



# Synchronous Shifts in Nutrients and Organic Carbon Responses Over the Diatom-to-Dinoflagellate Succession

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Phytoplankton blooms, including the diatom-to-dinoflagellate succession in coastal regions, are frequently observed by researchers through incubation experiments, albeit mainly in nutrient-enrichment conditions. This study, complementary to such previous experiments, aims at the dynamics of nutrients and organic matter during the natural community shift in a nutrient-constraint condition. A nutrient-constraint incubation experiment is conducted herein to investigate nutrient dynamics during a diatom-to-dinoflagellate succession and evaluate its effects on carbon cycling. The incubation successfully induced a transition from a diatom bloom dominated by *Skeletonema costatum* to a dinoflagellate bloom dominated by *Prorocentrum donghaiense*. Results showed that the DIP limitation triggers the succession. This experiment provides an excellent opportunity to examine how senescent bloom under nutrient stress affects the amount and bioavailability of dissolved organic matter (DOM) that is produced. Under the nutrient limitation, organic carbon production was significantly influenced by nutrient availability. Particulate organic carbon (POC) production is closely related to phytoplankton growth phases, while dissolved organic carbon (DOC) is related to nutrient structure. The relative partitioning of net production to POC is higher in the dinoflagellate-dominant stage than that in the diatom-dominant stage, which is driven by quick turnover of DOM related to nutrient structure in different stages. In terms of C:Chl a ratio, it was strongly dependent on growth phases and nutrient conditions. From the signatures of chromophoric dissolved organic matter (CDOM), the bioavailability of DOM decreased during the succession. In different phases, DOM composition varied, containing more protein-like components in the degradation phase than in the growth phases. Differences of DOM composition among varying community dominance were far smaller than different growth phases during this succession. Such an observation of a diatom-to-dinoflagellate succession and the related dynamics of nutrients and organic matter benefits the prediction of organic carbon export during community shifts in ecological models.

**Keywords:** diatom-to-dinoflagellate succession, nutrient structure, organic carbon dynamic, dissolved organic matter characteristics, Changjiang Estuary

## INTRODUCTION

A seasonal succession from diatoms to dinoflagellates has been widely observed in mid- to high-latitude marginal seas (Spilling et al., 2018; Zhang et al., 2019). This transition significantly influences the sustainability of ecosystems and biogeochemical processes, as well as carbon cycling in oceans (Irwin et al., 2015; Basu and Mackey, 2018). Response of the phytoplankton community to variable nutrient supplies (Behrenfeld et al., 2021) or organic carbon dynamics in different communities (Richardson and Jackson, 2007; Leblanc et al., 2018) have been investigated. However, nutrients and organic carbon dynamics during the phytoplankton community succession process remain unclear due to difficulties involved in field observations.

Phytoplankton communities are influenced by the interplay of constituent species traits and the environment conditions (Lima-Mendez et al., 2015; Lin et al., 2021). The nutrient inputs have a significant impact on the composition of phytoplankton communities and influence the subsequent nutrient dynamic (Altman and Paerl, 2012). Both field investigations and incubation experiments suggest that phytoplankton abundance and community structure can be modulated by nutrient inputs (Domingues et al., 2005; Furnas et al., 2005; Paerl, 2006). The competition of phytoplankton for nutrients not only drives the shift of community structure, but also generates corresponding changes in phytoplankton ecological stoichiometry (Tréguer et al., 2018; Zhou et al., 2021). As mentioned above, the shift of community composition is hardly observed in field campaigns. Incubation experiments, on the other hand, provide alternative opportunities to examine the details of such a succession. To study phytoplankton community structure and biodiversity responses to increasing nutrient disturbances, a series of incubation experiments with nutrient amendments have been designed and copious amounts of data were collected. For example, dissolved inorganic nitrogen (DIN) supply can alter the phytoplankton community structure and stimulate a regime shift from cyanobacteria to diatoms (Zhou et al., 2018). The results of the *Prorocentrum donghaiense* and *Skeletonema costatum* bi-algal culture experiments indicate that *S. costatum* dominates over *P. donghaiense* under high-nutrient conditions (Wang et al., 2013). Up to now, the majority of incubation experiments assume a high-nutrient input condition despite the relatively oligotrophic character of sea water. Such an idealistic approach is not conducive to numerical simulations due to the overestimated reaction rates from those high-nutrient incubation systems.

The community composition and nutrient availability are known as the main factors that influence the organic carbon production (Casareto et al., 2012). Production and partitioning of phytoplankton-derived organic matter are varied among species and growing phases (Kinsey et al., 2018). Previous studies in field and laboratory experiments show that a large fraction of organic carbon produced during phytoplankton blooms is partitioned into POC when diatoms dominate the community (Wetz and Wheeler, 2003; Hasegawa et al., 2010), and is partitioned into DOC when pico-plankton dominates (Teira et al., 2001). However, there are also studies indicate that the relative partitioning of net production to POC and DOC is

not related to phytoplankton communities but related to nutrients available (Conan et al., 2007). Investigations into the coupling between nutrients and organic carbon during these community shifts remain limited.

Dissolved organic matter (DOM) in the marine environment is a key component in the global carbon cycle (Carlson and Hansell, 2014). The optical properties of DOM (absorbance and fluorescence) have been widely used to determine the origin and fate of DOM, as they are linked to the inherent chemical characteristics of the DOM pool (Reader et al., 2015). One of the major sources of DOM is the autochthonous production by phytoplankton (Stedmon and Markager, 2005). Previous incubation experiments have relied on high nutrients to observe DOM formation by phytoplankton (Romera-Castillo et al., 2010), which demonstrate different components exudation by different phytoplankton species. Organic matter produced and released by phytoplankton is processed by heterotrophic bacterial communities that transform DOM into biomass and recycle inorganic nutrients (Kinsey et al., 2018S). The DOM characteristics in different phytoplankton growth phases are quite different due to the microbial reprocessing (Shields et al., 2019). Asmala et al. (2018) suggests that nutrient status is the major driver for DOM dynamic, whereas the characteristics of phytoplankton community seemed to have a minor role. Our understanding of DOM characteristics and transformation during the natural continuous process of diatom-to-dinoflagellate succession is still limited.

A seasonal shift of phytoplankton composition from a diatom- to a dinoflagellate-dominant community is observed in coastal regions influenced by the Changjiang Diluted Water (CDW) (Zhang et al., 2015). Many studies have discussed the influence of nutrient structure on the diatom-to-dinoflagellate succession. Dissolved inorganic phosphorus (DIP) limitation is regarded as the main limiting factor (Wang et al., 2003; Huang et al., 2012; Liu et al., 2016). However, the characteristics of organic matter dynamics in natural community succession influenced by nutrient structure is less studied in this area. A clear understanding of nutrient and organic carbon dynamics and their relationship in a diatom-to-dinoflagellate succession is still missing.

We present a hypothesis stating that depleted DIP triggers the diatom-to-dinoflagellate succession, and thereby significantly influences the organic carbon partitioning and composition. A nutrient-constraint incubation experiment (low DIP concentration) was conducted in this study, which successfully reproduced the transition from a diatom bloom dominated by *S. costatum* to a dinoflagellate bloom dominated by *P. donghaiense* in the observation field. The nutrient and organic carbon dynamics during this succession are determined and discussed in detail, and their biogeochemical implications are addressed.

## MATERIALS AND METHODS

Blooms were often observed around the Changjiang Estuary from April to July, with community succession from diatoms to dinoflagellates especially during the summer period. Upon

sampling on 8 July during the cruise of R/V *Zheyuke* in 2017, the dominant phytoplankton was *S. costatum* at the blooming station (122.67°E, 31.37°N, Chang et al., 2021). Approximately 120 L of surface water was collected with a pump and filtered with a 200  $\mu\text{m}$  mesh to remove the meso-zooplankton. The filtered water was then shipped to the laboratory immediately for the incubation experiments. The initial water chemical parameters are shown in **Table S1** (day 0), characterized as brackish water (salinity 14) with depleted DIP.

## Experiment Design

The experimental design is shown in **Figure S1**. The filtered water is mixed and transferred into twelve 10 L acid-washed transparent polycarbonate bottles (Nalgene, USA). This experiment was aimed to adjust nutrient concentrations by adding sodium silicate as the DSi source, and potassium dihydrogen phosphate as the DIP source. These 12 incubation bottles were then divided into four groups for testing the impact of increased nutrients in four different parametric cases, each with three bottles to be treated identically: (1) group A (control), the control group in which the water remains unaltered; (2) group B (+DSi), with an increase in DSi concentration by 16  $\mu\text{M}$ ; (3) group C (+DIP), with an increase in DIP concentration by 1  $\mu\text{M}$ ; (4) group D (+DSi, +DIP), with increases in DSi and DIP concentrations by 16  $\mu\text{M}$  and 1  $\mu\text{M}$ , respectively. More detailed nutrient structure information of the four groups is described in **Table S2**. The incubation bottles are exposed to natural light irradiation in an outdoor tank filled with tap water. Circulating water is used to keep the temperature at about 27–29°C consistent with offshore conditions (Jiang et al., 2021). The incubation experiments are simplified cultured systems without gas exchange most of the time, but are opened daily and gently shaken twice a day. The duration of the incubation experiments is 13 days. Aside from the initial gathering, samples are collected on days 1, 3, 6, 9, and 13.

## Samples Collection

Prior to sampling, the incubation bottles were stirred to ensure an even distribution of phytoplankton. The dissolved oxygen (DO) was directly measured *in situ* with a multiparameter probe (Multi 340i/SET, WTW, GmbH, Germany).

In the laboratory, the samples were filtered within several hours of collection. The samples for DOC, nutrients (inorganic nutrients,  $\text{NO}_3^-$ ,  $\text{NO}_2^-$ ,  $\text{NH}_4^+$ ,  $\text{PO}_4^{3-}$ , DSi; organic nutrients, dissolved organic nitrogen (DON) and phosphorous (DOP)) were filtered through 0.45  $\mu\text{m}$  nylon filters (Rephile, Shanghai, China). And then, saturated  $\text{HgCl}_2$  solution was added in each inorganic nutrient sample for further analysis. The samples for chromophoric dissolved organic matter (CDOM) were filtered through 0.22  $\mu\text{m}$  polyethersulfone filters (Millipore, Darmstadt, Germany). The samples for POC, total suspended particles (TSM) and chlorophyll *a* (Chl *a*) were filtered through pre-combusted (at 500°C for 5h) GF/F filters (pore size: 0.7  $\mu\text{m}$ , Whatman). The phytoplankton samples were preserved with formaldehyde solution. Nutrient samples and phytoplankton samples were room temperature preservation, while the

remaining samples were stored in a -20°C freezer prior to analyses.

## Chemical Analyses

Chlorophyll *a* (Chl *a*) concentration was measured using a Cary 100 Ultraviolet-visible (UV-Vis) spectrophotometer (Varian, CA, USA) after extraction with 90% acetone at 4°C for 24h in the dark with a precision of  $\pm 0.1 \mu\text{g/L}$  (Wang et al., 2017).

Dissolved oxygen (DO) was directly measured *in situ* with a multiparameter probe (WTW Multi 350i, Munich, Germany).

The phytoplankton species and abundance were identified using an imaging flow cytometer (FlowCAM 8400, Fluid Imaging Technologies, USA).

The concentrations of nutrients ( $\text{NO}_3^-$ ,  $\text{NH}_4^+$ ,  $\text{NO}_2^-$ ,  $\text{PO}_4^{3-}$  and  $\text{SiO}_3^{2-}$ ) were determined by performing a Continuous Flow Analysis (CFA) using a Skalar San<sup>plus</sup> system in the laboratory with colorimetric methods described by (Grasshoff et al., 1999). The analytical precision was 0.06  $\mu\text{M}$  for  $\text{NO}_3^-$ , 0.01  $\mu\text{M}$  for  $\text{NO}_2^-$ , 0.09  $\mu\text{M}$  for  $\text{NH}_4^+$ , 0.03  $\mu\text{M}$  for  $\text{PO}_4^{3-}$ , and 0.15  $\mu\text{M}$  for  $\text{SiO}_3^{2-}$ . Dissolved organic nitrogen (DON) and phosphorous (DOP) were quantified after alkaline digestion using potassium persulfate (Jiang et al., 2019).

Dissolved organic carbon (DOC) samples were assessed using a total organic carbon analyzer (Shimadzu TOC L-CPH, Japan) with the high-temperature catalytic oxidation method. The concentrations of POC were determined using an elemental analyzer (Flash EA 1112, Thermo Fisher, USA) after removing the carbonate fraction by acid fumigation with concentrated HCl. Repeated analyses of the intra-laboratory standard samples revealed a variation of < 5% (Wu et al., 2003).

The absorption spectra of chromophoric dissolved organic matter (CDOM) were measured with a Cary 100 Ultraviolet-visible (UV-Vis) spectrophotometer (Varian, CA, USA) with a wavelength range from 200 to 800 nm. Samples were measured in a 1 cm quartz cuvette, and Milli-Q water was used as the blank. The absorbance at each wavelength ( $A_\lambda$ ) was corrected for instrument drift by subtracting the mean absorbance for the 700–710 nm wavelength interval. The absorption coefficients ( $a_\lambda$ , in Napierian form, per meter) were calculated as described by Del Vecchio and Blough (2004):

$$a_\lambda = 2.303 \times A_\lambda / l$$

Where  $l$  is the path length in meters.

The CDOM concentration was quantified using the absorption coefficient at 350 nm ( $a_{350}$ ). The absorption spectra were characterized by fitting an exponential model with a nonlinear regression as described in Stedmon et al. (2000). A slope of these spectra,  $S$  coefficient for wavelengths 275–295 nm ( $S_{275-295}$ ) were calculated.  $\text{SUA}_{254}$  is defined as the UV absorbance at 254 nm divided by the DOC concentration (Weishaar et al., 2003).

The fraction of CDOM that emits induced fluorescent light is called fluorescent dissolved organic matter (FDOM). The emission excitation matrices (EEMs) for FDOM were measured with an F-4500 fluorescence spectrophotometer (Hitachi, Tokyo, Japan). The excitation wavelength was scanned from 250 to 478 nm (at 6 nm

intervals), and the emission spectra were collected every 1 nm from 300 to 550 nm. The scan speed was 1200 nm/min, and the excitation and emission slits were 5 nm. The spectra of Milli-Q water were subtracted as a blank. The fluorescence intensity was calibrated using Milli-Q water and reported in Raman units (R.U.) (Lawaetz and Stedmon, 2009). In brief, the calibration was performed by dividing the fluorescence intensity at a given emission wavelength by the water Raman scatter band collected at 350 nm excitation and integrated over 380–420 nm. PARAFAC was applied to decompose the EEMs into individual fluorescent components using the DOMFluor toolbox according to the method established by Stedmon and Bro (2008). OpenFluo was applied to search for matches of the spectra modeled by PARAFAC (Murphy et al., 2014). In our experiments, four fluorescent components were identified using a PARAFAC model with a split-half validation procedure: three humic-like components (C1, C2, and C4) and one protein-like component (C3). Due to the complex characteristics of dissolved organic matter (DOM), each component represented a group of fluorophores with similar fluorescence character. The details of their spectral properties are shown in **Table S3**.

## Data Analysis

The ratio of the net consumption rates for DSi and DIN ( $v(\text{DSi})/v(\text{DIN})$  ratio) was calculated based on the following equation:

$$v(\text{DSi})/v(\text{DIN}) = \frac{C(\text{DSi})_t - C(\text{DSi})_{t-1}}{C(\text{DIN})_t - C(\text{DIN})_{t-1}}$$

Here,  $C(\text{DSi})_t$ ,  $C(\text{DIN})_t$  are the DSi, DIN concentrations at day  $t$ , respectively.  $C(\text{DSi})_{t-1}$ ,  $C(\text{DIN})_{t-1}$  are the DSi, DIN concentrations at day  $t - 1$ , respectively.

## RESULTS

### The Initial Conditions of the Sampling Water

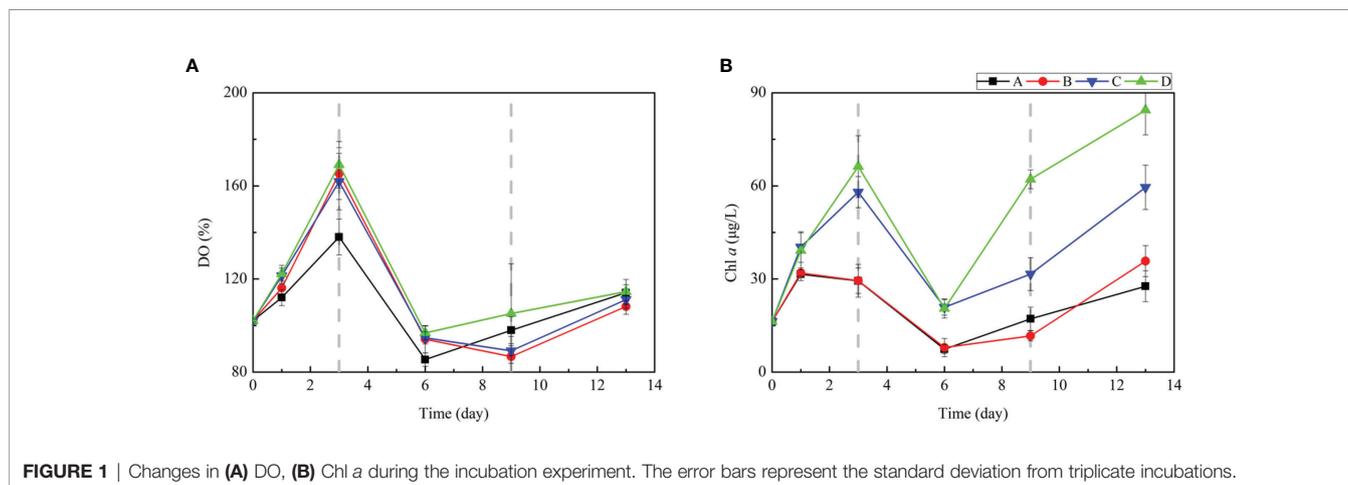
The initial concentrations for nutrient and organic carbon were shown in **Table S1**. In the initial water, a high Chl  $a$

concentration ( $16.31 \mu\text{g L}^{-1}$ ) revealed that the phytoplankton bloom (Chl  $a > 10 \mu\text{g L}^{-1}$ , Zhu et al., 2009) occurred in the sampling site. The DIP concentration was below the detection limit, indicating that the sampling water was a typical phosphorus limitation environment. The DSi and DIN concentration were  $64.53 \pm 1.12$  and  $68.75 \pm 1.71 \mu\text{M}$ , respectively, resulting in a low DSi/DIN ratio (0.94). Our nutrient treatments did not change the phosphorus limitation condition and the nutrient structure, as shown by the initial nutrient structures of the four groups. The DOC concentration was  $95.70 \pm 3.10 \mu\text{M}$ , the POC was  $0.53 \text{ mg/L}$ .

### Biological Response During the Incubation Experiments

The dissolved oxygen (DO) variation among the four groups was similar (**Figure 1A**), where they were observed to increase during the first 3 days and last 4 days, while observed to decrease during the middle period, from day 3 to day 9. In terms of Chl  $a$  concentration (**Figure 1B**), nutrient amendments increased the Chl  $a$  concentration to its peak value at day 3 in the incubation experiments of all groups, among which the values of groups with the DIP treatment (group C and D) always had higher concentrations than another two groups. The response of Chl  $a$  concentration suggested the transition of the phytoplankton community from growth to decline, and then to regrowth. Adding DIP significantly stimulates phytoplankton growth.

The phytoplankton community composition in four groups showed a similar succession (**Figure S2** and **Table 1**), but the biomass responses are different which are characterized by Chl  $a$  concentrations. In the first 3 days, the dominant phytoplankton community was *S. cotatum*. In the 3–9 day interval, diatoms still dominated the community despite the decline of *S. cotatum* and the significant increase of *Cyclotella*. In the period 9–13 day interval, the dominant phytoplankton community changed to dinoflagellate, to be *P. donghaiense*. The succession process reproduced the realistic algae succession in the Changjiang Estuary. By combining the Chl  $a$  and DO concentration variation with the transform of phytoplankton community composition, we defined three phases in our incubation



**TABLE 1** | The variation of phytoplankton abundance (cells ml<sup>-1</sup>) in sampling days of Group D.

Main species	0	1	3	6	9	13
<i>S. costatum</i>	12230	14560	21440	2360	/	/
<i>cyclotella</i>	/	80	240	1124	1120	/
<i>P. donghaiense</i>	40	120	/	1320	2580	4680

Because the phytoplankton assemblages made the inaccurate count of the abundance, not all sample data were recorded. We just identified the dominant species in four groups which showed the similar succession process.

"/" means it was not clearly found in the sample.

experimental period: the growth phase (GP) from day 0 to day 3, the declining phase (DP) from day 3 to day 9, and the regrowth phase (RP) from day 9 to day 13, together with two stages: the diatom-dominant stage, which includes the GP and DP, and the dinoflagellate-dominant stage which refers to the RP.

## Nutrient Variation During the Incubation Experiments

NO<sub>3</sub><sup>-</sup> concentrations showed similar trends in the four groups (Table S1), which decreased in the GP, held relatively stable in the DP, and then decreased again in RP. Notably, the groups with the DIP treatment showed higher NO<sub>3</sub><sup>-</sup> uptake rates compared to other groups. The NO<sub>2</sub><sup>-</sup> concentration was relatively low, approaching 1.2 μM in our experiment. The NH<sub>4</sub><sup>+</sup> concentration began to increase at incubations until the peak value at day 9, except for group D that peaked at day 6, after which they all decreased again. DON accumulated in the GP, and then decreased in the DP. In the RP, DON concentrations were relatively stable.

The DSi concentration decreased in the GP and reached its minimum value on day 3. The groups with DIP treatment showed the most consumption of all, which were ca. 27 μM in groups without DIP treatment (A and B) and 38 μM in groups with DIP treatment (C and D). Thereafter, the DSi concentration increased in the DP, and the concentration increases mainly occurred in the early stage of DP among all groups. The concentration variation of DSi indicated its reuse in the RP, and the amounts were much lower than those found in the GP.

The DIP concentrations were below the detection limit in all bottles, except the groups with the DIP treatment at day 0. The DOP showed different characteristics in four groups. For the DIP addition groups, the DOP concentrations were much higher, in which DOP increased in the GP along with the algae growth, and decreased in the DP. In the RP, DOP was relatively stable. In contrast, in the groups without the DIP addition, DOP had minimal accumulate over the study period.

## Organic Carbon Variation During the Incubation Experiments

The organic carbon variation was also linked to the phases of phytoplankton. POC concentration variation showed similar trend in four groups, which increased in the GP, decreased in the DP and then increased again in the RP (Figure S3A). The groups with DIP treatment (groups C and D) showed relatively higher values than that in groups without DIP treatment (groups A and B) in the late of the experiment (Table S1). However, there

was no significant difference among the different nutrient treatments in the GP (ANOVA,  $p > 0.05$ ).

The DOC concentration averaged 95.70 μM at the start of the experiment. The increasing trends of DOC were observed in all four groups and showed an insignificant difference (ANOVA,  $p > 0.05$ ). The accumulation rates in different stages varied (Figure S3B). In the diatom stage, there was a significantly higher accumulation rate of DOC than in the dinoflagellate stage.

The a<sub>350</sub> value showed an increasing trend, with insignificant difference among groups. The increasing trends of humic-like components (C1, C2, and C4) were observed in the growth phase of phytoplankton (GP and RP). The fluorescence of protein-like components (C3) in all groups increased steadily until day 6. After a slight decrease in the DP, it continuously increased in the RP.

## DISCUSSION

### Nutrient Dynamics in the DIP Depleted Condition During the Diatom-to-Dinoflagellate Succession

Previous studies usually consider the DIP limitation to be the major limiting factor in initiating a change in the major species from diatoms to dinoflagellates in the Changjiang Estuary (Wang et al., 2003; Ou et al., 2008; Huang et al., 2012; Wang et al., 2013; Liu et al., 2016; Mo et al., 2020). Our results also revealed the dominant role that DIP limitation played during the succession. In our DIP limitation incubation, increased NO<sub>3</sub><sup>-</sup> and DSi net consumption in all DIP treatment groups was associated with significant increases in diatom biomass (characterized by Chl *a*, Figure 1B), while the Chl *a* concentrations showed insignificant responses to the DSi treatment by the comparison between groups A and B (Figure 1B). This result indicated that DIP deficiency was the main limiting factor during this succession. However, small amounts of DIP enrichment did not change the phytoplankton community succession or delay the diatom community decline in our incubation experiments. Meanwhile, the DIP depletion in the initial did not stop the diatom blooms. This suggested that there may be another phosphate source to support phytoplankton growth. The DOP variation in our experiment also showed quick recycling of phosphate, which attests to the DOP's function in the incubation process as a phosphate source, especially among the groups without DIP treatment (Table S1). It has been confirmed that both *S. costatum* and *P. donghaiense* can use DOP efficiently,

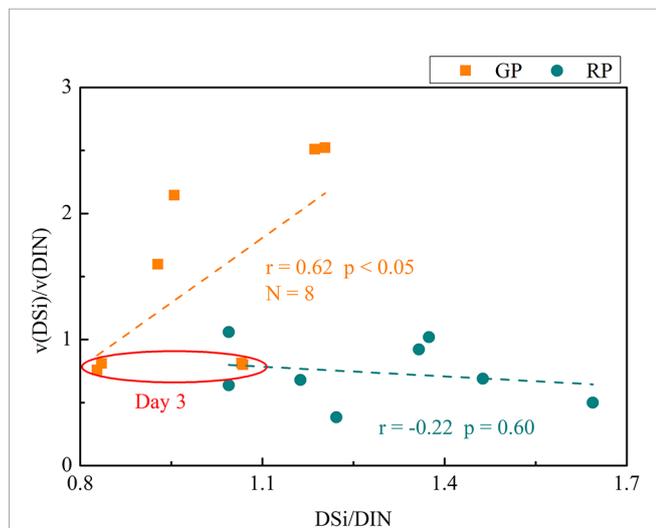
especially *P. donghaiense* in DIP depletion (Ou et al., 2015). Dinoflagellates are typically able to hydrolyze a variety of organic compounds and sustain their growth *via* the recycling of metabolic products under conditions of inorganic nutrient limitation (Glibert et al., 2012). Meanwhile, organic nutrients released into the water in the diatom phases can also stimulate the growth of dinoflagellate (Glibert et al., 2012). The advantage of *P. donghaiense* in utilizing DOP for growth is what allowed it to become abundant or even dominant in natural communities when DIP is depleted (Ou et al., 2015). This indicated that in natural diatom-to-dinoflagellate succession under DIP limitation, the role which DOP plays was indispensable (Huang et al., 2005; Nicholson et al., 2006; Fitzsimons et al., 2020).

The nutrient consumption during the succession process may be related to the ambient nutrient structure. The nutrient consumption structure was highly related to the nutrient structure in water (Figure 2). In the GP, the ratio of the consumption rates for DSi and DIN ( $v(\text{DSi})/v(\text{DIN})$  ratio) are positively correlated to the DSi/DIN ratio in all groups (Figure 2). The relative higher demands for DSi than DIN is owing to physiological change in diatoms under nutrient stress during photosynthesis (Sarmiento, 2006). Diatom cell growth under nutrient limitation have DSi/DIN ratios that can be many times higher than those from adequate nutrients (Claquin et al., 2002; Leynaert et al., 2004). This may cause a high  $v(\text{DSi})/v(\text{DIN})$  ratio which is higher than the Redfield ratio. On day 3 (the peak day of Chl *a* in the GP), the *S. costatum* biomass began to decline and gradually became an opportunistic species. Meanwhile, the  $v(\text{DSi})/v(\text{DIN})$  ratio was less than 1 as the DSi/DIN ratio declined. In the DP, extensive DSi regenerated from diatom detritus owing to the rapid and intense colonization of diatom detritus by bacteria (Amin et al., 2012). Large amounts of DSi were released into the water and the DSi/DIN ratio increased.

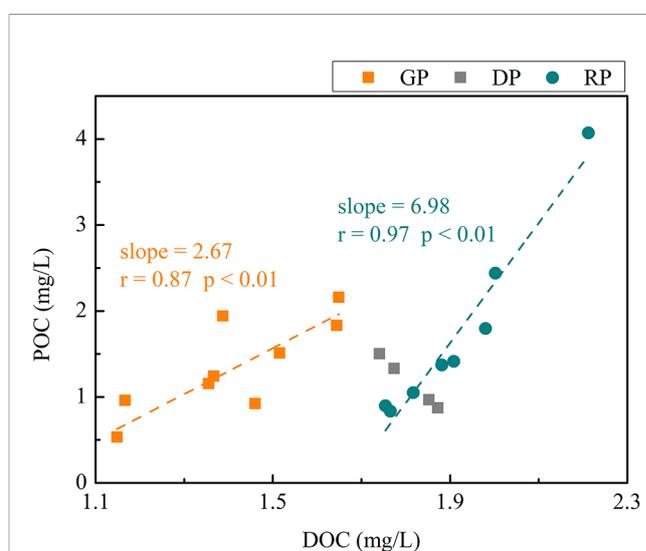
However, due to the greater adaptability of dinoflagellates compared to diatoms under DIP limitation and low DIN concentrations (Wang et al., 2013; Olofsson et al., 2019), dinoflagellates can easily become the dominant species when diatoms decay. Therefore, the *P. donghaiense* began to increase and started to dominate the phytoplankton community. It can be seen from the trend of DSi concentration that the DSi was re-used by residual diatoms in the RP. However, there was no significant relationship between the  $v(\text{DSi})/v(\text{DIN})$  ratio and DSi/DIN ratio, while the  $v(\text{DSi})/v(\text{DIN})$  ratio was always below 1 (Figure 2). Our results indicate that the  $v(\text{DSi})/v(\text{DIN})$  ratio tends to decrease during the succession. The significant variation of  $v(\text{DSi})/v(\text{DIN})$  ratio can be used to distinguish phytoplankton community dominated by diatoms from those dominated by non-siliceous species, with decreased ratios indicative of a succession from diatoms to non-siliceous species.

### Organic Carbon Dynamics During the Diatom-to-Dinoflagellate Succession on Bulk Level

Unlike wide studies on organic carbon dynamics which observe the phytoplankton community during the shift from a nutrient-replete to a nutrient-constrained condition, our experiments are designed under a nutrient-constrained condition throughout the community succession, which may obtain the new knowledge. Differences in predominant phytoplankton community members may lead to differences in organic carbon production (Conan et al., 2007). Our results also showed the different production of POC and DOC in varied communities (Figure S3). The accumulation rate of POC and DOC in the diatom-dominant stage was significantly higher than that in the dinoflagellate-dominant stage except group D during the succession process (Figures S3A, B). Meanwhile, the POC/DOC ratio (indicated by the slopes in Figure 3) indicated a



**FIGURE 2** | The relationship between the ratio of DSi and DIN consumption rate and DSi/DIN ratio. The points in red circle were the ratios of four group at the day 3.



**FIGURE 3** | The relationship between DOC concentration and POC concentration in different algae phases of the four groups.

significantly higher partition of net production into POC in the dinoflagellate-dominant stage than that in the diatom-dominant stage. These results were quite different from other results suggesting that DOC accounts for major fraction of organic carbon under nutrient depletion (Casareto et al., 2012). Many studies have suggested that the relative partitioning of net production to POC and DOC is not related to phytoplankton communities but related to nutrients available (Conan et al., 2007). The higher partitioning to DOC occurs in nutrient-limited condition (Conan et al., 2007; Casareto et al., 2012; Wyatt et al., 2014). These findings indicate that organic carbon dynamics is significantly different under different nutrient conditions. With the knowledge of either or both phytoplankton community composition and signatures of nutrient limitation, we can determine the environmental conditions that control the partitioning of organic carbon production. Although we cannot exclude the influence of phytoplankton communities, the inconsistency with previous studies may be related to nutrient-constraint conditions.

DOC production was related to nutrient conditions (Figure 4). Whereas our results indicated an association between DIP enrichment to the significant increase of phytoplankton biomass in groups B and D, the resulting DOC production associated therewith is insignificant (Figure 1 and Table S1). This result indicated that the increased biomass did not stimulate the DOC production. Enhanced production was likely closely coupled to the removal of organic carbon. The positive correlation between POC concentration and Chl *a* ( $r = 0.78$ ,  $p < 0.01$ ) indicates the POC production was related to phytoplankton growth. Combined with the weak relationship between DOC and Chl *a* ( $r = 0.33$ ,  $p > 0.05$ ), we suggested that the different partition of organic carbon production may be caused by the DOC recycling. Previous study has suggested that DOM plays an important role in nutrient regeneration (Zheng

et al., 2021). Hence, the quick turnover of DOM may be the reason for the less pronounced organic carbon accumulation and the lower DOC portioning of organic carbon in dinoflagellate-dominant stage.

To evaluate the DOC production in different dominant algae species stages, the values of DOC production in different stages of diatoms and dinoflagellates were normalized by the initial day of each state (day 0 and day 6 for the diatom and dinoflagellate states, respectively). We marked this value as  $\Delta\text{DOC}$ . The  $\Delta\text{DOC}$ s were strongly related to nitrogen structure, although they had significantly different behaviors in different stages (Figure 4). In the diatom-dominant stage (GP and DP), the  $\Delta\text{DOC}$  showed a positive correlation with the  $\text{NH}_4^+/\text{DIN}$  (Figure 4). The increase in  $\text{NH}_4^+/\text{DIN}$  indicated a rapid turnover of nitrogen compounds and the recycling of nitrogen, especially in the DP. In this stage, relatively abundant nutrients supported large amounts of  $\text{NH}_4^+$  regenerated from bacterial remineralization of DOM in waters (Killops and Killips, 2005). Along with the development of the algae succession, the DIN concentration gradually decreased and became an important factor modulating the growth of dinoflagellates. In the dinoflagellate-dominant stage (RP), although  $\Delta\text{DOC}$  showed a similar positive correlation with  $\text{NH}_4^+/\text{DIN}$ , the utilization of nitrogen was quite different (Figure 4). While DOC accumulated,  $\text{NH}_4^+/\text{DIN}$  decreased. Due to the low  $\text{NO}_3^-$  concentration and the obvious dinoflagellate preference for  $\text{NH}_4^+$  (Li et al., 2010; Yamamoto et al., 2017), the  $\text{NH}_4^+/\text{DIN}$  decreased. Low DON concentrations in the RP suggested quick DON recycling and subsequent difficulty in the accumulation due to the lack of nitrogen (Table S1). This also caused a lower accumulation rate of DOC in the RP. Our results show that the nutrient is the main driver for the organic carbon dynamics during the diatom-to-dinoflagellate succession.

The accumulation of POC in algal cells are influenced by phytoplankton growth phases and nutrient conditions, leading to the dynamics of carbon-to-chlorophyll ratios (C: Chl *a*)

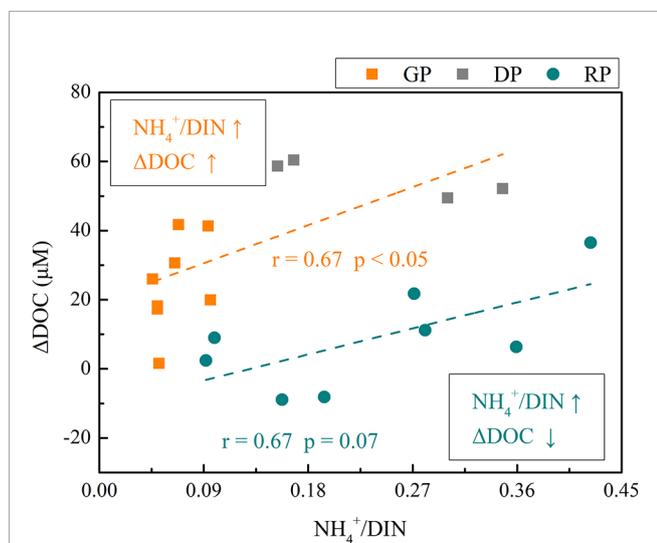


FIGURE 4 | The relationship between  $\text{NH}_4^+/\text{DIN}$  and  $\Delta\text{DOC}$  in different algae phases of the four groups.

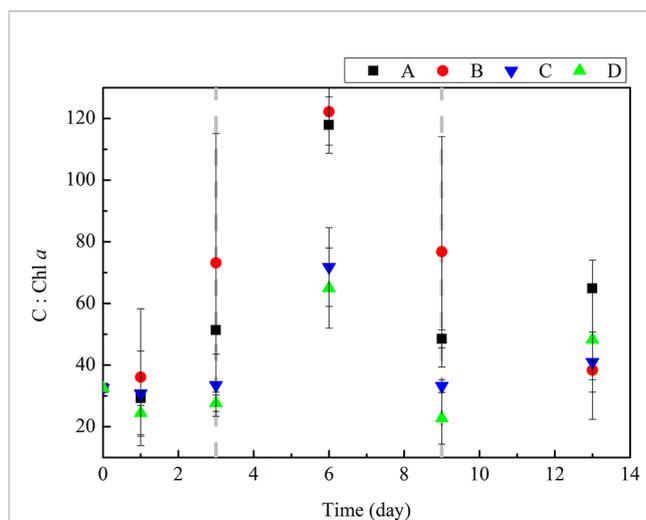
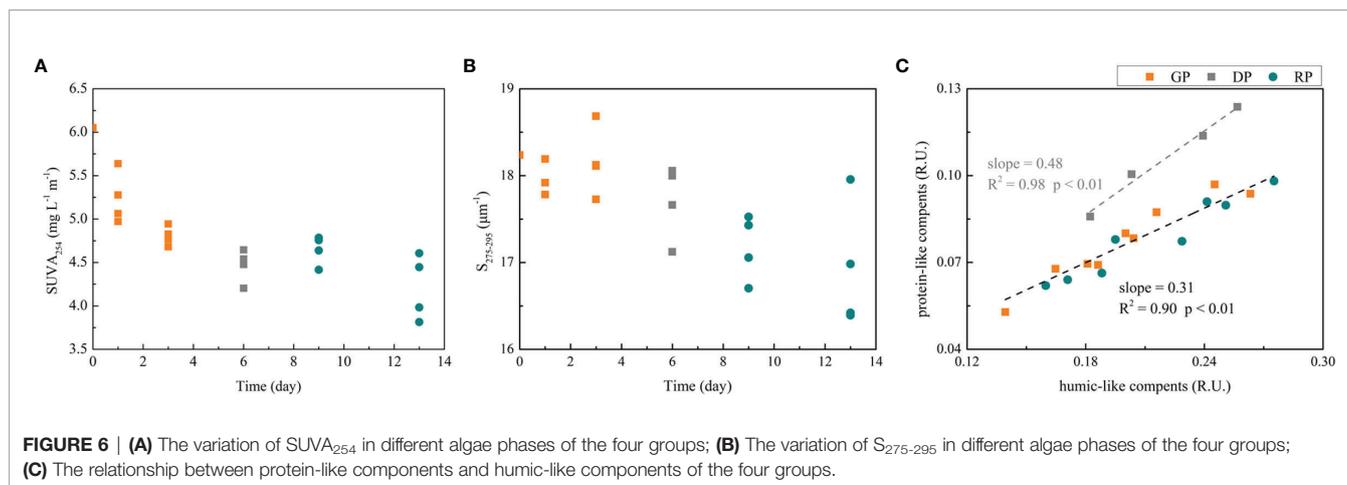


FIGURE 5 | Changes in C: Chl *a* during the incubation experiment.



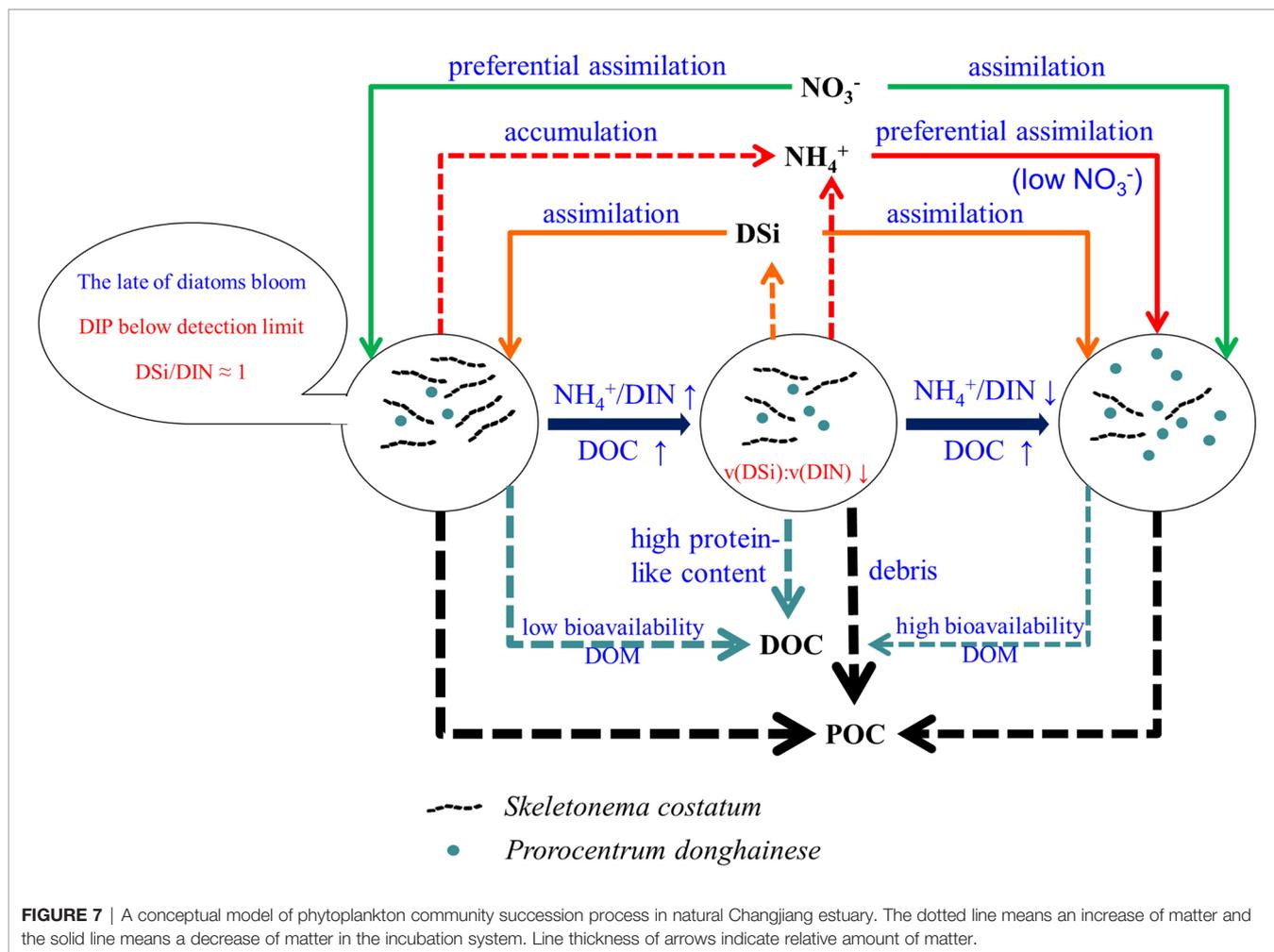
**FIGURE 6 | (A)** The variation of SUVA<sub>254</sub> in different algae phases of the four groups; **(B)** The variation of S<sub>275-295</sub> in different algae phases of the four groups; **(C)** The relationship between protein-like components and humic-like components of the four groups.

(Figure 5). C: Chl *a* ratios in our experiments showed that the highest ratios were presented in bloom decay phases (day 6). While C: Chl *a* ratios are relatively low during the phytoplankton growth phases, and the community composition had a minor effect (ANOVA,  $p > 0.05$ ). This result indicates that the C: Chl *a* is significantly influenced by phytoplankton growth phases but not species dominant stages under the nutrient limitation (Figure 5). The C: Chl *a* ratios in groups without DIP treatment are significantly higher than those in groups with DIP treatment during the late of the experiments (day 3-13) (ANOVA,  $p < 0.05$ ). This indicated the C: Chl *a* ratio increased under more restricted nutrient conditions, and the physiological base is the accumulation of excess carbon at low nutrient conditions (Jakobsen and Markager, 2016). C: Chl *a* ratio is governed by a combination of growth phases and nutrient availability effects. These transient, magnitude changes during the diatom-to-dinoflagellate succession within several days present so-far-overlooked challenges for models of phytoplankton acclimation and geographically extensive production estimates based on satellite remote sensing (Spilling et al., 2014).

## Bioavailability of DOM During the Diatom-to-Dinoflagellate Succession

Our experiments provide an excellent opportunity to examine how senescent bloom under nutrient stress affects the amount, bioavailability and fate of DOM that is produced. DOC accumulated in all phases during the incubation period, with compositional shifts indicated by CDOM parameters. The strong increase in DOC combined with insignificant trend in DON over RP suggests that the accumulated DOM became increasing C-rich as nutrients were depleted (Table S1). As the succession occurs, an increase in  $a_{350}$  as well as decreases in SUVA<sub>254</sub> and S<sub>275-295</sub> are observed (Table S1 and Figures 6A, B). This indicates a shift in DOM composition, specifically, an input of relatively fresh, high molecular weight (HMW) DOM (Del Vecchio and Blough, 2004; Berggren et al., 2009). In the Changjiang Estuary, substantial microbes are identified as heterotrophic functions (Wei et al., 2022). Heterotrophic

bacteria responded quickly to consume the DOC to replenish nutrients (Zheng et al., 2021). In the GP, the values of SUVA<sub>254</sub> and S<sub>275-295</sub> were higher than that in DP and RP, which suggested the less bioavailability of DOM produced. Previous studies have presented that less labile DOM largely originates during the nutrient-stressed and senescent phases of diatoms (Wear et al., 2015). In both the DP and RP, there were significant decreases of SUVA<sub>254</sub> and S<sub>275-295</sub>, indicating the production of a large amount of less aromatic and HMW DOC. The molecular size of DOM has implications for bacterial utilization of the DOM, as generally larger molecules suggest a high bioavailability of DOM and support higher bacterial growth and DOM utilization (Amon and Benner, 1996). This observation suggested that the newly produced DOM have a high potential for bacterial utilization of fresh allochthonous material (Asmala et al., 2013). The biological availability of DOM is a combination of the functioning of the microbes and environmental conditions, such as inorganic nutrient availability (Marín-Spiotta et al., 2014). The difference in DOM bioavailability in different phases may related to the nutrient condition. The more abundant nutrient condition (in the GP) allowed microbes to utilize CDOM more effectively. In the later stages of the experiments, the limitation of nutrients affected the microbial uptake of DOC due to a reduced assimilation of dissolved organic substrates when phosphorous was not available for microbes metabolism (Thingstad et al., 2008). On the other hand, the microbes shifts during the succession may also influence the DOM availability. Heterotrophic bacteria are the main consumers of the DOM produced by phytoplankton, and both diatoms and dinoflagellates have been associated to different bacterial communities (Camarena-Gómez et al., 2018). The growth of copiotrophic bacteria with high production rates in diatom-dominant communities may favor the recycling of carbon through the microbial loop (Camarena-Gómez et al., 2021), which may cause the low DOM bioavailability. In contrast, the increasing dinoflagellates seem to shift the bacterial community towards more oligotrophic generalist and reduce the bacterial production rates (Camarena-Gómez et al., 2021). Hence, the DOM



bioavailability variation is the result of the interaction of phytoplankton community, nutrient availability and bacterial community.

The FDOM components showed no significant differences in the two different community growth phases (GP and RP), but contained more protein-like components in the degradation phase (DP). The higher ratio of protein-like components to humic-like components in the DP implied a higher bioavailability of DOM upon its release during diatom degradation (**Figure 6C**). In the DP, the DOC increase was associated with cell lysis and the subsequent release of intracellular DOC into the water. On the contrary, in the GP and RP, the DOC was mainly attributed to the extracellular release from live phytoplankton cells. Intracellular DOC is more biolabile than extracellular DOC (Bittar et al., 2015). There was no significant difference in the GP and RP, suggesting that the difference in DOM dynamics and bioavailability was not directly linked to group-specific phytoplankton extracellular release (Asmala et al., 2018). This observation was also evident in Spilling et al. (2014), where it was concluded that during the buildup of the bloom, differences between varying community dominance were far smaller than anticipated from monoculture

studies. The quantity and characteristics of DOM varied differently in different phytoplankton incubation systems due to the nature of the bacterial community (Zhang et al., 2013; Kinsey et al., 2018; Shields et al., 2019). The observation of bacteria is not involved in this research; however, as it may provide more details to this phenomenon, such an observation is worthy of consideration in future research.

This study documents a novel relationship between instant nutrients and organic carbon responses derived from a natural diatom-to-dinoflagellate succession under nutrient-constrained condition in coastal regions as presented by a conceptual model in **Figure 7**. In coastal ecosystems, the coupling nutrients and organic carbon are important for ecosystem functioning. Changes in the phytoplankton communities may consequently lead to differences in the quantity and quality of organic matter export to the sea water, which in turn influences the biogeochemical cycling of nutrients. To have a better understanding of the seasonal phytoplankton community succession mechanisms, numerical modelling is an important approach to extract information from the complex field investigation data (Mutshinda et al., 2013). This study, which demonstrates synchronous shifts in organic carbon and nutrients

responses over the diatom-to-dinoflagellate succession in detail, will provide new insight to improve the numerical modelling. For example, the significant effect of direct DOP assimilation in supporting the phytoplankton community, especially in DIP depletion condition of late bloom, is rarely considered in many ecology models. The changing bioavailability of DOM in different growth phases makes it unreasonable to use a constant rate for DOM mineralization in ecological modelling, which may cause an inaccurate estimate of organic carbon dynamics.

## CONCLUSION

This incubation experiment successfully reproduced the succession of dominant phytoplankton species from diatoms to dinoflagellates in coastal regions receiving CDW. Nutrients and organic carbon, with concentrations of different chemical forms, in this process were well evaluated. The DIP limitation is the major factor of the diatom-to-dinoflagellate succession. In terms of consumption rates, the  $v(\text{DSi})/v(\text{DIN})$  ratio tends to decrease with the diatom decay. The organic carbon production was related to the phytoplankton community and nutrient structure. Due to the influence of nutrient availability, the relative partitioning of net production to DOC is higher in the diatom-dominant stage than that in the dinoflagellate-dominant stage. The relative partitioning of net production to POC is higher in the dinoflagellate-dominant stage than that in the diatom-dominant stage, which is driven by quick turnover of DOM related to nutrient structure in different stages. The production of DOC was influenced by nutrient structure. The accumulation of DOC was more closely related to the  $\text{NH}_4^+/\text{DIN}$  ratio and its fractional abundance in different phases was quite different. C: Chl *a* ratio is governed by a combination of growth phases and nutrient availability effects. As the succession occurs, DOM became more bioavailable as the result of the interaction of phytoplankton community, nutrient availability and bacterial community. DOM composition varied in different phases. The DOM released during diatom degradation containing more protein-like components than the growth phases. Differences of organic carbon production among varying community dominance were far smaller than different growth phases.

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## DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding author.

## AUTHOR CONTRIBUTIONS

XW: conceptualization, investigation, formal analysis, data curation, writing - original draft, and visualization. SJ: conceptualization, investigation, and writing - review & editing. YWu: conceptualization, resources, writing - review & editing, supervision, funding acquisition, and project administration. YWa: investigation. 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/fmars.2022.845372/full#supplementary-material>

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