originating from the aerosol in the incubated seawater

	NH_4^+	NO_3^-	Na^+	K^+	Cl^-	SO_4^{2-}
Expt 1	12.39	10.34	3.49	1.11	1.39	31.65
Expts 2 and 3	15.88	11.82	3.27	1.44	1.90	53.08

RESULTS

Phytoplankton biomass

The 3 experiments were conducted in summer 2011, autumn 2012 and winter 2013. Initial seawater concentrations of $NO_2 + NO_3$ were below 3 µM in all 3 experiments (Table 1). The added aerosol resulted in about 1 µM external inorganic nitrogen (N) released into the seawater (Table 2). Initial chl *a* concentrations were ~3.8, ~1.0 and ~12.2 µg l^{-1}, with micro-sized phytoplankton accounting for 53, 69 and 71% of total chl *a* in Expts 1, 2 and 3, respectively (Table 1, Fig. 2). High chl *a* concentration but undetectable $NO_3 + NO_2$ concentration indicated a post-bloom stage in Port Shelter during Expt 3. Addition of atmospheric aerosol induced significant increases of total chl *a* concentration to ~1.8-, ~2.0- and ~2.4-fold of control values during incubation, with 2.0-, 2.7- and 2.5-fold increases in chl *a* corresponding to the micro-sized cells, and 1.3-, 1.5- and 1.9-fold increases in chl *a* derived by the pico- and nano-sized cells in Expt 1, 2 and 3, respectively (Fig. 2). After the 2 d incubation, micro-sized phytoplankton accounted for 66, 45 and 66% in controls, but had increased to 74, 60, and 75% in the aerosol treatments in Expt 1, 2 and 3, respectively.

The abundance of Syn was 4 × 10^5 cells ml^{-1} in Expt 1 (conducted in summer), which was about 1 and 2 orders of magnitude higher than that in Expts 2

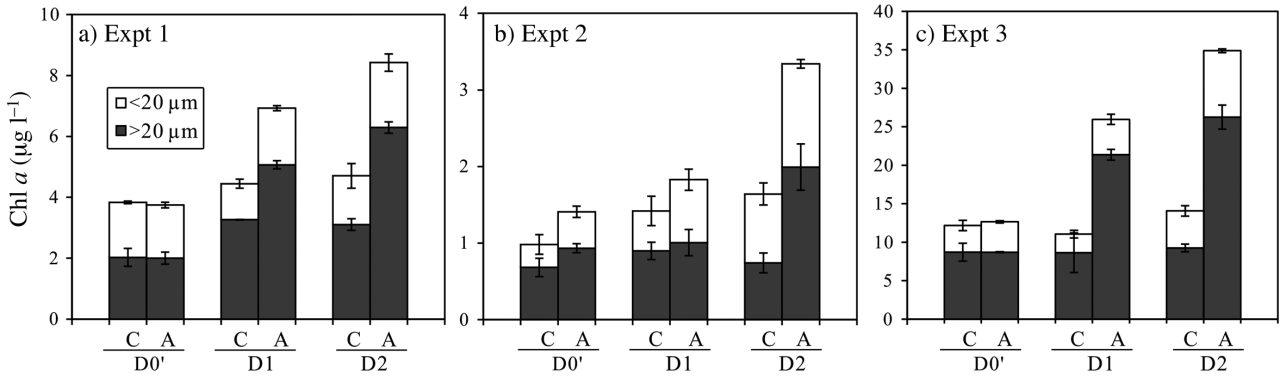

Fig. 2. Effect of aerosol addition on chl *a* concentrations in 2 size fractions of phytoplankton in (a) Expt 1, (b) Expt 2, and (c) Expt 3. Error bars represent the standard deviation of size-fractioned chl *a* concentrations. Note the different scale in *y*-axis. C: control; A: aerosol treatment; D0′: 3 h after incubation; D1: 24 h after incubation; D2: 48 h after incubation. >20 µm: micro-sized phytoplankton cells; <20 µm: nano- and pico-sized phytoplankton cells

and 3 (conducted in winter) (Fig. 3). Contrary to the significant increase in total chl *a* concentration, the abundance of picoplankton populations exhibited only a moderate increase, and sometimes even a decrease, in the aerosol treatment. After 1 d incubation, the abundance of Syn in aerosol treatments was 1-, 1.3- and 0.7-fold the control values, and the abundance of Peuk was 0.8-, 0.8- and 0.9-fold the control values in Expts 1, 2 and 3, respectively.

Growth rate (μ) and grazing mortality (*m*)

Generally, aerosol addition resulted in a clear increase in both μ and *m* of bulk phytoplankton biomass based on total chl *a* concentration. Estimates of μ were 0.9, 0.8 and 0.6 d^{-1} in the controls, and 1.3, 1.2 and 1.2 d^{-1} in the aerosol treatments, increasing by 1.4-, 1.5- and 1.9-fold in Expts 1, 2, and 3, respectively (Fig. 4). μ increased by 1.5-, 1.4- and 2.2-fold for micro-sized phytoplankton, and by 1.1-, 2.0- and 1.5-fold for pico- and nano-sized phytoplankton in the aerosol treatments compared to the controls (Fig. 4). Syn and Peuk μ was generally lower than that of total phytoplankton. After 1 or 2 d of incubation in the control treatments, μ was 0.2, 0.3 and 0.5 d^{-1}, and 0.6, 0.6 and 0.3 d^{-1} for Syn and Peuk, respectively, and the rate was 1.2-, 1.8- and 1.7-fold, and 1.8-, 1.5- and 1.9-fold in the aerosol treatments compared with the controls in Expts 1, 2 and 3, respectively (Fig. 4).

Based on total chl *a* measurement, *m* was 0.6, 0.3 and 0.3 d^{-1} in the controls after incubation in Expts 1, 2 and 3, respectively, with higher or similar values of small phytoplankton (<20 μm) compared with large phytoplankton (>20 μm) (Fig. 5). Aerosol addition induced 1.2-, 1.9- and 2.3-fold increases in *m* in total phyto-

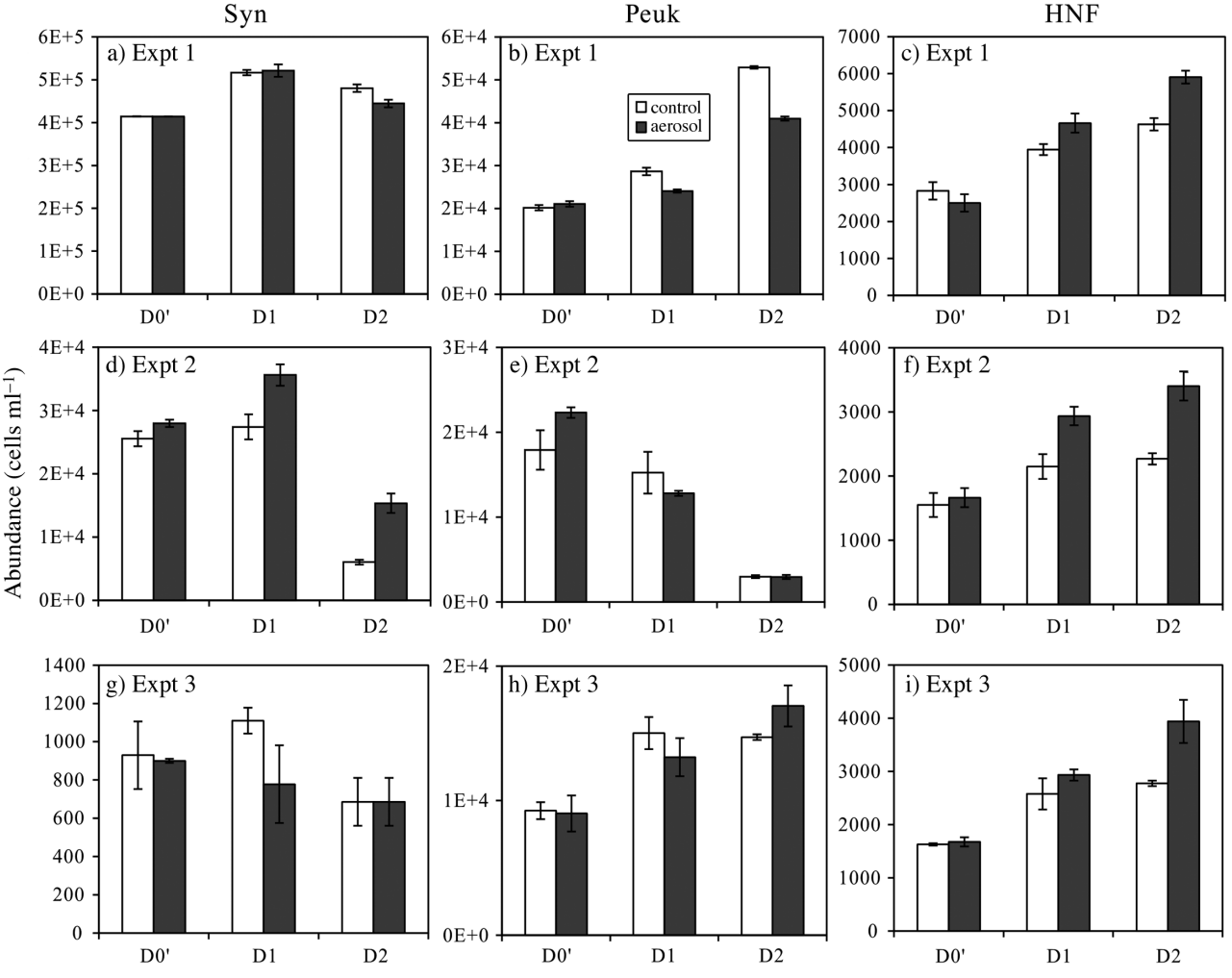

Fig. 3. Response of (a,d,g) Syn, (b,e,h) Peuk and (c,f,i) HNF abundances to aerosol addition in (a–c) Expt 1, (d–f) Expt 2, and (g–i) Expt 3. Error bars represent the standard deviation of replicates. Note the different scale in *y*-axis in all 3 experiments. Syn: *Synechococcus*; Peuk: picoeukaryotes; HNF: heterotrophic nanoflagellates; D0′: 3 h after incubation; D1: 24 h after incubation; D2: 48 h after incubation

plankton compared with controls in Expts 1, 2 and 3, respectively. The grazing rate increased by 1.3-, 1.6- and 2.0-fold for micro-sized phytoplankton, and by 1.2-, 2.2- and 2.8-fold for nano- and pico-sized phytoplankton in Expts 1, 2 and 3, respectively (Fig. 5). In the controls after 1 or 2 d incubation, m was 0.1, 1.1 and 0.9 d^{-1}, and 0.3, 0.8 and 0.3 d^{-1} of Syn and Peuk, respectively; the rate increased by 4.2-, 1.4- and 1.2-fold, and 1.2-, 1.6- and 1.7-fold in the aerosol treatments compared with controls in Expts 1, 2 and 3, respectively (Fig. 5).

Response of m/μ and $\mu - m$

The m/μ ratio, which indicates the proportion of microzooplankton consumption to phytoplankton production, was 0.7, 0.4 and 0.5 (D0′ and D2) and 0.6 in the controls, and 0.6, 0.5 and 0.5 (D0′ and D2) and 0.6 in the aerosol treatments in Expts 1, 2 and 3, respectively (Table 3). However, the ratio showed different responses to aerosol addition for phytoplankton of different size-fractions. For nano- and pico-sized phytoplankton, the m/μ ratio increased from 0.6, 0.3 and 0.8 (D0′ and D2) and 0.3 to 0.7, 0.5 and 0.9 (D0′ and D2) and 0.6, respectively, for the 3 experiments after aerosol addition; whereas for micro-sized phytoplankton, the ratio generally decreased or maintained similar levels from 0.7, 0.4 and 0.3 (D0′ and D2) and 0.7 to 0.6, 0.4 and 0.4 (D0′ and D2) and 0.7, respectively (Table 3). For picoplankton, the m/μ ratio was 3.4-, 0.8- and 0.7-fold that of control values for Syn, and 1.5-, 1.1- and 0.9-fold that of control values for Peuk after aerosol addition in Expts 1, 2 and 3, respectively (Table 3).

The net growth rate, calculated as $\mu - m$, increased by 1.9-, 1.2- and 2.5-fold for total phytoplankton in the aerosol treatments compared with controls in Expts 1, 2 and 3, respectively (Table 3).

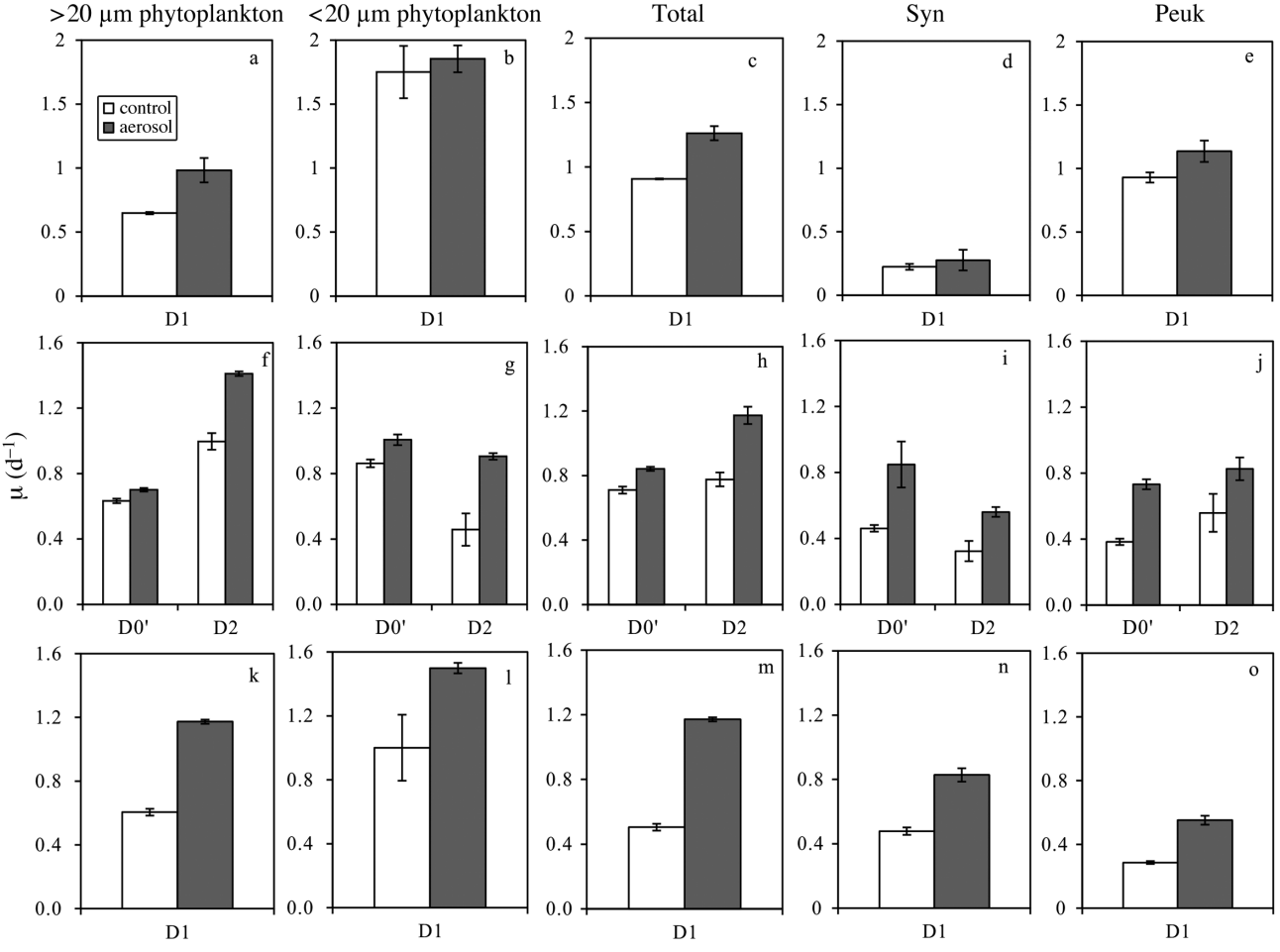

Fig. 4. Phytoplankton growth rate (µ, d^{-1}) of (a,f,k) >20 µm phytoplankton, (b,g,l) <20 µm phytoplankton, (e,h,m) total phytoplankton community, (d,i,n) Syn and (e,g,o) Peuk in response to aerosol addition in (a–e) Expt 1, (f–j) Expt 2 and (k–o) Expt 3. Error bars represent the standard deviation of replicates. Syn: *Synechococcus*; Peuk: picoeukaryotes; D0′: 3 h after incubation; D1: 24 h after incubation; D2: 48 h after incubation

The net growth rate of micro-sized phytoplankton increased by 2.2-, 1.3- and 3.0-fold after aerosol addition, which is higher than the values of the 0.8-, 1.0- and 0.9-fold increase of nano-and pico-sized phytoplankton compared to the control in the 3 experiments (Table 3). The response of net growth rate of picoplankton to aerosol addition was variable in the different experiments: it decreased in the aerosol treatment in Expts 1 and 2 (only D2 for Syn), but increased slightly in Expt 3 compared with that of the control.

DISCUSSION

A shift in phytoplankton size composition from small cells to larger cells was observed in our previous aerosol experiment conducted in the South China Sea (Guo et al. 2012), and this observation has been further verified in this study. Since the N/P ratio of the seawater was <16 at the experimental site, the nutrients released from the aerosol (N: ~1 µM) relieved N limitation. The growth rate of both size fractions of phytoplankton as well as individual picoplankton populations exhibited clear increases, with a more significant increase in growth rate occurring in larger cells (>20 µm) than in small cells (<20 µm) in aerosol-amended waters in Expts 1 and 3. It has been well demonstrated that large cells tend to be competitively superior in nutrient-replete environments because they are better equipped and more able to rapidly respond to nutrient injections (Goldman 1993), whereas the smaller cells tend to be more capable of meeting their nutrient demands in nutri-

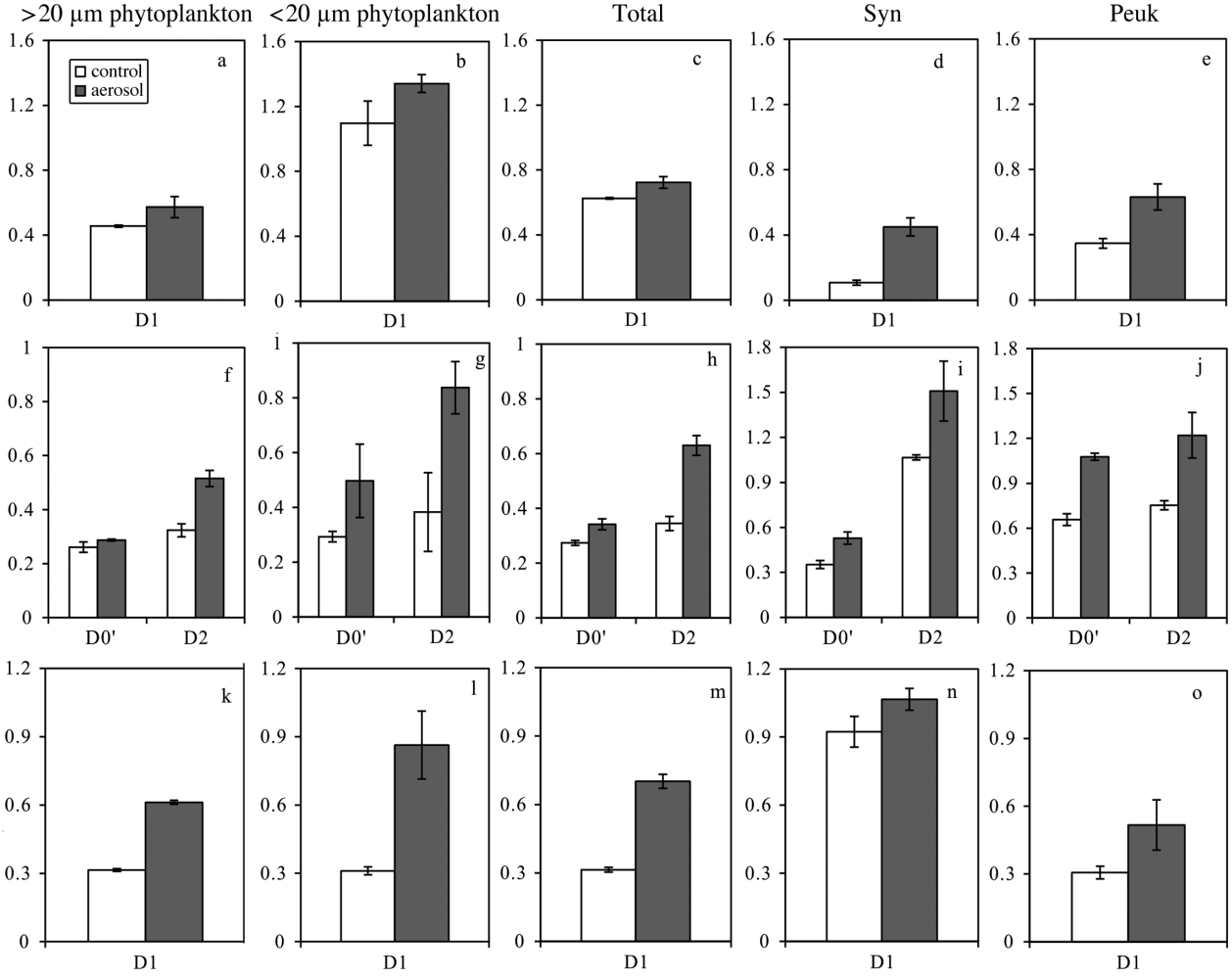

Fig. 5. Phytoplankton grazing mortality (*m*, d^{-1}) of (a,f,k) >20 µm phytoplankton, (b,g,l) <20 µm phytoplankton, (e,h,m) total phytoplankton community, (d,i,n) Syn and (e,g,o) Peuk in response to aerosol addition in (a–e) Expt 1, (f–j) Expt 2 and (k–o) Expt 3. Error bars represent the standard deviation of replicates. Syn: *Synechococcus*; Peuk: picoeukaryotes; D0′: 3 h after incubation; D1: 24 h after incubation; D2: 48 h after incubation

Table 3. Response of grazing mortality to growth rate ratio (m/μ) and net growth rate ($\mu - m$) to aerosol addition. C: control; A: aerosol treatment; Syn: *Synechococcus*; Peuk: picoeukaryotes; m: grazing mortality; μ: growth rate; D1: 24 h after incubation; D0′: 3 h after incubation; D2: 48 h after incubation

			>20 µm phytoplankton		<20 µm phytoplankton		Total phyt. community		Syn		Peuk	
			C	A	C	A	C	A	C	A	C	A
Expt 1	D1	m/μ	0.70	0.58	0.63	0.72	0.69	0.57	0.48	1.62	0.37	0.56
		$\mu - m$	0.19	0.41	0.65	0.51	0.28	0.54	0.12	−0.17	0.58	0.50
Expt 2	D0′	m/μ	0.41	0.41	0.34	0.50	0.39	0.41	0.77	0.62	1.36	1.47
		$\mu - m$	0.37	0.41	0.57	0.51	0.44	0.50	0.11	0.32	−0.17	−0.34
	D2	m/μ	0.32	0.37	0.82	0.92	0.45	0.54	3.30	2.69	1.35	1.48
		$\mu - m$	0.67	0.89	0.07	0.07	0.43	0.54	−0.74	−0.95	−0.19	−0.39
Expt 3	D1	m/μ	0.74	0.65	0.32	0.58	0.62	0.60	1.94	1.29	1.07	0.93
		$\mu - m$	0.11	0.33	0.69	0.64	0.19	0.47	−0.44	−0.24	−0.02	0.03

ent-depleted environments due to their larger surface area to volume ratio (Raven 1998, Chisholm 2000). However, the different physiological characteristics and growth ability between larger and smaller phytoplankton cells alone could not explain the dramatic biomass difference between the 2 size fractions, and particularly the often observed negative response of pico- and nano-phytoplankton to aerosol addition. Results of this study confirm the active role of microzooplankton grazing in controlling the picoplankton biomass, leading to an increase of the proportion of large cells in the phytoplankton community after a major atmospheric deposition event.

With increased phytoplankton growth rate, a significant and immediate increase of microzooplankton grazing mortality on both sizes of phytoplankton and picoplankton populations was induced by aerosol addition across all 3 experiments, suggesting an internal regulation of grazing activity by the growth state of phytoplankton prey. Higher grazing rates on prey cells with higher growth rates has been obtained in many experiments at both the community and individual level (Strom 2002 and reference therein). Field investigations using dilution assays have also showed close trophic feedbacks between the production and consumption of phytoplankton within the microzooplankton community (Chen et al. 2009). This positive feedback could be one of the strategies that enable microzooplankton to maximize their chances of prey encounters and utilization, and thus allow them to survive in the ocean (Strom 2002). Changes in prey physiological characters, such as cell size, cell surface properties and nutritional content could also regulate the feeding behavior of microzooplankton (Christaki et al. 1998, Monger et al. 1999). In our study, the increased nutrient concentrations due to aerosol addition improved the nutritional quality and physiological condition of the phytoplankton cells, and thus accelerated the grazing activity of microzooplankton. Rapid increases in grazing rates of microzooplankton after nutrient addition have also been reported in previous studies (Landry et al. 2000, Suzuki et al. 2002, Worden & Binder 2003).

However, the regulation of microzooplankton grazing in response to aerosol addition is uneven among different size classes of phytoplankton. The degree of increase in grazing mortality of small cells (<20 µm) was slightly higher than that of larger cells (>20 µm) after aerosol addition. Higher microzooplankton grazing impacts on smaller phytoplankton (e.g. cyanobacteria and prymnesiophyceae) than larger prey (e.g. diatoms) has been reported (Gaul & Antia 2001, Liu & Dagg 2003, Huang et al. 2011). Previous studies have reported that grazing on phytoplankton <20 µm in size was closely related to phytoplankton growth rate in the same size classes; whereas grazing on phytoplankton >20 µm was largely dependent of the biomass of large ciliates and dinoflagellates, but was unrelated to phytoplankton growth rate in this size class (Liu & Dagg 2003, Strom et al. 2007). The relative constancy of small phytoplankton in control and aerosol treatments in our study further proved the closely balanced phytoplankton growth and grazing loss at lower food web levels, even under conditions of nutrient perturbation (Landry et al. 2000). In the time-series dilution experiments conducted in Expt 2, we observed that the grazing mortality of small cells in the aerosol treatment was significantly higher compared with that in controls just 3 h after aerosol amendment (D0′), and the difference became more apparent after 2 d of incubation (D2). This quick response indicated that significant increase of growth

rate and nutritional condition of small cells after aerosol addition immediately stimulated the grazing activity of small protists. On the other hand, the grazing mortality of large phytoplankton did not increase as quickly as that of the small cells: it did not exhibit noticeable changes between control and treatment at D0′, but showed a sharp increase in the aerosol treatment at D2 (Fig. 5f). The relatively slow response on grazing of large phytoplankton is possible due to the lower growth rate of large grazers (e.g. dinoflagellates and large ciliates) compared with the small protists (e.g. nanoflagellates and small ciliates) that mainly feed on small phytoplankton. The dramatic accumulation of large phytoplankton biomass also suggests their advantage in escaping from grazing and other loss processes.

Due to the different degree of enhancement in growth and grazing rates of small and large phytoplankton, as discussed above, the proportion of microzooplankton consumption on phytoplankton production was different for the different sizes of phytoplankton. Generally, 40 to 70% of phytoplankton daily production passed through the trophic level of microzooplankton in the microcosm experiments, which is in agreement with the global average reported by Calbet & Landry (2004). Without aerosol addition, a similar percentage of microzooplankton consumption on the production of small and large cells was obtained in Expts 1 and 2 (D0′), while the percentage for micro-phytoplankton was much higher than that for nano- and pico-phytoplankton in Expt 3. The different conditions in Expt 3 were probably because the experiment was conducted at a post-bloom stage, with high chl *a* concentration (~12 µg l^{-1}) mainly composed of diatoms and mixotrophic dinoflagellates (data not shown). However, after aerosol enrichment, a consistent trend of change was observed across all 3 experiments: microzooplankton consumption of nano- and pico-phytoplankton increased, but decreased on mico-phytoplankton (Table 3). Such response, in addition to the stronger growth stimulation for larger phytoplankton than for smaller phytoplankton, further enlarges the size shift from smaller to larger phytoplankton cells. Therefore, the uneven stimulation in growth and grazing mortality of phytoplankton in different size fractions leads to a compositional shift in the phytoplankton community, with typically little change in picoplankton abundance but with a larger change in larger cells — especially diatoms: the 'winners' after nutrient input in both artificial enrichment experiments (Landry et al. 2000, Vuorio et al. 2005, Boyd et al. 2007) and natural processes (Paerl 1997, Blain et al. 2007).

CONCLUSIONS

In the present study, the response of microzooplankton grazing to atmospheric dust input was directly measured for the first time in a subtropical coastal environment to confirm the speculation that atmospheric deposition not only enhance phytoplankton growth, but also microzooplankton grazing. In addition to the significant increases in both phytoplankton growth and microzooplankton grazing after aerosol addition, there was a clear difference in the level of stimulation across the size spectrum and among different populations within the prey and predator communities. Microphytoplankton showed the greatest increase in growth rate, but pico- and nano-phytoplankton bore the greatest increase in mortality due to microzooplankton grazing. Such size- and species-dependent and uneven response in growth and grazing mortality will cause a shift in the phytoplankton community from a small cell- to a large cell-dominated system after a strong atmospheric deposition event, which will have significant implications for the dynamics of the marine plankton food web and carbon flux.

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