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Effects of daily nitrogen and phosphorus input on planktonic community metabolism in a semi-enclosed bay by mesocosm experiment

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Abstract

Planktonic metabolism plays an important role in affecting the energy transportation and carbon cycle of the marine ecosystem. However, its regulation mechanism remains unclear under the continuously exogenous nutrient inputs in nearshore waters. In this study, a mesocosm experiment was conducted in a semi-enclosed bay, the Daya Bay, to explore the responses of plankton metabolic balance and community structure to a concentration gradient of daily nitrogen and phosphorus inputs. The results showed that nutrient enrichments promoted phytoplankton biomass, total primary production, and community respiration, and the promoting effect enhanced alongwith the increase of nutrient concentration. However, the net community production fluctuated more violently between autotrophic and heterotrophic with the increase of nutrient inputs and tended to be more heterotrophic from the 5th day to the 10th day of the experiment. In addition, the daily flux of nitrogen and phosphorus, 2 μmol/(L·d) and 0.066 μmol/(L·d), respectively, could be regarded as a potential threshold for ecosystem stability and health, since most of the ecological characteristics related to plankton structure and function have undergone significant changes when the nutrient level is higher than that. In the nearshore enclosed or semi-enclosed waters, nutrient from continuous terrigenous input is likely to be concentrated and exceed this level, indicating the ecological risks due to the metabolic unbalance and the deterioration of plankton community structure.

Key words: primary production, community respiration, community metabolism, continuous nutrient input, mesocosm experiment, Daya Bay

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1 Introduction

The planktonic metabolism, which is mainly decided by gross primary production (GPP) and community respiration (CR), is an important progress affecting the oceanic carbon cycle. The net community production (NCP) is the balance between GPP and CR, and is crucial to identify the state of metabolism of marine plankton community (autotrophic or heterotrophic) and estimate the ability of carbon fixation of the pelagic ecosystem (del Giorgio and Duarte, 2002; Duarte and Regaudie-de-Gioux, 2009; Finkel et al., 2004; del Giorgio and Williams, 2005; Rochelle-Newall et al., 2007; Smith and Kemp, 2003). Both GPP and CR depend on the growth and reproduction of organisms, but they are two different ecological processes with different responses to environmental changes. GPP is mainly derived from photosynthetic autotrophic activities of phytoplankton and therefore is mainly regulated by irradiance and nutrient availability. CR is closely related to energy consumption processes such as respiration and is influenced by temperature, dissolved organic carbon, and also nutrients (Rochelle-Newall et al., 2007). Therefore, the different responses of GPP and CR may lead to uncertainties in their equi-

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librium relationship, i.e., NCP. The ecosystem tends to be autotrophic when the promotion effect on GPP is stronger than CR, while the ecosystem tends to be heterotrophic when CR is stronger than GPP. Among various environmental factors, the impact of nutrients on aquatic metabolism has been given much attention since eutrophication is a global ecological issue. Some studies found that the increase of nutrient contents leads to autotrophic planktonic metabolism, promoting dissolved inorganic carbon uptake and organic matter output, especially in eutrophic waters (Cotovicz et al., 2015; López-Sandoval et al., 2019). However, Wilson et al. (2017) observed that the planktonic metabolism tends to shift toward heterotrophic under nutrient inputs, especially when the terrigenous input of organic carbon was included (Smith and Mackenzie, 1987). It suggests that large amounts of organic inputs can cause metabolic balance shift toward heterotrophs, e.g., the case of Mediterranean coastal areas (Duarte et al., 2004; Vidussi et al., 2011), while a large amount of inorganic nutrient input will lead to a more autotrophic ecosystem (Agustí et al., 2004). The above viewpoints are also used to explain the contrast between continental shelf as a sink and nearshore ecosystem as a source of atmospheric CO_2 since nearshore waters are fueled by large amounts of terrestrial-derived organic carbon (Cai, 2011; Chen and Borges, 2009). However, some cases have shown that inorganic nutrient supplementation can also lead to a decrease in NCP, or even a negative value (Lagaria et al., 2011), which makes the nutrient effect on the metabolic balance of plankton community more complicated.

Coastal systems carry significant socioeconomic benefits as aquaculture farms, port, and aquatic resource conservation (Boesch, 2002; Conley et al., 2002). Consequently, most coastal waters are subject to eutrophication under the influence of terrestrial inputs derived from urbanization and industrialization, and excess nutrient input has become one of the most critical environmental factors that influence the metabolic balance of coastal ecosystems (Rochelle-Newall et al., 2007; Yang et al., 2020). Many studies have demonstrated the changes of phytoplankton community with nutrient concentrations and ratios of nutrient elements, such as inorganic nitrogen (N) and phosphorus (P). Although the increase of exogenous nutrients promotes phytoplankton biomass and primary production (Song et al., 2009), the biomass of heterotrophic bacteria is enhanced (Huete-Stauffer and Morán, 2012). Taken together, a comprehensive study of the balance of primary production and respiration (i.e., NCP), which also indicates the ecosystem carbon flux under N and P nutrient inputs, is lacking. In the global promotion of carbon neutrality, besides the direct carbon emissions to the atmosphere, the contribution of ecosystem metabolism to the coastal carbon budget is especially drawing more and more attention.

Furthermore, many studies on nutrient enrichments are based on batch-culture experiments, but the coastal waters are experiencing continuous nutrient inputs. Thus, the current nutrient concentration might not reflect the riverine nutrient enrichment conditions and its potential impact on the balance of autotrophic and heterotrophic activities of planktonic community. In addition to that, some studies explore the relationship of nutrient condition and plankton community structure and function according to statistical analysis of various *in situ* observations (Agusti et al., 2018). However, it may not reflect the actual nutrient input levels in the waterbody and the nutrient preference and intracellular storage in the planktonic community. In general, the volatility patterns of planktonic metabolism and the underlying regulatory mechanism under different levels of continuous nutrient input are still unclear (Agusti et al., 2018; Cotovicz et al., 2015; Song et al., 2015).

In this study, a mesocosm experiment was conducted in the semi-enclosed coastal area of the Daya Bay to explore the comprehensive responses of planktonic metabolism, including GPP, CR, and NCP, and other ecological coupling characteristics to daily input of different inorganic nutrient concentrations. We primarily focus on (1) whether different levels of inorganic nutrient input will lead to different metabolic states; (2) whether there are thresholds of nutrient input intensity that significantly change the balance of GPP and CR and other ecological characteristics of the phytoplankton community; (3) whether the dynamics of metabolism can be applied to indicate the potential ecological risks caused by continuous nutrient inputs.

2 Materials and methods

2.1 *Experimentation setups*

The Daya Bay is one of the largest semi-enclosed bays along the coast of Guangdong, China. Its hydrological conditions are influenced by the subtropical climate, with the northeast monsoon prevailing from October to April of the next year, and the southwest monsoon predominates from May to September (Ke et al., 2019). With the increase of population and rapid economic development, it has shifted from oligotrophic to mesotrophic, and even partly eutrophic in the last decades (Song et al., 2015; Wang et al., 2008; Wu and Wang, 2007; Wu et al., 2020; Yang et al., 2020). The mesocosm experiment was conducted in Dapeng Cove, the southwestern part of the Daya Bay (Fig. 1), from June 28 to July 8, 2018. This area is prone to eutrophication and algal bloom under continuous nutrient input from rivers and mariculture, and the relatively low water exchange rate of the semi-enclosed Daya Bay makes eutrophication more serious (Song et al., 2009). We chose an abandoned fish raft located in the nearshore water region but not directly affected by the river input and used waterproof canvas material to separate 18 pools with a volume of 13.5 m^3 (3 m×3 m×1.5 m) following previously established methods (Song et al., 2019). The waterproof canvas was suspended from the floating fish raft and slowly submerged as seawater flowed inside each pool. Then transparent plastic films were built at 1.5 m top above the pools to prevent precipitation.

This mesocosm experiment was designed to determine the effect of continuously nutrient inputs on the planktonic ecology and explore the potential nutrient concentration threshold for changing the structure and function of planktonic community. Nitrate $\left(NO_3^-\right)$ and phosphate $\left(PO_4^{3-}\right)$ have proven to be the most effective nutrient combination in promoting phytoplankton in this region and are the primary nutrient components of river inputs (Song et al., 2019). In this study, N (KNO_3) and P (NaH_2PO_4) were kept at a 30:1 molar ratio and delivered together daily to mimic continuous inputs of exogenous nutrients (Table 1). The molar ratio and concentration gradient of N and P in each treatment were determined according to previous annual observations in the Daya Bay (Jiang et al., 2016; Wang et al., 2009; Wu et al., 2017). The experiment did not enrich dissolved silicate because previous *in situ* observations and incubation experiment results have shown that it is not a limiting factor for the growth of phytoplankton in the Daya Bay (Song et al., 2019; Zhao et al., 2019). N and P were pre-weighted for each treatment, then dissolved by seawater and evenly spread into the pool at 9:00 every morning right after the physical-chemical and biological samples were collected. The control did not receive any nutrient enrichments during the incubation. The mesocosms lasted ten days,

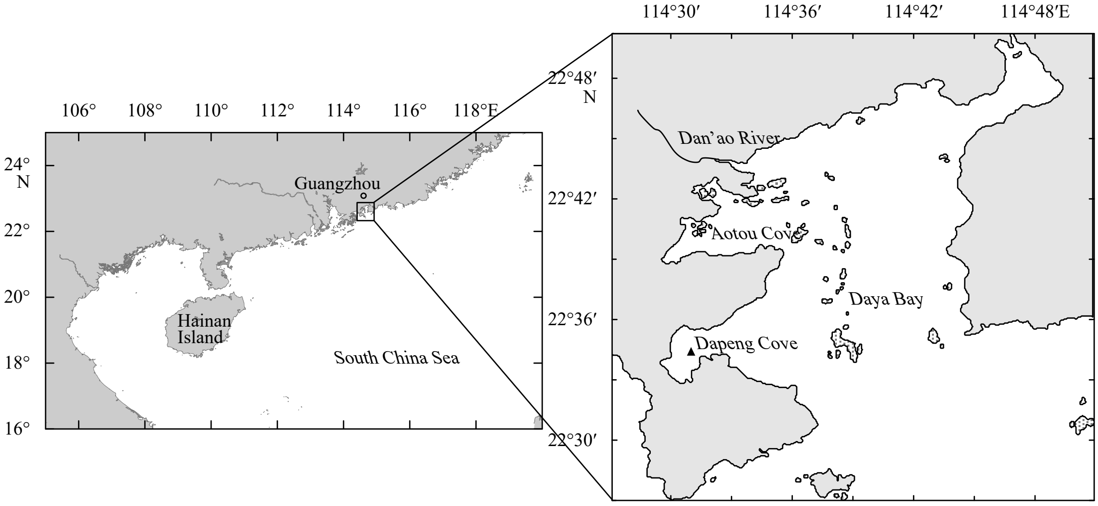

Fig. 1. Location of the Daya Bay and mesocosms site (▲).

Table 1. Concentrations of nutrient enrichments of each treatment

Treatments	KNO_3-N/(μmol·L^{-1}·d^{-1})	NaH_2PO_4-P/(μmol·L^{-1}·d^{-1})
Control	0	0.000
N_1+$P_{1/30}$	1	0.033
N_2+$P_{2/30}$	2	0.066
N_4+$P_{4/30}$	4	0.132
N_8+$P_{8/30}$	8	0.264
N_{16}+$P_{16/30}$	16	0.528

Note: Subscript represents preset molar concentration of KNO_3 and NaH_2PO_4.

and each treatment included three replicates.

2.2 *Sample collection and measurements*

2.2.1 *Physical and chemical parameters*

Temperature, salinity, and dissolved oxygen (DO) were measured and recorded by a multi-parameter water quality monitor Sonde (YSI6600, Yellow Springs Instruments, USA). Water samples were frozen and stored under −20°C, and the dissolved inorganic nutrient concentrations in seawater were measured by AA3 nutrients-autoanalyzer (Seal Instruments, Germany) in the laboratory following the method by Grasshoff et al. (1983). DIN is the sum of nitrate, nitrite, and ammonia.

2.2.2 *Phytoplankton biomass and size structure*

Water samples were sequentially filtered through 20 μm, 3 μm, and 0.7 μm pore size membrane filters to collect filter samples for micro-, nano- and pico-sized chlorophyll *a* (Chl *a*), respectively. Total Chl *a* concentration is the sum of micro-, nano- and pico-Chl *a* (Qiu et al., 2010; Xiang et al., 2019). Filters were wrapped in aluminum foil, stored in liquid N, and extracted with 90% acetone at 4°C for 24 h, then measured by Turner Designs fluorometer 10-AU (Parsons et al., 1984).

2.2.3 *Microscopic analysis of phytoplankton abundance and community structure*

One-liter seawater was collected and fixed with Lugol's solution, then concentrated to 40 mL by siphoning after 24 h of precipitation (Parsons et al., 1984). The concentrated sample was identified under an inverted microscope (Utermöhl, 1958). Phytoplankton diversity was presented as the Shannon-Wiener diversity index (H').

$$H' = -\sum_{i=1}^{S} \frac{n_i}{N} \ln\left(\frac{n_i}{N}\right),$$

where n_i is the abundance of the i species in the sample; N is the total abundance of all species in the sample; S is the number of species in the sample.

2.2.4 *Picoplankton abundance*

The seawater samples were prefiltered through a 20 μm pore-size mesh, and then fixed in 2 mL cryotubes with 2% final concentration of formaldehyde, and then stored in liquid N. *Synechococcus* (*Syn*) and picoeukaryotes (Euk) cells abundances were measured by Accuri C6 flow cytometer (Becton-Dickinson, USA) under FL2 (orange fluorescence) vs. FL3 (red fluorescence) and side light scatter (SSC) vs. FL3 signals (Jiang et al., 2017). Heterotrophic bacteria (HBA) cells abundance was measured by Accuri C6 flow cytometer (Becton-Dickinson, USA) based on their SSC, FL1 (green fluorescence) and FL3 signals. Water samples of HBA were stained with the nucleic acid dye SYBR Green I (Sigma-Aldrich Co., USA) (final dilution 10^{-4}, v/v) in the dark for 15 min before analysis (Jiang et al., 2017). Unfortunately, samples of picoplankton from the 6th day to the 10th day (D6–D10) (in the N_{16}+$P_{16/30}$ was D4–D10) were damaged during storage.

2.2.5 *Plankton community metabolism*

Planktonic metabolism was measured by the light-dark bottle method (Serret et al., 1999, 2015). Seawater was transferred slowly through silicon tubing and overflowed at least half the volume of a bottle from each pool to two light bottles (340 mL borosilicate glass bottle) and two dark bottles (light bottle covered by aluminum foil). The bottles were suspended in the seawater next to the mesocosm for the *in situ* environment and incubated for 24 h. DO was measured at the 0 h and 24 h of incubation period by Presens precision sensing Microx 4 Oxygen meter with a detection limit of 0.01 mg/L and detection accuracy of ±0.025 mg/L. NCP was calculated from the differences of DO concentration after 24 h incubation of light bottle. CR was calculated from the difference of DO concentration after 24 h incubation of dark bottle. GPP was the sum of NCP and CR. The units of CR, GPP and NCP were converted from DO to carbon, assuming a respiratory quotient of 1.0 (Ducklow and Doney, 2013; Nixon et al., 2008).

2.2.6 *Data analysis*

Two-way repeated-measures analysis of variance was conducted to test the difference of metabolic and biotic parameters between treatments over time. Pearson's correlation coefficient was calculated to test the relationship of metabolic parameters. One-way analysis of variance was conducted to determine whether the average of environmental and biotic parameters changed significantly between different concentrations of nutrient inputs. All the analyses are performed using SPSS 18.0.

3 Results

3.1 *Environmental conditions*

The environmental conditions of each experimental group are shown in Fig. 2. The temporal variability of seawater temperature and salinity were consistent among all treatments during the 10 d incubation (Figs 2a, b). The concentration of DO changed significantly [F(50, 100)=7.21, p<0.000 1] and was generally higher than that of the control under N and P additions. DO concentration in the $N_{16}+P_{16/30}$ treatment was the highest among all treatments during D0–D2, reaching a peak of (10.41±0.89) mg/L on D2, then continued to drop to (2.90±0.36) mg/L at D10. The DO concentration in the $N_1+P_{1/30}$, $N_2+P_{2/30}$, and $N_4+P_{4/30}$ treatments continuously increased to more than 10 mg/L and even higher than that in the $N_8+P_{8/30}$ and $N_{16}+P_{16/30}$ treatment at the end of the experiment.

The temporal variability of dissolved inorganic nutrient concentrations (DIN, PO_4^{3-} and SiO_3^{2-}) showed great differences between the control and the $N_8+P_{8/30}$, $N_{16}+P_{16/30}$ treatments during D2–D10 (Figs 2d, e and f). The highest DIN concentrations appeared in the $N_{16}+P_{16/30}$ treatment, ranging from 6.04 μmol/L to 107.56 μmol/L with an average of (55.80±26.07) μmol/L. The second highest DIN showed in the $N_8+P_{8/30}$ treatment, ranging from 4.68 μmol/L to 47.39 μmol/L with an average of (25.79±9.96) μmol/L. The PO_4^{3-} concentration in the $N_{16}+P_{16/30}$ treatment was also the highest among all treatments, with a range of 0.18 μmol/L to 1.14 μmol/L and an average of (0.58±0.32) μmol/L. The vari-

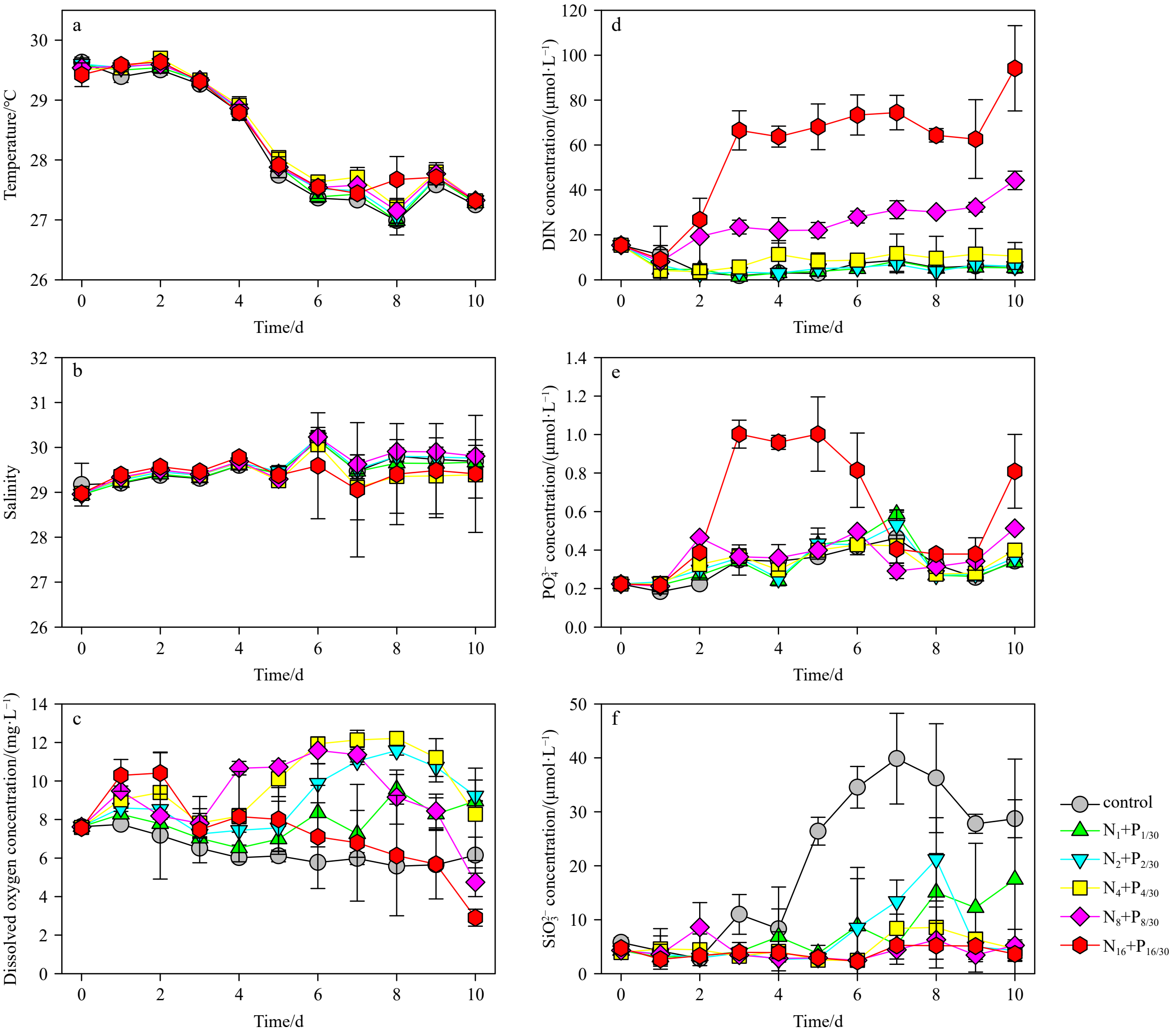

Fig. 2. Changes of environmental conditions in the mesocosms (vertical bars indicate the standard deviations from triplicate pools of each mesocosms, n=3). The implications of the figure legends are listed in Table 1.

ation pattern of SiO$_3^{2-}$ was opposite to that of DIN and PO$_4^{3-}$. The concentration of SiO$_3^{2-}$ was always the highest in the control group, ranging from 2.08 μmol/L to 45.84 μmol/L with an average of (18.45±13.78) μmol/L, but kept low in the N$_{16}$+P$_{16/30}$ treatment and averaged (3.53±1.48) μmol/L during the incubation.

3.2 *Plankton community metabolism*

The responses of GPP, CR, and NCP to the concentration gradient of N and P additions are shown in Fig. 3. The initial metabolic state was autotrophic, and the GPP, CR, and NCP were (718.23±104.80) mg/(m^3·d) (in terms of C), (368.90±56.24) mg/(m^3·d) (in terms of C), and (343.38±116.21) mg/(m^3·d) (in terms of C), respectively. Both GPP and CR were promoted by N and P additions, and the promotion effect increased with the rising nutrient input concentrations. Differences were statistically significant for both GPP [$F(50, 100)=10.76$, $p<0.000\,1$] and CR [$F(50, 100)=13.72$, $p<0.000\,1$] under different treatments during incubation period. The highest GPP and CR were observed in the N$_{16}$+P$_{16/30}$ treatment, ranging from 603.75 mg/(m^3·d) (in terms of C) to 3 900 mg/(m^3·d) (in terms of C) [average value (1 882.71±840.73) mg/(m^3·d) (in terms of C)] and 386.25 mg/(m^3·d) (in terms of C) to 3 667.50 mg/(m^3·d) (in terms of C) [average value (1 595.38±787.71) mg/(m^3·d) (in terms of C)], respectively. The second greatest GPP and CR appeared in the N$_8$+P$_{8/30}$ treatment, ranging from 622.5 mg/(m^3·d) (in terms of C) to 2 120.63 mg/(m^3·d) (in terms of C) [average value (1 376.93±393.32) mg/(m^3·d) (in terms of C)] and 281.25 mg/(m^3·d) (in terms of C) to 2 261.25 mg/(m^3·d) (in terms of C) [average value (1 386.14±496.86) mg/(m^3·d) (in terms of C)], respectively. GPP and CR in the N$_1$+P$_{1/30}$, N$_2$+P$_{2/30}$, and N$_4$+P$_{4/30}$ treatments changed gently and kept lower than those in the N$_8$+P$_{8/30}$ and N$_{16}$+P$_{16/30}$ treatments during the 10 d incubation. GPP in the control gradually decreased from (584.38±21.67) mg/(m^3·d) (in terms of C) to (248.13±23.03) mg/(m^3·d) (in terms of C) at D10, while CR remained relatively stable and ranged between 333.69 mg/(m^3·d) (in terms of C) and 377.28 mg/(m^3·d) (in terms of C) across the incubation.

The concentration gradient of nutrient inputs also caused statistically significant different for NCP [$F(50, 100)=21.13$, $p<0.000\,1$]. However, NCP fluctuated alternately between positive and negative values after nutrient enrichment, and the fluctuation range increased with the increase of N and P concentrations. For example, NCP varied between −982.50 mg/(m^3·d) (in terms of C) and 2 220.00 mg/(m^3·d) (in terms of C) in the N$_{16}$+P$_{16/30}$ group, while it fluctuated moderately in the treatments of N$_1$+P$_{1/30}$, N$_2$+P$_{2/30}$ and N$_4$+P$_{4/30}$, with the ranges of −135.00 mg/(m^3·d) (in terms of C)–686.25 mg/(m^3·d) (in terms of C), −476.25 mg/(m^3·d) (in terms of C)–525.00 mg/(m^3·d) (in terms of C) and −652.50 mg/(m^3·d) (in terms of C) –723.75 mg/(m^3·d) (in terms of C), respectively. NCP in the control was kept stable and ranged from −161.25 mg/(m^3·d) (in terms of C) to 292.50 mg/(m^3·d) (in terms of C).

3.3 *Chl a concentration and size structure*

The response of total Chl *a* across the concentration gradient of daily nutrient inputs is shown in Fig. 4a, it showed significant differences along the concentration gradients of N and P inputs during the experiment [$F(50, 100)=11.11$, $p<0.000\,1$]. Total Chl *a* concentration was promoted by N and P addition, and the promotion effect was more significant with increasing nutrient concentration. The total Chl *a* of the N$_{16}$+P$_{16/30}$ treatment was the highest among all experimental treatments, varying from 1.49 μg/L to 15.95 μg/L (average value (6.41±4.86) μg/L), reaching a peak value of (15.95±1.83) μg/L on D8. Total Chl *a* concentration in the control decreased from (2.32±1.02) μg/L to (0.35±0.14) μg/L across the incubation period. The size structure of Chl *a* after 10 d incubation under different concentrations of nutrient input are shown in Table 2. The initial total Chl *a* was almost equally contributed by micro- (33.78%), nano- (29.79%), and pico-fraction (36.43%). But at the end of the culture experiment, the contribu-

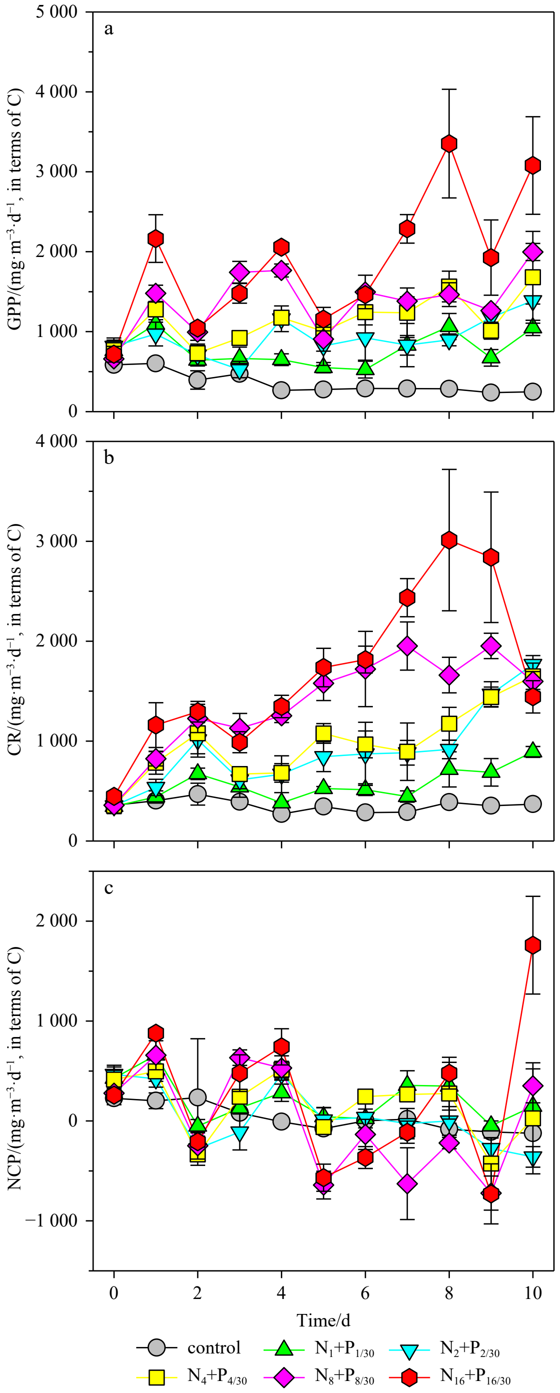

Fig. 3. Changes of planktonic metabolism of gross primary production (GPP, a), community respiration (CR, b) and net community production (NCP, c) in the mesocosms (vertical bars indicate the standard deviations from triplicate pools of each mesocosms, $n=3$). The implications of the figure legends are listed in Table 1.

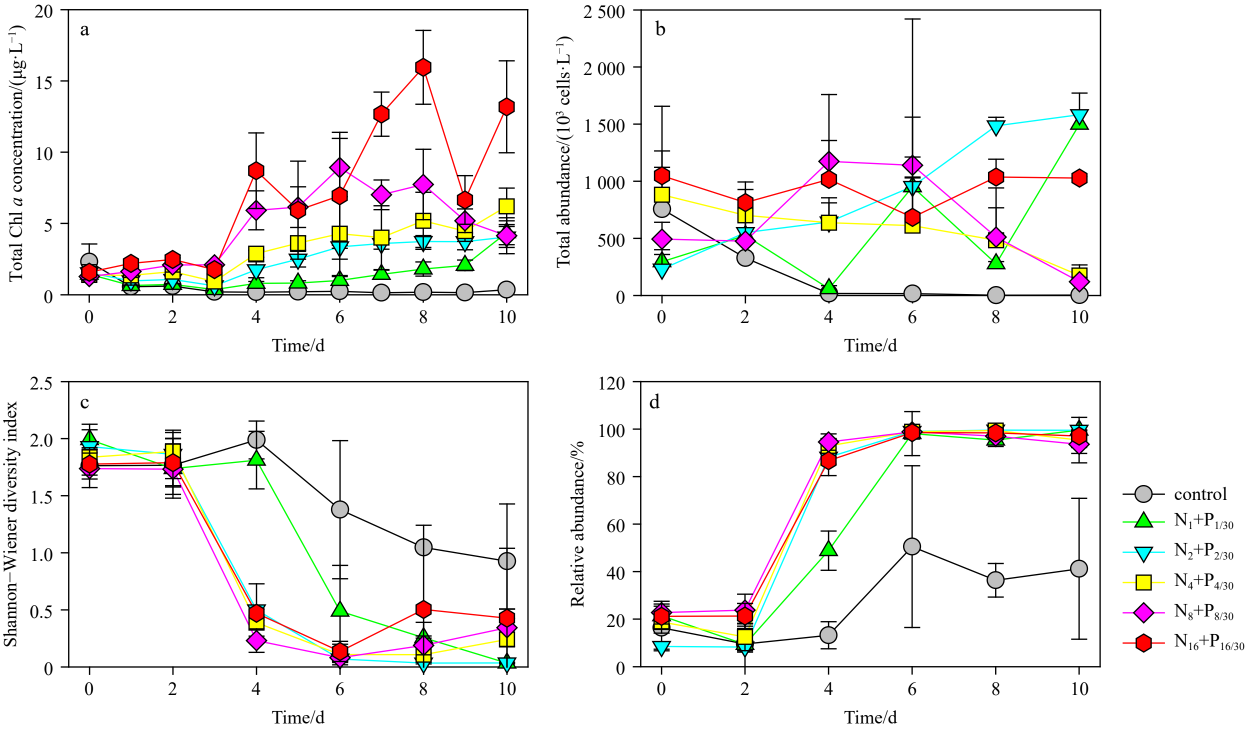

Fig. 4. Changes of total chlorophyll *a* (Chl *a*) concentration (a), micro-phytoplankton total abundance (b), Shannon-Wiener diversity index (c), and relative abundance of *Rhizosolenia sinensis* (d) in the mesocosms (vertical bars indicate the standard deviations from triplicate pools of each mesocosms, *n*=3). The implications of the figure legends are listed in Table 1.

Table 2. Chlorophyll *a* size structure in the mesocosms after the 10 d incubation

Treatments	Micro/%	Nano/%	Pico/%	<20 μm/%
Control	12.72±8.03	29.04±7.84	58.24±14.75	87.28±8.03
N_1+$P_{1/30}$	17.18±8.81	41.16±4.06	41.66±7.63	82.82±8.81
N_2+$P_{2/30}$	5.47±1.95	47.55±13.86	46.98±12.37	94.53±1.95
N_4+$P_{4/30}$	2.65±1.66	30.33±12.67	67.02±14.29	97.35±1.66
N_8+$P_{8/30}$	8.89±4.51	47.82±3.35	43.28±1.98	91.11±4.51
N_{16}+$P_{16/30}$	10.15±3.45	43.25±9.58	46.6±9.05	89.85±3.45

Note: The implications of the subscripts are listed in Table 1.

tion of nano- and micro-fraction (<20 μm) increased by more than 40%, accounting for over 90% of the total Chl *a* when the nutrient input was higher than the level of N_2+$P_{2/30}$, which was higher than the control, and the portion of micro-phytoplankton decreased to less than 10% (Table 2).

3.4 *Phytoplankton community structure*

The phytoplankton communities in all treatments were primarily contributed by diatoms, accounting for 94.38%±15.79% of the total abundance during the incubation period (Fig. S1a). The initial phytoplankton community was predominated by *Asterionella glacialis*, *Cylindrotheca closterium* and *Rhizosolenia sinensi*. The contribution of *Rhizosolenia sinensis* to the total phytoplankton abundance increased from 18.34%±6.70% on D0 to 84.36%±27.63% on D10 under nutrient enrichment conditions (Fig. 4d), while in the control, *Chaetoceros* sp. and *Thalassionema nitzschioides* became the most abundant species at the end of the experiment. *Rhizosolenia sinensis* was the most abundant species in the N_8+$P_{8/30}$ and N_{16}+$P_{16/30}$ treatments on D10, and the abundance of dinoflagellate also increased (majorly *Scrippsiella trochoidea*) in these two treatments (Fig. S1b, Table S1), which might contribute to the rebound of the diversity index during D8–D10 of incubation (Fig. 4c).

The response of micro-phytoplankton abundance and community diversity to nutrient enrichments is shown in Figs 4b and c. The total abundance of micro-phytoplankton was promoted by N and P additions (Fig. 4b), but the community structure was simplified with the decrease of the *H*′ (Fig. 4c). In the N_8+$P_{8/30}$ and N_{16}+$P_{16/30}$ treatments, the abundance increased and peaked at (1 174.17×10^3±477.33×10^3) cells/L and (1 016.33×10^3±278.33×10^3) cells/L on D4, respectively, then it started decreasing or remained stable. But in the treatments with lower concentration of nutrient inputs (the N_1+$P_{1/30}$ and N_2+$P_{2/30}$), the abundance increased across the incubation period and peaked at (1 499.17×10^3±100.64×10^3) cells/L and (1 582.67×10^3±154.38×10^3) cells/L on D10, respectively. The micro-phytoplankton diversity index (*H*′) in the N_{16}+$P_{16/30}$ treatment decreased dramatically from 1.79±0.17 on D2 to 0.14±0.07 on D6, but *H*′ remained higher in the N_1+$P_{1/30}$ treatment than that of the other treatment with nutrient addition until D6. Although the total abundance of the control group decreased during the incubation, the diversity index kept higher than that of the other treatments with nutrient inputs.

3.5 *Euk, Syn and HBA*

The responses of Euk, *Syn* and HBA to the N and P concentration gradient are shown in Fig. 5. The abundances of Euk, *Syn* and HBA in the treatments with nutrient enrichments were promoted and remained higher than those in the control. The promotion effect increased with the increase of nutrient input concentrations. Euk responded quickly, and the highest abundance ((40.00±0.83) cells/μL) was recorded in the N_{16}+$P_{16/30}$ treatment on D1. The abundance of *Syn* peaked in the N_4+$P_{4/30}$ treatment on D3 ((1 164.00±657.86) cells/μL) and the N_8+$P_{8/30}$ treatment on

D4 ((1 087.00±300.02) cells/μL), respectively. The abundance of HBA peaked at (574.00±55.15) cells/μL in the $N_8+P_{8/30}$ treatment and (641.00±97.09) cells/μL in the $N_{16}+P_{16/30}$, respectively, on D2.

4 Discussion

4.1 *Response of community metabolism to daily nutrient inputs*

The results of this study show that the community metabolism was initially autotrophic in the study region, and inorganic nutrient enrichments promoted both GPP and CR. However, NCP fluctuated between positive and negative values during the incubation period. Furthermore, the oscillation amplitude of NCP was stronger in the treatments with higher concentration of nutrient inputs (the $N_8+P_{8/30}$ and the $N_{16}+P_{16/30}$ treatments).

The fluctuation of NCP is attributed to the different responses of GPP and CR to nutrient addition. Our correlation analysis further clarifies the complexity of NCP regulation between GPP and CR with the increase of nutrient input (Table 3). NCP was positively correlated with GPP when nutrient input was absent or weak, but it became more negatively correlated with CR when nutrient inputs increased. However, no significant correlation between NCP and GPP or CR was observed under the highest concentration of nutrient input (the $N_{16}+P_{16/30}$ treatment) during the incubation, indicating that the metabolic balance was disturbed with increasing eutrophication levels.

Table 3. Pearson's correlations between the net community production (NCP), the gross primary production (GPP) and the community respiration (CR)

Treatment	GPP and NCP	CR and NCP
Control	0.898**	0.011
$N_1+P_{1/30}$	0.707*	−0.474
$N_2+P_{2/30}$	−0.092	−0.801*
$N_4+P_{4/30}$	0.263	−0.666*
$N_8+P_{8/30}$	0.464	−0.694*
$N_{16}+P_{16/30}$	0.525	−0.358

Note: Asterisks represent the level of significance: *p<0.05, **p<0.01. The implications of the subscripts are listed in Table 1.

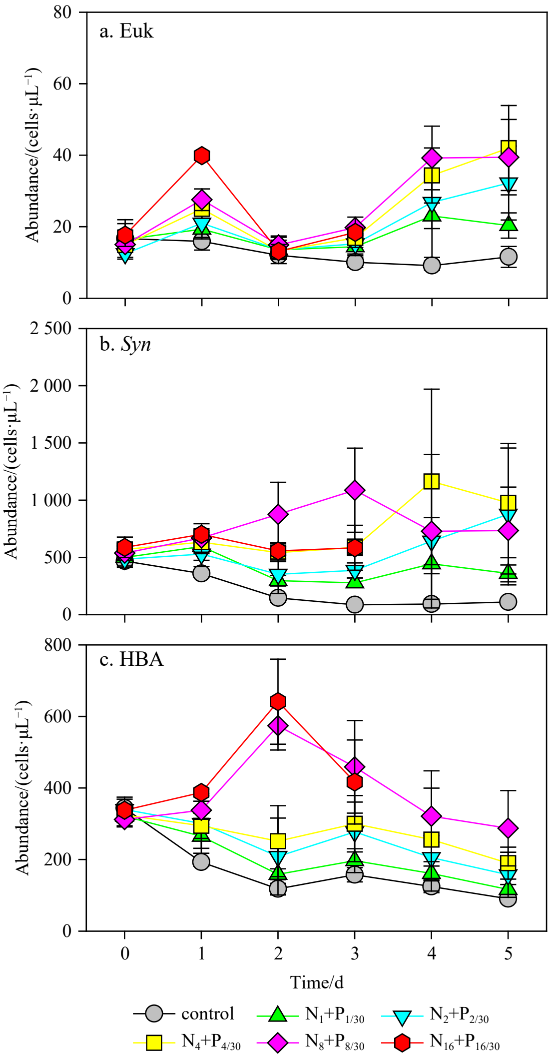

Fig. 5. Changes of Euk (a), *Syn* (b) and HBA (c) abundance in the mesocosms (vertical bars indicate the standard deviations from triplicate pools of each mesocosms, n=3). The implications of the figure legends are listed in Table 1.

GPP and CR are affected by different ecological processes, and their responses to environmental changes are not always synchronized or in the same amplitude level, which may result in a complicated mechanism for regulating NCP (Rochelle-Newall et al., 2007). GPP is mainly contributed by phytoplankton and promoted by nutrient supplementation (Krause-Jensen et al., 2012; Xie et al., 2019). The promotion of nutrient inputs on GPP or phytoplankton biomass has been reported in river-coastal systems (Caffrey et al., 2014; Kemp et al., 1997; Malone et al., 2015). For example, GPP in the coastal region was the highest when upwelling prevailed, and the ecosystem shifted from heterotrophic to autotrophic (Arbones et al., 2008). However, the predomination of GPP over CR cannot be sustained and generally occurred in a short period (3–4 d) under continuous nutrient inputs. It may be because that GPP does not increase linearly with the increase of nutrient concentration, since there is a saturation concentration of nutrient for phytoplankton growth and utilization (Morel, 1987), and the carbon fixation efficiency is also impacted by the variation of species composition and size structure of phytoplankton community (Marañón, 2015; Zwart et al., 2015). In our study, the phytoplankton size structure shifted toward a smaller size (<20 μm) after incubation, especially the fraction of nano-Chl *a* (Table 2). Furthermore, we estimated the size-fractioned primary production according to the assimilation coefficient of phytoplankton with different cell sizes in the same research area (Zhu et al., 2008), and also found that nano-phytoplankton was the major contributor of the community primary production after incubation under high concentration nutrient input (Fig. S2b).

The community respiration is generally contributed by bacteria respiration and various planktonic heterotrophic actives. The accumulation of organic matters during the planktonic growth and metabolism progress could inhibit primary production while enhancing CR (Joint et al., 2002). Brussaard et al. (2005) has demonstrated that organic matters released during phytoplankton bloom could activate bacterial metabolism and supply 85% of the carbon demand of bacteria. Some studies also observed that ecosystems were heterotrophic in the growing season (del Giorgio and Peters, 1994). CR is also contributed by various heterotrophic actives associated with the microbial food web, including microzooplankton and protist grazing (Strayer, 1988; Vázquez-Domínguez et al., 2007). For example, some small-sized mixotrophic protists are able to obtain energy from phagocytosis and cause grazing pressure on small primary producers (Huang et al., 2008; Yu et al., 2007; Zhang et al., 2017), and their photosynthetic efficiency is weakened in a mixotrophic strategy (Adolf et al., 2006). Li et al. (2021) also found that plankton of 1–200 μm was the main contributor to CR in the Daya Bay in summer, accounting for 65.52% of the total CR, while bacterial metabolism only contributed 23.61%. Therefore, these heterotrophic activities may have a cascade effect of GPP, which causes a time lag between GPP and CR (Dodds and Cole, 2007; Sommer and Lewandowska, 2011; Stange et al., 2017; Wiltshire et al., 2008).

4.2 *Biogeochemical feedback of planktonic metabolism*

Planktonic metabolism has a complex relationship with the environment. It is regulated by multiple environmental parameters in the ocean, while the metabolic activities could conversely influence the environment, such as DO and nutrient concentration in the water. Planktonic metabolism can affect the DO concentration directly. For example, the excessive organic matters accumulation and community respiration caused large oxygen-consuming under a high concentration of nutrient inputs (Malone, 1991). In the present study, the higher GPP over CR induced by nutrient input causes a rapid increase of DO during the first two days of incubation, while the increasing CR and negative NCP values are closely related to heterotrophic activities, leading to a decrease in DO concentration after D6 in the N_8+$P_{8/30}$ and the N_{16}+$P_{16/30}$ treatments of the mesocosm experiment. The concentration of DO was lower than that of the control under intense nutrient input (the N_8+$P_{8/30}$ and the N_{16}+$P_{16/30}$ treatment) after 10-d incubation, even dropping to (2.90± 0.36) mg/L under the highest concentration of nutrient inputs (the N_{16}+$P_{16/30}$ treatment), which is lower than the water quality standard (5 mg/L) and the requirements in sustaining aquatic life (Davis, 1975; USEPA, 2000), suggesting the risk of intensified hypoxia under higher concentration of nutrient inputs with accumulatively heterotrophic feedback of plankton metabolism.

The biochemical cycle of N and P is closely determined by the planktonic metabolism, and the utilization and storage ability of plankton. In our study, the nutrient concentrations kept low and showed no significant changes when the nutrient inputs were less than or equal to the N_4+$P_{4/30}$ treatment due to a relative higher nutrient uptake rate by phytoplankton production (Figs 6K, L). However, the nutrient concentrations increased significantly under a higher level of nutrient inputs, indicating that the exogenous nutrient has exceeded the ability of phytoplankton on nutrient utilization and caused irreversible damage to the planktonic ecosystem (Dodds et al., 1989; Scheffer et al., 2001).

4.3 *Indicative function of metabolic disorders in ecosystem instability and potential risks*

Coastal waters are facing various ecological risks under the interference of anthropogenic activities. The most common ecological risks include algal blooms, especially harmful algal blooms, and hypoxia (Ganf and Viner, 1973), which are associated with plankton metabolisms. In this study, the variation of GPP was coupled with phytoplankton biomass, and the boost of GPP was accompanied by the predominance and high abundance of certain phytoplankton species, such as *R. sinensis*, and noticeable decline in biodiversity, which indicated a phytoplankton bloom event (Fig. 4). A similar situation has been observed that the nearshore waters are often lower in biodiversity index but higher in GPP than that of the offshore waters of the Daya Bay, which is possibly related to the long-term impact of river input and mariculture (Song et al., 2009; Sun et al., 2006). Our study demonstrated that higher nutrient inputs caused the coupling events of the drastic increase of GPP, the outbreak of *R. sinensis* bloom and the further decreased species diversity. Furthermore, some small dinoflagellates, including *S. trochoidea* and *Protoperidinium* sp. were promoted, and the proportion of dinoflagellate to the whole phytoplankton biomass also increased after D8 of the mesocosm experiment. *Scrippsiella trochoidea* is a common causative bloom species in eutrophic coastal waters (Song et al., 2009; Yang et al., 2020; Yin et al., 2008). Dinoflagellates may become dominant and even induce bloom after an outbreak of diatom species under continuous N and P inputs (Song et al., 2019), which further reduces the stability of plankton communities and the health of ecosystems.

The eutrophic level of aquatic ecosystem is conventionally assessed by detecting the *in situ* environmental and biological parameters and comparing them with water quality standards for the early warning ecological risks. However, as we suggested above, the ecosystem health is more closely related with the dynamic community metabolism since the environmental and biological parameters are often associated with the biogeochemical feedback of these metabolic progresses, such as the algal bloom induced by a rapid increase in GPP, or hypoxia induced by accumulative heterotrophic metabolism. For example, Chl *a* of 10 μg/L is generally regarded as the criterion for phytoplankton blooms (Watson et al., 2017). In this study, Chl *a* exceeded this value after 4 d incubation under the highest level of nutrient inputs (Fig. 4a). However, our results show that NCP could indicate an abnormal signal by its intensified fluctuation faster than the observed Chl *a* meets this criterion value. GPP has been suggested as an index of coastal eutrophication (Liu et al., 2013), while a comprehensive consideration of GPP and CR would be more effective to reflect phytoplankton physiological processes in the changing environment according to our experiment. The metabolic balance can be used to assess the stability of an ecosystem, i.e., in a relatively stable and healthy ecosystem, NCP does not deviate too far from the zero level, or in some studies, the GPP to CR ratio is approximately equal to 1 (Duarte et al., 2013; Williams et al., 2013). Similar recommendations were made by Ferreira et al. (2020) for ecosystem assessment by reviewing the published survey results, and our research further proved this viewpoint through *in situ* experiments. In summary, we suggest the community metabolic characteristics, including GPP, CR and NCP, as effective indicators for ecosystem health and warning of potential ecological risks since they can provide a wealth of information as mentioned above.

4.4 *Potential nutrient input threshold associated with ecosystem health and metabolic balance*

The nutrient criteria of eutrophication vary widely in different water regions (Table S2). It is difficult to find a general threshold that can reflect the qualitative impact of nutrient addition on ecosystem stability and health due to a complex relationship between nutrient input and ecological response. In this study, the experimental conditions are more similar to nutrient inputs in natural nearshore waters. On this basis, and including the indicative role of plankton metabolism on ecosystem health as mentioned above, the daily input nutrients of 2 μmol/(L·d) (in terms of N) and 0.066 μmol/(L·d) (in terms of P) can be regarded as the threshold, since GPP, CR, and most biotic factors changed significantly when daily nutrient inputs exceed this level according to the statistical result in both D0–D5 period (Fig. 6) and the whole 10 d incubation period (Fig. S3). Although the environmental nutrient concertation did not change significantly until the inputs exceeded 4 μmol/(L·d) (in terms of N) and 0.066

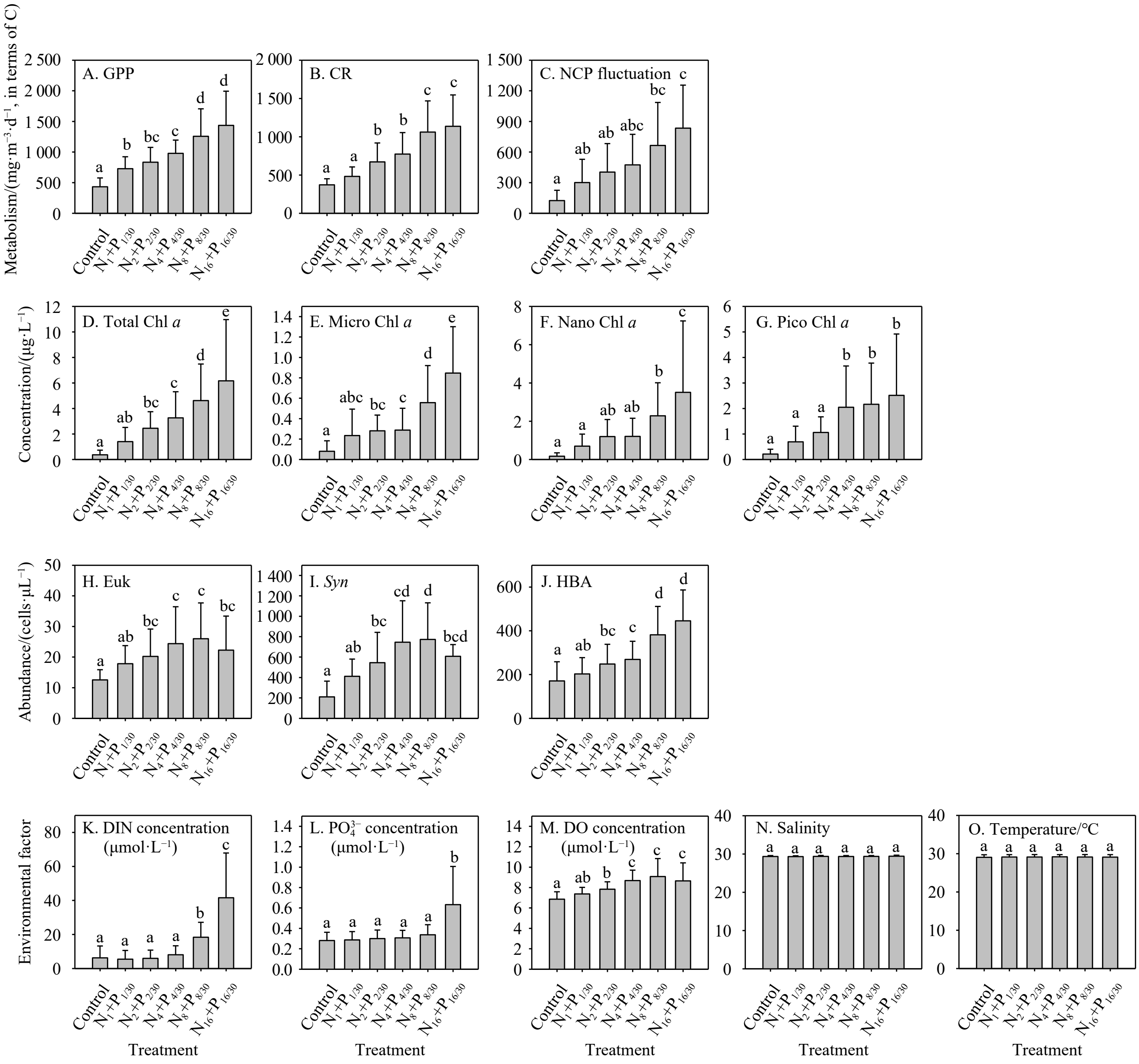

Fig. 6. Comparison of the ecological responses to nutrient input of different concentrations (different lowercase, a to e, indicate statistically significant differences between treatments at $p<0.05$; vertical bars indicate the standard deviations from triplicate pools of each mesocosms; data from the D0–D5 period). The implications of the subscripts are listed in Table 1.

μmol/(L·d) (in terms of P), the significant change of metabolism, biotic factors, and phytoplankton diversity has indicated a severe disorder of metabolic balance and disruption of the planktonic ecosystem. Therefore, planktonic metabolism is suggested as a more indicative index than environmental nutrient concentration to evaluate ecosystem health, and 2 μmol/(L·d) (in terms of N) and 0.066 μmol/(L·d) (in terms of P) could be considered the critical nutrient thresholds for ecosystem health management in the Daya Bay.

The total dissolved N and P fluxes of major rivers in the Daya Bay are 3 690.39 t/a and 80.63 t/a respectively (averaged 0.109 μmol/(L·d) (in terms of N) and 0.002 μmol /(L·d) (in terms of P), respectively) (Huang et al., 2019), which are much lower than the potential criteria indicated in this study (Table 4). However, eutrophication along the nearshore regions is much more severe than the average level of the whole bay. The Daya Bay is a subtropical drowned valley bay, and its hydrological conditions vary significantly between the monsoonal wet and the dry season. For example, more than 70% of annual precipitation occurs during the wet season (March to August) (Ke et al., 2019), which causes excessive nutrients from riverine input. Therefore, the semi-enclosed Aotou Cove receive 4.45 μmol/(L·d) (in terms of N) and 0.08 μmol/(L·d) (in terms of P) (Table 4) due to high nutrient inputs from the Dan'Ao River and its neighboring terrestrial sources in the northwest nearshore of the Daya Bay, and the seasonal water stratification and the lack of seawater-exchange fur-

Table 4. Dissolved nitrogen (N) and phosphorus (P) fluxes from major rivers and sewage outlets in the Daya Bay

Water region	Period	Daily input/ μmol·(L·d)⁻¹		Reference
		N	P	
Aotou Cove	yearly	2.845	0.051	Huang et al. (2019)
	wet season	4.45	0.080	Huang et al. (2019)
Whole Daya Bay	yearly	0.109	0.002	Huang et al. (2019)

Note: The water volume of the Daya Bay is 6.6 km³ (600 km²×11 m), and that of Aotou Cove is 0.195 km³ (39 km²×5 m).

ther prolong the residence time of exogenous nutrients (Sun et al., 2011). Since in the Daya Bay, the plankton community is more sensitive to N or N+P enrichment than P enrichment (Song et al., 2019), the excessive N input (more than 2 μmol/(L·d)) mentioned above emphasize the instability and ecological risk of the nearshore aquatic ecosystems. Frequently reported harmful algal blooms and hypoxia events also proved the vulnerability of nearshore waters affected by continuously exogenous nutrient inputs in this bay (Song et al., 2004, 2009).

5 Conclusions

Based on a mesocosm experiment with daily nutrient inputs in a semi-enclosed bay, this study indicates the potentially different responses of GPP and CR to nutrient input at various concentration levels. Continuously high nutrient inputs caused drastic fluctuation of metabolism balance with frequent shifts between autotrophic and heterotrophic states, which further affected the functional stability of the planktonic ecosystem and increased the risk of algal bloom and hypoxia event in semi-enclosed and stable waters. Moreover, continuous nutrient inputs minimized and simplified phytoplankton communities in the nearshore waters. This study also indicates that nutrient inputs of 2 μmol/(L·d) (in terms of N) and 0.066 μmol/(L·d) (in terms of P) could be considered as a potential criterion for eutrophication management in nearshore waters with insufficient water exchange, as significant changes of planktonic metabolism, community structure and biomass appeared when nutrient input exceeded this level. It is suggested that the metabolic responses of plankton community to dynamic nutrient inputs have important implications for further assessment of coastal ecosystem health and carbon budget.

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Supplementary information:

Fig. S1. Changes of diatom (a) and dinoflagellate (b) abundances in the mesocosms.

Fig. S2. Size structure of Chl *a* (a) and primary production (b) in the mesocosms on D10.

Fig. S3. Comparison of the ecological responses to nutrient input of different concentrations.

Table S1. Changes of dominant phytoplankton species (the three most dominant) before (the initial) and after 10 d incubation in different treatments.

Table S2. Nutrient criteria for eutrophication in different regions.

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