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# Impact of atmospheric wet deposition on phytoplankton community structure in the South China Sea



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#### ABSTRACT

The South China Sea (SCS), which is the largest marginal sea in East Asia, plays a significant role in regional climate change. However, research on the phytoplankton community structure (PCS) response to atmospheric wet deposition remains inadequate. In this study, field incubation experiments were performed to survey the impact of atmospheric wet deposition on the PCS in the SCS in December 2013. Results indicate that the mean dissolved inorganic nitrogen/dissolved inorganic phosphorous (DIN/DIP) ratio in rainwater was 136, which was higher than that in seawater. Under low initial nutrient concentrations, rainwater inputs not only significantly increased total chlorophyll a (Chl a) concentrations but also potentially altered the PCS. The total Chl a concentration increased 1.7-, 1.9-, and 1.6-fold; microphytoplankton increased 2.6-, 3.2-, and 1.7-fold with respect to their initial values in the 5%, 10% addition, and 10% addition (filtered) treatment samples, respectively. Finally, microphytoplankton contributed 61% to the total Chl a concentration in 10% addition treatment samples. Differences in the nutrients induced by atmospheric wet deposition resulted in a shift in the advantage from picophytoplankton. Diatoms became the predominant species, accounting for 55% of the total abundance after rainwater addition.

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#### 1. Introduction

Atmospheric deposition is considered a significant source of nutrients in the marine ecosystem (Van Jaarsveld, 1993; Zhang, 1994; Migon and Sadroni, 1999; de Leeuw et al., 2003; Jickells et al., 2005; Boulart et al., 2006; Okin et al., 2011). Atmospheric nutrient inputs, which are normally equal to or greater than river inputs, are recognized as the second largest source of major and trace elements in seawater (Duce and Tindale, 1991; Jickells, 1995). Atmospheric wet deposition is the main source of nutrients, especially in oligotrophic oceans (Owens et al., 1992; Jickells, 1995). A previous study showed that the atmospheric nutrient inputs are estimated to increase in the future (Duce et al., 2008). Marine phytoplankton mainly includes cyanophyta, bacillariophyta, pyrrophyta, euglenophyta, and chrysophyta. Phytoplanktons are classified into three size classes: microphytoplankton (>20 µm), nanophytoplankton (2–20 µm), and picophytoplankton (<2 µm) (Paerl et al., 1990, 2002; Markaki et al., 2003). In general, the size of

\* Corresponding author. Tel./fax: +86 532 66782506. *E-mail address:* jtwang@ouc.edu.cn (J.-T. Wang). cyanophyta is less than 2  $\mu$ m, whereas diatoms and dinoflagellates are larger 20  $\mu$ m (Brotas et al., 2013). Atmospheric wet deposition provides external nutrients that support marine phytoplankton growth, impacts primary production (Zou et al., 2000; Martínez-García et al., 2015), and may even trigger biogenic blooms in oceans (Huo et al., 2001; Wang et al., 2011). New nutrients derived from atmospheric wet deposition can promote marine phytoplankton biomass and nitrogen fixation and enhance marine capacity in absorbing CO<sub>2</sub>. Atmospheric wet deposition could also change the paths of carbon and nitrogen cycles in oceans and potentially impact regional environmental change (Paerl, 1997; Bishop et al., 2002; Jo et al., 2007; Duce et al., 2008; Guo et al., 2012; Shi et al., 2012).

The South China Sea (SCS), which is located in the tropical—subtropical rim of the Northwest Pacific Ocean, is one of the largest marginal seas in the world (Chen et al., 2001). The climate in the SCS is part of the Asian monsoon system. In this system, the northeast monsoon prevails in winter and spring, whereas the southwest monsoon occurs in summer and autumn (http://www. sciencedirect.com/science/article/pii/S0967064513003512Liu et al., 2002). The SCS is essentially oligotrophic, especially in the central basin. In the SCS, chlorophyll *a* (Chl *a*) concentration is usually low, and concentrations of N and P in the euphotic layer are also under detection limits (Ning et al., 2004). Thus, the new nutrients imported by atmospheric deposition may be an important environmental factor to control phytoplankton growth (McGillicuddy et al., 2003; Chen et al., 2006). Guo et al. (2012) used a direct experimental approach to test the effects of atmospheric dry deposition on the PCS and photosynthetic efficiency. However, data that could reveal the response of phytoplankton growth to atmospheric wet deposition in the SCS are limited. This paper aims to evaluate the effects of atmospheric wet deposition on the PCS in the SCS.

#### 2. Materials and methods

#### 2.1. Rainwater and seawater sample collection

Rainwater addition experiments were conducted aboard on December 18–25, 2013 in the SCS.

Rainwater samples were collected within 32 h before the experiments in the areas extending from  $2^{\circ}59''$  N,  $109^{\circ}55''$  E to  $4^{\circ}11''$  N,  $110^{\circ}43''$  E (Fig. 1). A wet deposition collector made of a polyethylene bottle (10 L) was connected to a polyethylene funnel placed on the front deck at 7 m above sea level. Samples were frozen at -20 °C, and subsequently thawed and mixed with seawater samples before the experiments. A 200 mL subsample was stored for chemical analysis. Surface seawater samples were collected using acid-cleaned bottles below the surface (~0.5 m depth) and then screened through a 200 µm mesh to exclude most zooplankton. The *in situ* temperature was 27.5 °C.

#### 2.2. Experimental design

The rainwater addition experimental design included an incubation series for the following treatment samples in10 L bottles prepared in duplicate: (1) control treatment: no rainwater addition; (2) 5% addition: 5% (v/v) rainwater addition; (3) 10% addition: 10% (v/v) rainwater addition; (4) 10% addition (filtered): 10% (v/v) addition of filtered rainwater through nuclepore filters (pore size: 0.7  $\mu$ m). The exact initial volume for each experimental treatment was 8 L. Experimental bottles were placed in plastic containers filled with *in situ* surface seawater to control the incubation temperature. The experimental samples were incubated under *in situ* light–dark conditions for 8 days.



100° 102° 104° 106° 108° 110° 112° 114° 116° 118° 120°

Fig. 1. The sampling areas in the SCS. Quadrangle represents seawater collection site and circles represent the rainwater collection area in December 2013.

#### 2.3. Nutrient analysis

The filtered water sample were collected in 50 mL polyethylene bottles and immediately frozen at -20 °C until subsequent analysis for nutrients. Sampling was conducted once every two days during the incubation period. Concentrations of ammonium (NH<sup>+</sup><sub>4</sub>), nitrite (NO<sup>-</sup><sub>2</sub>), nitrate (NO<sup>-</sup><sub>3</sub>), phosphate (dissolved inorganic phosphorous, DIP), and silicate (SiO<sup>4</sup><sub>4</sub>) were analyzed by spectrophotometry using a Bran + Luebbe Auto Analyzer 3 (Zhu et al., 2006). The detection limits of NH<sup>+</sup><sub>4</sub>, NO<sup>-</sup><sub>2</sub>, NO<sup>-</sup><sub>3</sub>, DIP, and SiO<sup>4</sup><sub>4</sub> were 0.04, 0.003, 0.015, 0.024, and 0.03 µM, respectively. Dissolved inorganic nitrogen (DIN) included NH<sup>+</sup><sub>4</sub>, NO<sup>-</sup><sub>2</sub>, and NO<sup>-</sup><sub>3</sub>. For the rainwater analysis to determine total nitrogen (TN) and total phosphorus (TP), sample was collected in polyethylene bottles and analyzed using persulfate digestion and standard colorimetric methods (Zhu et al., 2006). The precision values of TN and TP determinations were 3% and 5%, respectively.

#### 2.4. Chl a analysis

Water samples each with a volume of 250 mL were taken once every four days during the incubation period. However, Chl a, a proxy for phytoplankton biomass, presents certain problems (Domingues et al., 2008); nevertheless, it was used as the biomass index in numerous references (Hall et al., 2013; Baek et al., 2015; Jakobsen et al., 2015). Chl a was determined in accordance with the work of http://www.sciencedirect.com/science/article/pii/ S0304420399000420 Parsons et al. (1984). For the sizefractionted microphytoplankton (20 - 200)μm), nanophytoplankton (2–20  $\mu$ m) and picophytoplankton (0.7–2  $\mu$ m), the samples were filtered sequentially through 20, 2, and 0.7  $\mu$ m filters, respectively. The filters were immediately wrapped in tin foil and then frozen at -20 °C. Pigments were extracted with 25 mL of 90% acetone (v/v) at 4 °C in the darkroom overnight and then measured using a fluorescence spectrophotometer (Hitachi F-4500). The fluorescence spectrophotometer was calibrated with a Chl a standard (Aladdin, HPLC). The detection limit of Chl *a* was 0.01  $\mu$ g L<sup>-1</sup>.

#### 2.5. Phytoplankton analysis

For microphytoplankton, and nanophytoplankton, 500 mL water samples were fixed with Lugol's iodine soultion. The samples were analyzed under inverted microscope by the Utermöhl method (Edler and Elbrächter, 2010). For picophytoplankton, 4.5 mL of samples were fixed with P+G (1% paraformaldehyde + 0.05% glutaraldehyde final), followed by deep-freezing in liquid nitrogen. Picophytoplankton analysis was conducted using the protocol proposed by Maria et al. (2000) in the laboratory.

#### 2.6. Data analysis

Nutrient consumption rate was calculated according to Xu et al. (2008) as following: nutrient consumption rate  $(\mu) = (N_t - N_0)/t$ , where  $N_t$  and  $N_0$  are nutrient concentrations at days t and 0. In addition to Chl *a*, net phytoplankton growth rate is also the important parameter to judgment of phytoplankton growth state. The phytoplankton growth rate (R) was calculated according to Tang (2010) as:  $R = ln([Chl a]_t/[Chl a]_0)/t$ , where [Chl  $a]_t$  and [Chl  $a]_0$  are Chl *a* concentrations at days t and 0. The specific growth rate (r) was determined according to the following equation:  $r = (lnX_t-lnX_0)/t$ , where  $X_t$  and  $X_0$  are cell numbers at days t and 0. Significant differences between treatments were determined using ANOVA with Duncan comparisons (software used: *SPSS* 19.0) and were accepted at p < 0.05.

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