Effect of Anthropogenic Aerosol Addition on Phytoplankton Growth in Coastal Waters: Role of Enhanced Phosphorus Bioavailability

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Atmospheric deposition can supply nutrients to induce varying responses of phytoplankton of different sizes in the upper ocean. Here, we collected surface and subsurface chlorophyll a maximum (SCM) seawaters from the Yellow Sea and East China Sea to conduct a series of onboard incubation experiments, aiming to explore the impact of anthropogenic aerosol (AR, sampled in Qingdao, a coastal city in Northern China) addition on phytoplankton growth using schemes with (unfiltered seawater, UFS) and without (filtered seawater, FS) microsized (20–200 µm) cells. We found that AR addition stimulated phytoplankton growth obviously, as indicated by chlorophyll a (Chl a) in surface incubations, and had stimulatory or no effects in SCM incubations, which was related to nutrient statuses in seawater. The high ratio of nitrogen (N) to phosphorus (P) in the AR treatments demonstrated that P became the primary limiting nutrient. The alkaline phosphatase activity (APA), which can reflect the rate at which dissolved organic P (DOP) is converted into dissolved inorganic P, was 1.3–75.5 times higher in the AR treatments than in the control, suggesting that AR addition increased P bioavailability in the incubated seawater. Dinoflagellates with the capacity to utilize DOP showed the dominant growth in the AR treatments, corresponding to the shift in phytoplankton size structure toward larger cells. Surprisingly, we found that nanosized (2–20 µm) and picosized (0.2–2 µm) Chl a concentrations in UFS were generally higher than those in FS. The APA in UFS was at least 1.6 times higher than in FS and was proportional to the contribution of microsized cells to the total Chl a, suggesting that microsized cells play an important role in the increase in APA, which contributes to the growth of nanosized and picosized phytoplankton. Current work provides new insight into the increase of P bioavailability induced by atmospheric deposition and resultant ecological effect in coastal waters.

Keywords: atmospheric deposition, nutrients, phytoplankton, size structure, China coastal waters, alkaline phosphatase
INTRODUCTION

Atmospheric deposition can supply a considerable amount of nutrients, including macronutrients such as nitrogen (N) and phosphorus (P), and micronutrients such as iron (Fe) and zinc (Zn), to the ocean (Jickells et al., 2005; Hooper et al., 2019), and affect the phytoplankton size structure and community composition (Okin et al., 2011; Marañón et al., 2015; Zhang et al., 2019). The deposition of a large amount of N promoted the growth of diatoms and inhibited the growth of diazotrophs in the Bay of Bengal and the Arabian Sea (Krishnamurthy et al., 2007). Dust additions increased the N:P ratios in the seawater and induced the dominant growth of nanosized (2–20 µm) phytoplankton in the East China Sea (ECS) (Zhang et al., 2019). The shift in phytoplankton size structure is always accompanied by the competition for nutrients among phytoplankton of different sizes (Stolte and Riegman, 1996; Hutchins et al., 1999). Large phytoplankton (≥2 µm in cell size) have a greater capacity for biomass accumulation and anti-predator defense (Agusti and Kalff, 1989; Finkel, 2007), leading to an advantageous growth in eutrophic seawater. With minimal diffusion boundary layer thickness and a larger specific surface area (Pasciak and Gavis, 1974; Finkel, 2007; Marañón, 2015; Wei et al., 2019), picosized (0.2–2 µm in cell size) phytoplankton have a competitive advantage in oligotrophic seawater (Chisholm, 1992; Finkel, 2007). However, this consensus is roughly defined, and the nutrient competition mechanism between phytoplankton of different sizes is rather complicated in realistic conditions, where the trophic status is not ideally eutrophic or oligotrophic (Chisholm, 1992; Finkel, 2007). For example, in contrast to diatoms, dinoflagellates have a growth advantage in high-nitrate and low-phosphate seawaters due to their acclimatization to high ratios of N:P (Moore et al., 2013; Zhang et al., 2019), even if there is an overlap in the size structure of diatoms and dinoflagellates. In high nutrient low chlorophyll (HNLC) and coastal seawaters, dust additions can induce the rapid growth of different kinds of diatoms covering nanosized and microsized cells (Boyd et al., 2007; Zhang et al., 2018). Our quantitative knowledge of the relationship between nutrient uptake and the growth of different sized phytoplankton is still inadequate.

The impact of atmospheric deposition on primary productivity is generally associated with the substantial supply of N and/or Fe nutrients, whereas few studies focus on P due to its negligible supply relative to N and Fe (Okin et al., 2011; Kim et al., 2014; Wu et al., 2018). Although some studies pointed out that atmospheric deposition can promote the utilization of DOP to relieve P limitation by providing cofactors such as Fe and Zn in open oceans primarily characterized by oligotrophy (Mahaffey et al., 2014; Browning et al., 2017), there are few studies reported in coastal waters characterized by mesotrophy and even eutrophy. In the context of the overwhelming input of N relative to P through various ways such as riverine input and atmospheric deposition, the phenomenon of P limitation becomes increasingly prevailing in coastal waters (Zheng and Zhai, 2021). The impact of atmospheric deposition on marine phytoplankton is not only confined to the traditional relationship between supply (e.g., N and Fe supply) and demand (e.g., N and Fe limitation), but also considering the acclimatization mechanism to copy with the potential P deficiency. A few studies have deduced that atmospheric deposition might enhance the utilization of DOP in P-deficient environments by calculating the P budget in the system and setting up model parameters (Chu et al., 2018; Zhang et al., 2018). However, there is still a lack of direct evidence to verify this hypothesis in coastal waters, and the resultant ecological effect is still poorly understood.

The Yellow Sea (YS) and ECS adjacent to the East Asian continent are marginal seas of the northwestern Pacific Ocean and are obviously influenced by anthropogenic air pollutants from the surrounding continent (Wang et al., 2000; Zhang and Gao, 2007). A series of studies reported that anthropogenic aerosols can transport a long distance to reach coastal seas and even open oceans (Fu et al., 2015; Kang et al., 2017; Xiao et al., 2018). The source apportionment results also showed that particles collected in the YS were full of secondary, biomass burning, and soot-like particles, indicating that marine aerosols are strongly affected by anthropogenic activities (Du et al., 2012; Fu et al., 2015; An et al., 2019). The N:P ratio in anthropogenic aerosol (AR) is generally much higher than the phytoplankton stoichiometry (i.e., Redfield ratio: N:P = 16:1). In the Jiaozhou Bay of the YS, the N:P ratio of atmospheric dry deposition is higher than 100 and even exceeds 1,000 in some specific conditions (Xing et al., 2017; Wu et al., 2018). It has been reported that anthropogenic N deposition has the potential to change nutrient structure in the seawater (Kim et al., 2014). Atmospheric N deposition is regarded as an important factor that induces phytoplankton blooms (Tan and Shi, 2012; Tan and Wang, 2014). On the other hand, under the impact of vertical water mixing, the nutrients in atmospheric deposition can be transferred to the subsurface layers. Model studies have shown that the supplementation of N in surface waters to the lower layer is an important reason for the formation of subsurface chlorophyll a maximum (SCM) (Hodges and Rudnick, 2004; Gong et al., 2017). In contrast to the surface layer, few studies focused on the impact of atmospheric deposition on phytoplankton in the SCM layer.

In this study, we carried out three onboard incubation experiments enriched with AR using surface and SCM seawaters in the YS and ECS. The unfiltered and filtered (through 20-µm membrane) seawaters were used to illustrate the effects of microsized (20–200 µm) phytoplankton on the growth and nutrient uptake of nanosized (2–20 µm) and picosized ones. Based on this, our study intended to (1) reveal the difference in phytoplankton response to AR addition in surface and SCM seawaters; (2) identify the main factor of AR addition that affects the growth and community structure succession of phytoplankton; and (3) explore the interaction between different sized phytoplankton under the effects of AR addition.

MATERIALS AND METHODS

Incubation Experiments

The AR samples used for incubation experiments were collected with a cellulose acetate filter membrane (Whatman 41) on the Laoshan campus of Ocean University of China (36°9’39"N,
(30°29'29"E) on 30 June 2019. During the sampling period, the AQI was 57–83 µg m⁻³, indicating that the air quality is moderate. The detailed air quality conditions are shown in Table 1.

In the summer of 2019, three onboard microcosm experiments were conducted during the cruise of R/V Beidou in the YS and the northern part of the ECS (Figure 1A). The initial seawater at U1, U2 and U3 was collected from surface layers (~3–5 m below the water surface) and SCM layers (captured by the CTD profile data) using Niskin bottles with Sea Bird CTD-General Oceanic Rosette assembly (Table 2). The site name is abbreviated as Uisur and Uiscm, respectively, and i refers to the site number, i.e., 1/2/3.

Based on the content of N in AR aerosols, three treatments were conducted in triplicate for the incubation experiments: (1) control, no AR addition; (2) low AR addition, the added amount of AR was expressed in the unit of N (i.e., 1 µmol N L⁻¹) at U2, indicating that the added amount of AR contains 1 µmol N L⁻¹; (3) high AR addition, 1.7 µmol N L⁻¹ at U3, and 2 µmol N L⁻¹ at U1. AR sample was first ultrasonically extracted in deionized water at 0°C for 1 h, and the leaching solution including particles was added to the incubation bottles directly (Guo et al., 2013). Apart from inorganic nutrients, the aerosol additions could stimulate mixotrophic dinoflagellates by promoting the utilization of organic matter (Granéli et al., 1999; Heisler et al., 2013). Apart from inorganic nutrients, the aerosol additions could stimulate mixotrophic dinoflagellates by promoting the utilization of organic matter (Granéli et al., 1999; Heisler et al., 2013).

### Table 1: Air quality conditions during the anthropogenic aerosol (AR) sampling period.

| Parameter          | Concentration |
|--------------------|---------------|
| Humidity (%)       | 62–91         |
| AQI (µg·m⁻³)       | 57–83         |
| PM₂.₅ (µg·m⁻³)    | 29–61         |
| PM₁₀ (µg·m⁻³)      | 63–94         |
| NH₄⁺ (µmol·m⁻³)    | 0.17          |
| NO₃⁻ + NO₂⁻ (µmol·m⁻³) | 0.43 |
| PO₄³⁻ (nmol·m⁻³)  | 7.37          |
| Fe (nmol·m⁻³)      | 31.88         |
| Zn (nmol·m⁻³)      | 2.31          |
| Al (nmol·m⁻³)      | 72.24         |
| Mn (nmol·m⁻³)      | 0.98          |
| Cu (nmol·m⁻³)      | 0.19          |
| Cd (nmol·m⁻³)      | 0.01          |
| Ni (nmol·m⁻³)      | 0.13          |
| Pb (nmol·m⁻³)      | 0.13          |
| Co (nmol·m⁻³)      | 0.01          |

Humidity data during sampling were obtained from National Meteorological Information Center (NMIC, https://data.cma.cn/), AQI, PM₂.₅, and PM₁₀, were obtained from China National Environmental Monitoring Center (CNEMC, http://www.cnemc.cn/). The soluble nutrients and total trace metal concentrations in aerosols were sampled and determined in the laboratory (Zhao et al., 2015).

### Measurements of Chlorophyll a, Nutrients, and the Phytoplankton Community Structure

#### Chlorophyll a

Approximately 150 ml of seawater from incubated bottles was sampled at ~07:00 a.m. every day during the incubations. The sampled seawater was subsequently filtered through 20-, 2-, and 0.2-µm filters, to obtain microsized, nanosized, and picosized cells. After 20–24 h of extraction by 90% acetone in darkness at ~20°C, the pigments collected by different filters were measured using a Trilogy fluorometer (Turner Designs). The total Chl a concentration was obtained by summing three size-fractionated Chl a concentrations.

### Nutrients

An ultrasonic method was used to leach nutrients in AR samples. Briefly, AR samples were ultrasonically extracted in deionized water at 0°C for 1 h. The leaching solution was then filtered through a 0.45-µm polyethersulfone syringe filter (Shi et al., 2010). The filtrates were used for the determination of soluble nutrients from aerosols, including NO₃⁻, NO₂⁻, NH₄⁺, Si(OH)₄, and PO₄³⁻. In addition, ~200 ml of incubated seawater (sampled every day) was filtered through acid-washed cellulose acetate membranes into 125-ml acid-washed high-density polyethylene bottles (pre rinsed with the filtrates three times). The water samples were frozen at -20°C immediately prior to the determination of NO₃⁻ + NO₂⁻, PO₄³⁻, and Si(OH)₄ in the university laboratory. All nutrient samples were measured with a QuAAtro continuous-flow analyzer (SEAL Analytical). The detection limits for NH₄⁺, NO₃⁻, NO₂⁻, PO₄³⁻, and Si(OH)₄ were 0.04, 0.02, 0.005, 0.01, and 0.03 µmol L⁻¹, respectively. For convenience, NO₃⁻ + NO₂⁻ is abbreviated to N + N.
The concentrations of total trace metals were analyzed by inductively coupled plasma mass spectrometry (ICP-MS) (Shi et al., 2012). The 8-cm² cellulose acetate filter was put into the Teflon high pressure vial, with 2 ml of 69% HNO₃ and 0.5 ml of 40% HF. After digestion at 180°C for 48 h, the solution was evaporated at 160°C, the residue was dissolved with 2% HNO₃ and diluted to 50 ml for determination.

**Alkaline Phosphatase Activity**

About 45 ml of seawater was sampled from the incubated bottles and mixed with 0.5 ml of fluorogenic substrate 4-methylumbelliferone phosphate (MUF-P) as the mixed substrate. After the addition of the mixed borax-sodium carbonate buffer solution (pH ≈ 11) and the mixed substrate to the sample tube, the fluorogenic substrate MUF-P hydrolyzed by AP was converted into equimolar phosphate group and 4-methylumbelliferone (MUF). The fluorescence value of MUF was measured and recorded by a Trilogy fluorometer (Turner Designs) at 0, 0.5, and 1 h (Sebastián et al., 2004). The slope was calculated as the hydrolysis rate, which reflect the alkaline phosphatase activity (APA).

**High-Throughput Sequencing**

Approximately, 1 L of seawater from incubated bottles was filtered using 0.22-μm Whatman polycarbonate filters under gentle vacuum pressure (≤ 0.02 MPa). Filters were stored immediately in liquid nitrogen until DNA extraction and high-throughput sequencing (Shanghai Personal Biotechnology Co., Ltd., Shanghai, China). The V4 hypervariable region was selected as the target region of the 18S rDNA (Liu et al., 2021).

### TABLE 2 | Background conditions of seawater at the experimental sites.

| Site | Incubation dates (2019) | Water layer | Water depth (m) | Temperature (°C) | Salinity | NO₃⁻ + NO₂⁻ (μmol·L⁻¹) | PO₄³⁻ (μmol·L⁻¹) | Si(OH)₄ (μmol·L⁻¹) | N:P (µmol:µmol) | APA (nmol·(L·h)⁻¹) | Chl a (µg·L⁻¹) | Micro Chl a (%) | Nano Chl a (%) | Pico Chl a (%) | Dinophyceae (%) |
|------|-------------------------|-------------|----------------|-----------------|----------|-------------------------|-----------------|-----------------|----------------|-----------------|---------------|----------------|--------------|--------------|----------------|
| U1   | Aug 16-21               | Surface     | 3              | 24.2            | 31.5     | 0.19                    | 2.85            | 35.3            | 0.38           | 9               | 18            | 73             | 60           | 9            | 60             |
| U2   | Sep 3-8                 | Surface     | 19             | 28.6            | 31.8     | 1.70                    | 4.78            | 3.4             | 1.19           | 26              | 22            | 52             | 95           | 93           | 95             |
| U3   | Aug 22-28               | Surface     | 3              | 26.7            | 31.4     | 0.08                    | 1.34            | 2.2             | 0.55           | 8               | 8             | 65             | 85           | 8            | 85             |
|      |                         | SCM         | 34             | 27.7            | 32.8     | 2.59                    | 4.90            | 3.7             | 0.95           | 8               | 8             | 62             | 89           | 9            | 89             |

ND, not detectable.

**FIGURE 1** | Water sampling stations used for the microcosm incubation experiments in the Yellow Sea and East China Sea (A) and the treatment procedure of the incubations (B). Where micro refers to the contribution of microsized Chl a to total Chl a.
primers for polymerase chain reaction (PCR) were forward primer 582F, 5'-CCAGGASYGCGGTATCTCC-3' and reverse primer V4R, 5'-ACTTTCGTTCTTGATYRA-3' (Hernández-Ruiz et al., 2020). The Illumina NovaSeqPE250 platform was used for paired-end sequencing of community DNA fragments. First, we demultiplexed the raw sequence data and then invoked QIIME cutadapt trim-paired to cut the primers (Martin, 2011). Quality control of these sequences was performed using the DADA2 plugin with QIIME dada2 denoise-paired (Callahan et al., 2016). Then, we merged amplicon sequence variants (ASVs) and removed singleton ASVs. A pretrained naive Bayes classifier plugin was used to annotate the species for each ASV using QIIME2 software (2019.4) (Bokulich et al., 2018). The SILVA database (Release132)1 was used for species annotation. The microbiome bioinformatics of communities was analyzed using QIIME2 (2019.4). The accession number in NCBI Sequence Read Archive was PRJNA835313.

Data Analysis
We used one-way ANOVA to assess whether there was a significant difference in the Chl a concentration between the control and treatments (Andersen et al., 2020) and evaluated the nutrient limitation in surface seawater at the three sites. Statistical analysis was performed using IBM SPSS Statistics 20 (SPSS 20.0). CANOCO software (version 5.0) was used to analyze the relationships between environmental factors and phytoplankton. The detrended correspondence analysis (DCA) used species-sample data showed that the first axis of gradient was less than 3. Therefore, redundancy analysis (RDA) was the better choice.

RESULTS
Overview of Original Seawater in Surface and Subsurface Chlorophyll a Maximum Layers
In general, trophic statuses in surface seawater at U1Sur, U2Sur, and U3Sur and in SCM seawater at U3SCM were lower than those in SCM seawater at U1SCM and U2SCM. Phytoplankton at U1-3Sur were colimited by N and P based on the significant increase in Chl a after N + P addition relative to the control treatment (on days 4–6, \( p < 0.05 \), Supplementary Figure 1).

At U1Sur, the concentrations of N + N, PO\(_4^{3-}\), and Si(OH)\(_4\) were 0.19, 0.01, and 2.85 \( \mu \)mol L\(^{-1} \), respectively. APA was less than 18.4 nmol (L h\(^{-1} \)), and the

1http://www.arb-silva.de
Changes in Inorganic Nutrients

The N:P ratio after AR addition increased from 11:1-20:1 to 44:1-54:1 at all sites in the surface incubated seawater and at U3SCM in the SCM incubated seawater. Due to the sufficient nutrient stock in the original seawater at U1SCM and U2SCM, the N supplied by AR addition only increased the N:P ratio from 10:1-24:1 to 16:1-28:1. Because of the low contents of PO$_4^{3-}$ and Si(OH)$_4$ in the AR, the changes in the concentrations of PO$_4^{3-}$ and Si(OH)$_4$ in AR-amended seawater were slight at all sites.

During the incubations at U1-3Sur and U3SCM, the concentrations of N + N did not change significantly in the control and AR treatments (Figure 2). In the AR treatments, the N + N concentrations remained relatively stable and were significantly higher than those in the control treatments. In contrast, the concentrations of PO$_4^{3-}$ were close to the detection limit in all treatments. The maximum consumption of Si(OH)$_4$ in the control and AR treatments was less than 13% at U1-3Sur and U3SCM. At U1SCM and U2SCM, the concentrations of N + N and PO$_4^{3-}$ decreased gradually by more than 85% in the control and AR treatments relative to the original values. The concentrations of Si(OH)$_4$ decreased sharply in the control (>28%) and AR treatments (>69%) on days 3-5 at both sites (Figure 2 and Supplementary Figure 2).

There were no obvious differences in the concentrations of N + N, PO$_4^{3-}$, and Si(OH)$_4$ between FS and UFS in the control and AR treatments at U1-3Sur and U3SCM. In the FS at U1SCM and U2SCM, the consumption of N + N and Si(OH)$_4$ in AR treatments
was lower than that in the UFS at the end of the incubations ($p < 0.05$, Figure 2 and Supplementary Figure 2).

**Changes in Alkaline Phosphatase Activity**

In the UFS, the APA in the AR treatments was 1.3-75.5 times higher than that in the control at the end of the incubations ($p < 0.05$, Figure 3), and this phenomenon could be observed in both surface and SCM incubations. In contrast, there was almost no difference in APA between control and AR treatments on the last day of FS incubations at all sites except U1\_SCM. For the AR treatments, the APA in the UFS at all sites was generally higher (1.6-7.3 times) than that in the FS (Figure 3).

**Changes in Total and Size-Fractionated Chlorophyll a**

At U1\_3Sur, the concentration of the total Chl $a$ in the AR treatments was generally higher than that in the control. At U1\_SCM, the total Chl $a$ concentration in the AR treatments was more than 1.2 times higher than that in the control on days 2-5. At U2\_SCM and U3\_SCM, there was no significant difference in Chl $a$ between the control and AR treatments (Figure 4). The responses of phytoplankton of different sizes varied with AR addition. At U1\_Sur, the dominant size of phytoplankton changed from picoseize to nanosize (46% contribution to total Chl $a$). A similar pattern in the size shift toward larger cells also occurred at U2\_Sur and U3\_Sur, although picosized cells always dominated the contribution to total Chl $a$ (Figure 5). At U1\_SCM and U2\_SCM, the dominant contributor of phytoplankton in AR treatments was picosized cells on days 1-2 and shifted to microsized cells ($\geq$49% contribution to total Chl $a$) on day 5. At U3\_SCM, the dominant contributor was always picosized phytoplankton during the incubations (Figure 5).

For AR treatments, the concentrations of nanosized and picosized Chl $a$ in UFS were generally higher than those in FS at U1\_Sur and U3\_Sur. Specifically, at U1\_Sur, nanosized and picosized Chl $a$ concentrations in the UFS enriched with AR were 2.4 and 1.8 times higher than those in the FS on day 5. At U3\_Sur, the picosized Chl $a$ concentration in UFS enriched with AR was 2.7 times higher than that in FS on day 6. Similar to the incubations with surface seawater, the picosized Chl $a$ concentration enriched with AR in FS (0.10 $\mu$g L$^{-1}$) at U3\_SCM was lower than that in UFS (0.25 $\mu$g L$^{-1}$) on day 6. In contrast, at U1\_SCM, nanosized and picosized Chl $a$ concentrations in FS (4.72 and 0.36 $\mu$g L$^{-1}$) were higher than those in UFS (2.89 and 0.22 $\mu$g L$^{-1}$) on day 5.
U2SCM, there was no significant difference in the concentrations of size-fractionated Chl a between UFS and FS (Figure 4).

**Changes in the Phytoplankton Community**

The ASVs at all sites assigned to phytoplankton could be classified into 25 groups of eukaryotic microalgae at class level (level 3). The ASV richness of Dinophyceae (dinoflagellates) accounted for ≥60% of phytoplankton in the original seawater at each site. In terms of UFS, Dinophyceae dominated the phytoplankton community in the AR treatments at all sites (Figure 6). The dominant class changed from Dinophyceae to Chloropiconophyceae (71%) in the control on day 5 of the incubations (corresponding to the maximum Chl a concentration) at U1Sur. Chloropicon spp. was the main component of Chloropiconophyceae (Supplementary Figure 3). With AR addition, the relative abundance of Dinophyceae increased to 46% being the dominant phytoplankton (Figure 6). The succession of phytoplankton communities at U3SCM was similar to that at U1Sur. Dinophyceae maintained the dominant status in the control and AR treatments at the rest of the incubation sites (Figure 6).

**DISCUSSION**

Based on the distinct nutrient concentrations (N, P, and Si) in the original seawaters, the sites used for incubations were classified into two types: U1-3Sur and U3SCM with lower trophic status, where the concentrations of N + N, PO₄³⁻, and Si(OH)_4 did not exceed 0.50, 0.02, or 3.00 µmol L⁻¹, respectively; U1SCM and U2SCM with higher trophic status, where the concentrations of N + N, PO₄³⁻, and Si(OH)_4 exceeded 1.50, 0.10, and 4.50 µmol L⁻¹, respectively.

**Distinct Responses of Phytoplankton to Anthropogenic Aerosol Additions in Surface and Subsurface Chlorophyll a Maximum Seawaters**

Aerosol additions generally stimulated phytoplankton growth and shifted the phytoplankton size structure toward larger cells
in surface incubated seawaters (Figure 5). This is justified because of the established supplementary relationship between nutrients (primarily N) supplied by AR and phytoplankton requirements. Such fertilization effect has also been widely reported in the previous studies (Cottingham, 1999; Liu et al., 2013; Zhang et al., 2019). In contrast, AR additions had a limited effect on phytoplankton size structure at U3_{SCM}, although its trophic status was similar to those in surface seawaters (Figure 5). This is ascribed to the photo-acclimation of phytoplankton under the condition of low irradiance in SCM layer (Fujiki and Taguchi, 2002; Fu et al., 2018). With the abrupt enhancement of light intensity (from SCM to surface), phytoplankton in the incubated seawater need to readjust to the new environment and thus showed a limited response to AR additions.

Interestingly, AR addition had a significant fertilization effect on phytoplankton growth at U1_{SCM} (Figure 4), which was characterized by the higher trophic status among these sites. Note that there was a shift in dominant phytoplankton from picosized cells to large cells during the incubations at U1_{SCM}, which was different from the sustaining dominance of picosized cells at U3_{SCM} (Figure 5). Moreover, large phytoplankton can better acclimate to the abrupt increase in light intensity compared with picosized cells, due to their stronger self-shading capacity by the pigment (package effect) to reduce light absorption (Marañón, 2015). At U2_{SCM}, the stimulation effect of AR addition was not as obvious as that at U1_{SCM} (Figure 4). This is because there was an obvious shift of dominant algae from Coscinodiscophycceae in the control to Dinophycceae in the AR treatments at U2_{SCM} (Figure 6). The obvious succession in phytoplankton community while slight change in Chl a under the condition of aerosol enrichment was also observed in eutrophic seawaters of the ECS (Meng et al., 2016). The substantial input of N relative to P supplied by AR addition increased the N:P ratio from 24:1 in the original seawater to 28:1 in the AR treatments at U2_{SCM}, which was more favorable for the growth of dinoflagellates (Zhang et al., 2019; Table 2). At U1_{SCM}, in contrast, the N:P ratio ranged between 10:1 and 16:1 in the control and AR treatments, leading to the increase in relative abundance of diatoms (primarily Coscinodiscophycceae) (Zhang et al., 2019). Collectively, in contrast to the consistent phytoplankton response to AR addition in surface seawater, the impact of AR addition in SCM seawater is complicated, which is closely related to nutrient concentration and structure in seawater.

Utilization of Dissolved Organic P Enhanced by Anthropogenic Aerosol Addition

The substantial N supplied by AR had the potential to alleviate and even alter N pressure of phytoplankton in the incubated...
seawater (Figure 2). As a result, the relatively P-deficient environment created by AR additions made it possible for phytoplankton to induce acclimatization mechanisms to cope with P stress (Moore et al., 2013). As shown in Figure 3, the APA value in the AR treatments was higher than that of the control at the end of the incubations at all sites, indicating that AR could enhance the utilization of DOP to increase P bioavailability in the incubated seawater. Such phenomenon was supported by the good correlation between PO$_4^{3-}$ and APA (Figure 7). In part from the establishment of P-deficient environment, AR additions also provided a considerable amount of soluble Fe and Zn, which acted as cofactors of phosphohydrolytic
enzymes (Mills et al., 2004; Mahaffey et al., 2014). The promotion effect of AR addition on the utilization of DOP shows the acclimation of phytoplankton to the overwhelming N input relative to P in coastal waters (Zhang et al., 1999; Zamora et al., 2010; Xing et al., 2017) and is conducive to understanding P biogeochemical cycles in the perspective of atmospheric deposition.

In terms of the phytoplankton community composition, Dinophyceae generally dominated the community in UFS enriched with AR at all sites (Figure 6). With relatively high tolerance to nutrient-deficient environments and the potential to utilize DOP by inducing the expression of the gene for the synthesis of AP (Lin et al., 2012), dinoflagellates showed an advantageous growth in the AR treatments. This is also the reason why nutrient limitation had a slight impact on the growth of Dinophyceae based on RDA (Figure 7). In addition, due to the selective feeding of micrograzers, picosized phytoplankton suffer from a higher grazing pressure (Cottingham, 1999; Strom et al., 2007). In contrast, large dinoflagellates have the ability to keep themselves away from the prey of the dominant zooplankton species Paracalanus parvus (Hexanauplia) through particle rejection behavior (reject particles as food, Supplementary Figure 4; Huntley et al., 1986; Tiselius et al., 2013).

Role of Microsized Phytoplankton in Affecting the Growth of Nanosized and Picosized Phytoplankton

As described in “Changes in Total and Size-Fractionated chlorophyll a” section at U1_Sur, U3_Sur, and U3_SCM characterized by lower trophic statuses, the nanosized and picosized Chl a concentrations in FS enriched with AR were lower than those in UFS. In contrast, we did not observe similar results at U1_SCM and U2_SCM characterized by higher trophic statuses (Figure 4).

Nutrients, irradiance, and temperature are considered the three major factors that affect phytoplankton growth (Laws et al., 2000; Litchman, 2007). There was no difference in light and temperature between UFS and FS, and thus, nutrients play a key role in causing the lower nanosized and picosized Chl a concentrations in FS. At U1-3_Sur and U3_SCM, there were no obvious differences in N + N and PO4_3− between UFS and FS (Figure 2). Meanwhile, we found that the APA values in UFS were 1.6-7.3 times higher than those in FS (Figure 3), indicating that microsized cells played an important role in increasing P bioavailability in the incubated seawater (Sebastián et al., 2004). This was supported by the positive linear relationship between APA and the contribution of microsized cells to the total Chl a (Figure 8A). As an extracellular enzyme, AP enters the environment through autolyzing or organisms excreting (Štrojsová et al., 2003). Besides, in contrast to FS, nanosized and picosized Chl a concentration in UFS increased linearly with relative change of APA (Figure 8B). Therefore, under the impact of AR addition, microsized cells have the ability to favor the growth of nanosized and picosized cells by increasing P bioavailability in seawater. The result at U1_SCM characterized by higher trophic statuses could also support this argument. On days 1-2 of the incubations, there was no difference in the nanosized and picosized Chl a concentrations in the AR treatments between UFS and FS when PO4_3− was sufficient in the seawater, but lower nanosized and picosized Chl a concentrations in FS were measured when PO4_3− was exhausted on day 3 (Figures 2, 4). Besides, dinoflagellates and green algae with the capacity of utilizing DOP also showed advantageous growth in the P-deficient condition (Figure 6).

There are other factors that might have caused the mismatch between UFS and FS in the concentration of nanosized and picosized Chl a. For example, the biodiversity of the community decreased after removal of microsized cells, which increased the difficulty for the community to reestablish a new balance (Dyke et al., 2007). Nanosized and picosized phytoplankton may adopt a strategy to survive in unstable habitats, e.g., by producing spores that can be dormant temporarily and revive at an appropriate time (Nayaka et al., 2017). However, these inferences cannot account for the change in nanosized and picosized Chl a at U1_SCM and U2_SCM characterized by higher trophic statuses (Figure 4). Therefore, our study provides a new clue from the perspective of nutrient utilization to illustrate how microsized phytoplankton affect the growth of nanosized and picosized ones.

CONCLUSION

In this study, we conducted three onboard incubation experiments using surface and SCM seawaters under the condition of sea surface light intensity in the Yellow Sea and East China Sea. AR addition generally stimulated phytoplankton growth in surface incubations and had a stimulatory or slight impact in SCM incubations, which primarily depends on the nutrient concentration and structure in seawater. We also found that AR addition could alleviate P limitation by promoting the utilization of DOP in both surface and SCM incubations. Specifically in seawater with lower trophic status, microsized cells have the ability to promote the growth of nanosized and picosized cells by increasing P bioavailability in the incubated seawater. Considering the lower contribution of microsized cells in the oligotrophic areas of the open oceans (López-Urrutia and Morán, 2015; Marañón et al., 2015), such a promotion effect of microsized cells induced by anthropogenic aerosol deposition may focus on coastal waters and thus can be regarded as a result of anthropogenic influences to a large extent. With the enhanced influence of human activities in the recent years, atmospheric deposition characterized by high N:P ratios has intensified the prevailing P limitation in offshore waters (Harrison et al., 1990; Xú et al., 2008). The acclimation mechanism of different sized phytoplankton to P limitation under the influence of atmospheric deposition deserves to be further investigated.
DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

AUTHOR CONTRIBUTIONS

QW: conceptualization, investigation, methodology, data curation, formal analysis, visualization, software, and writing – original draft. CZ: conceptualization, investigation, writing – review and editing, data curation, and funding acquisition. HJ: investigation. YC and XY: writing – review and editing. HG: supervision, methodology, resources, writing – review and editing, and funding acquisition and also was responsible for ensuring that the descriptions are accurate and agreed by all authors. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2022.915255/full#supplementary-material

REFERENCES

Agusti, S., and Kalf, J. (1989). The influence of growth conditions on the size dependence of maximal algal density and biomass. Limnol. Oceanogr. 34, 1104–1108. doi: 10.4319/lo.1989.34.6.1104
An, Z., Huang, R., Zhang, R., Tie, X., Li, G., and Cao, J. (2019). Severe haze in northern China: a synergy of anthropogenic emissions and atmospheric processes. Proc. Natl. Acad. Sci. U.S.A. 116, 8657–8666. doi: 10.1073/pnas.1900125116
Andersen, I. M., Williamson, T. J., González, M. J., and Vanni, M. J. (2020). Nitrate, ammonium, and phosphorus drive seasonal nutrient limitation of chlorophytes, cyanobacteria, and diatoms in a hyper-eutrophic reservoir. Limnol. Oceanogr. 65, 962–978. doi: 10.1002/lno.1363
Bokulich, N. A., Kacker, B. D., Rideout, J. R., Dillon, M., Bolyen, E., and Knight, R. (2018). Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2’s q2-feature-classifier plugin. Microbiome 6:90. doi: 10.1186/s40168-018-0470-z
Boyd, P. W., Pitchkell, T., Law, C. S., Blain, S., Boyle, E. A., and Buesseler, K. O. (2007). Mesoscale iron enrichment experiments 1993–2005: synthesis and future directions. Science 315, 612–617. doi: 10.1126/science.1131669
Browning, T. J., Achterberg, E. P., Yong, J. C., Rapp, I., Utermann, C., and Engel, A. (2017). Iron limitation of microbial phosphorus acquisition in the tropical North Atlantic. Nat. Commun. 8:15465. doi: 10.1038/ncomms15465
Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., and Callahan, B. J., Mcmurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., and Holmes, S. P. (2016). DADA2: high-resolution sample inference from Illumina amplicon data. Nat. Methods 13, 581–583. doi: 10.1038/nmeth.3869
Chisholm, S. W. (1992). “Phytoplankton Size, “ in Evolution of Modern Marine Food Webs, in Evolution of Primary Producers in the Sea, eds P. G. Falkowski and A. H. Knoll (Burlington: Academic Press), 333–350.
Fu, H., Zheng, M., Yan, C., Li, X., Gao, H., and Yao, X. (2015). Sources and characteristics of fine particles over the Yellow Sea and Bohai Sea using online single particle aerosol mass spectrometer. J. Environ. Sci. China 29, 62–70. doi: 10.1016/j.jes.2014.09.031
Fu, M., Sun, P., Wang, Z., Wei, Q., Pu, Z., and Zhang, X. (2018). Structure, characteristics and possible formation mechanisms of the subsurface chlorophyll maximum in the Yellow Sea Cold Water Mass. Cont. Shelf Res. 165, 93–105. doi: 10.1016/j.csr.2018.07.007
Fujiki, T., and Taguchi, S. (2002). Variability in chlorophyll a specific absorption coefficient in marine phytoplankton as a function of cell size and irradiance. J. Plankton Res. 24, 859–874. doi: 10.1016/j.jplankres.2010.12.009
Gong, X., Jiang, W., Wang, L., Gao, H., Boss, E., and Yao, X. (2017). Analytical solution of the nitracline with the evolution of subsurface chlorophyll maximum in stratified water columns. J. Geophys. Res. Biogeosci. 14, 2371–2386. doi: 10.1111/1542-4974.12955
Granéli, E., Carlsson, P., and LeGrand, C. (1999). The role of C, N and P in dissolved and particulate organic matter as a nutrient source for phytoplankton growth, including toxic species. Aquat. Ecol. 33, 17–27. doi: 10.1023/A:1009925510509
Guo, C., Jing, H., Kong, L., and Liu, H. (2013). Effect of East Asian aerosol enrichment on microbial community composition in the South China Sea. J. Plankton Res. 35, 485–503. doi: 10.1016/j.jplankres.2010.12.009
Harrison, P. J., Hu, M. H., Yang, Y. P., and Lu, X. (1990). Phosphate limitation in estuarine and coastal waters of China. J. Exp. Mar. Biol. Ecol. 140, 79–87. doi: 10.1016/0022-0981(90)90083-O
Heisler, J., Gilbert, P. M., Burkholder, J. M., Anderson, D. M., Cochlan, W., and Dennison, W. C. (2008). Eutrophication and harmful algal blooms: a scientific consensus. Harmful Algae 8, 3–13. doi: 10.1016/j.hal.2008.06.006
Hernández-Ruiz, M., Barber-Lluçà, E., Prieto, A., Logares, R., and Teira, E. (2020). Response of pico-nano-eukaryotes to inorganic and organic nutrient additions. Estuar. Coast. Shelf. Sci. 235:106565. doi: 10.1016/j.ecss.2019.106565
Hodges, B. A., and Ruddy, D. L. (2004). Simple models of steady deep maxima in chlorophyll and biomass. Deep Sea Res. Part I Oceanogr. Res. Pap. 51, 999–1015. doi: 10.1016/j.dsr.2004.02.009
Hooper, J., Mayewski, P., Marx, S., Henson, S., Potocki, M., and Sneed, S. (2019). Examining links between dust deposition and phytoplankton response using ice cores. Aeol. Res. 36, 45–60. doi: 10.1016/j.aeolres.2018.11.001
Hunter, M., Sykes, P., Rohan, S., and Marin, V. (1986). Chemically-mediated rejection of dinoflagellate prey by the copepods Calanus pacificus and Paracalanus parvus: mechanism, occurrence and significance. Mar. Ecol. Prog. Ser. 28, 105–120. doi: 10.3354/meps028105
Hutchins, D. A., Witter, A. E., Butler, A., and Luther, G. W. (1999). Competition among marine phytoplankton for different chelated iron species. Nature 400, 858–861. doi: 10.1038/23680

Jickells, T. D., An, Z. S., Andersen, K. K., Baker, A. R., Bergametti, G., Brooks, N., et al. (2005). Global iron connections between desert dust, ocean biogeochemistry, and climate. Science 311, 67–71. doi: 10.1126/science.1105959

Kang, M., Yang, F., Ren, H., Zhao, W., Zhao, Y., and Li, L. (2017). Influence of continental organic aerosols to the marine atmosphere over the East China Sea: insights from lipids, PAHs and phthalates. Sci. Total Environ. 60, 339–350. doi: 10.1016/j.scitotenv.2017.06.214

Kim, T., Lee, K., Duce, R., and Liss, P. (2014). Impact of atmospheric nitrogen deposition on phytoplankton productivity in the South China Sea. Geophys. Res. Lett. 41, 3156–3162. doi: 10.1002/2014GL061965

Krishnamurthy, A., Moore, J. K., Zender, C. S., and Luo, C. (2007). Effects of atmospheric inorganic nitrogen deposition on ocean biogeochemistry. J. Geophys. Res. Biogeoosci. 112:G02019. doi: 10.1029/2006G000334

Laws, E. A., Falkowski, P. G., Smith, W. O. Jr., Ducklow, H., and Mccarthy, J. J. (2000). Temperature effects on export production in the open ocean. Global Biogeochem. Cy. 14, 1231–1246. doi: 10.1029/1999GB001229

Lin, X., Zhang, H., Cui, Y., and Lin, S. (2012). High sequence variability, diverse subcellular localizations, and ecological implications of alkaline phosphatase in Dinoflagellates and other eukaryotic phytoplankton. Front. Microbiol. 3:235. doi: 10.3389/fmicb.2012.00235

Litchman, E. (2007). “Chapter 16 - Resource competition and the ecological success of phytoplankton,” in Evolution of Primary Producers in the Sea, eds P. G. Falkowski and A. H. Knoll (Burlington: Academic Press), 351–375.

Liu, Q., Zhao, Q., Minmtn, A., Yang, E. I., and Jiang, Y. (2021). Planktonic microbial eukaryotes in polar surface waters: recent advances in high-throughput sequencing. J. Mar. Sci. Technol. 3, 94–102. doi: 10.1007/s42995-020-00062-y

Liu, Y., Zhang, T. R., Shi, J. H., Gao, H. W., and Yao, X. H. (2013). Responses of chlorophyll a to added nutrients, Asian dust, and rainwater in an oligotrophic zone of the Yellow Sea: implications for promotion and inhibition effects in an incubation experiment. J. Geophys. Res. Biogeoisci. 118, 1763–1772. doi: 10.1002/2013JG002329

López-Urrutia, Á. and Morán, X. A. G. (2015). Temperature affects the size-structure of phytoplankton communities in the ocean. Limnol. Oceanogr. 60, 733–738. doi: 10.1002/lno.10049

Mahaftey, C., Reynolds, S., Davis, C. E., and Lohan, M. C. (2014). Alkaline phosphatase activity and its relationship to inorganic phosphorus among marine phytoplankton. EMBrNet J. 17, 10–12. doi: 10.14806/ ej.17.1.200

Meng, X., Chen, Y., Wang, B., Ma, Q. W., and Wang, F. I. (2016). Responses of phytoplankton community to the input of different aerosols in the East China Sea. Geophys. Res. Lett. 43, 7081–7088. doi: 10.1002/2016GL069068

Mills, M. M., Ridame, C., Davey, M., La Roche, J., and Geider, R. J. (2004). Iron and nitrogen co-limitation of grazing and related parameters in the East China Sea. Environment 58, 1657–1666. doi: 10.1016/j.marenvres.2003.08.007

Moran, E. (2015). Cell size as a key determinant of phytoplankton metabolism and community structure. Ann. Rev. Mar. Sci. 7, 241–264. doi: 10.1146/annurev-marine-011814-015955

Morán, E., Cerveño, P., Latasa, M., and Tatadlaké, R. D. (2015). Resource supply alone explains the variability of marine phytoplankton size structure. Limnol. Oceanogr. 60, 1848–1854. doi: 10.1002/lno.10138

Martin, M. (2011). Impacts of atmospheric deposition on phytoplankton community structure in the Yellow Sea. EMBrNet J. 17, 10–12. doi: 10.14806/ ej.17.1.200

Meng, X., Chen, Y., Wang, B., Ma, Q. W., and Wang, F. I. (2016). Responses of phytoplankton community to the input of different aerosols in the East China Sea. Geophys. Res. Lett. 43, 7081–7088. doi: 10.1002/2016GL069068

Mills, M. M., Ridame, C., Davey, M., La Roche, J., and Geider, R. J. (2004). Iron and phosphorus co-limit nitrogen fixation in the eastern tropical North Atlantic. Nature 429, 292–294. doi: 10.1038/nature02550

Moore, C. M., Mills, M. M., Arrigo, K. R., Berman-Frank, I., Bopp, L., Boyd, P. W., et al. (2013). Processes and patterns of oceanic nutrient limitation. Nat. Geosci. 6, 701–710. doi: 10.1038/ngeo1765

Nakaya, S., Toppo, K., and Verma, S. (2017). “Adaptation in algae to environmental stress and ecological conditions,” in Plant Adaptation Strategies in Changing Environment, eds V. Shukla, S. Kumar, and N. Kumar (Berlin: Springer), 103–115.
the Pearl River. *Deep Sea Res. Part I Oceanogr.* 55, 1330–1342. doi: 10.1016/j.dsr.2008.05.007

Zamora, L. M., Landolfi, A., Oschlies, A., Hansell, D. A., Dietze, H., and Dentener, F. (2010). Atmospheric deposition of nutrients and excess N formation in the North Atlantic. *J. Geophys. Res. Biogeosci.* 7, 777–793. doi: 10.5194/bg-7-777-2010

Zhang, C., Gao, H., Yao, X., Shi, Z., Shi, J., and Yu, Y. (2018). Phytoplankton growth response to Asian dust addition in the northwest Pacific Ocean versus the Yellow Sea. *J. Geophys. Res. Biogeosci.* 15, 749–765. doi: 10.5194/bg-15-749-2018

Zhang, C., He, J., Yao, X., Mu, Y., Guo, X., and Ding, X. (2020). Dynamics of phytoplankton and nutrient uptake following dust additions in the northwest Pacific. *Sci. Total Environ.* 739:139999. doi: 10.1016/j.scitotenv.2020.139999

Zhang, C., Yao, X., Chen, Y., Chu, Q., Yu, Y., and Shi, J. (2019). Variations in the phytoplankton community due to dust additions in eutrophication, LNLC and HNLC oceanic zones. *Sci. Total Environ.* 669, 282–293. doi: 10.1016/j.scitotenv.2019.02.068

Zhang, J., Chen, S. Z., Yu, Z. G., Wang, C. S., and Wu, Q. M. (1999). Factors influencing changes in rainwater composition from urban versus remote regions of the Yellow Sea. *J. Geophys. Res. Atmos.* 104, 1631–1644. doi: 10.1029/1998JD100019

Zhao, R., Han, B., Lu, B., Zhang, N., Zhu, L., and Bai, Z. (2015). Element composition and source apportionment of atmospheric aerosols over the China Sea. *Atmos. Pollut. Res.* 6, 191–201. doi: 10.5094/APR.2015.025

Zheng, L., and Zhai, W. (2021). Excess nitrogen in the Bohai and Yellow seas, China: distribution, trends, and source apportionment. *Sci. Total Environ.* 794:148702. doi: 10.1016/j.scitotenv.2021.148702

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