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LETTER

Primary productivity coupled to oxic methane production in coastal waters of southern China

Yuming Rao [1], **Guang Gao** [2], **Mina Bizic** [3], **Ilana Berman-Frank** [4], **Kunshan Gao** [2,5]*

[1]State Key Laboratory of Marine Environmental Science, College of the Environment and Ecology, Xiamen University, Xiamen, China; [2]State Key Laboratory of Marine Environmental Science, College of Ocean and Earth Science, Xiamen University, Xiamen, China; [3]Environmental Microbiomics, Technische Universität Berlin, Berlin, Germany; [4]Department of Marine Biology, Leon H. Charney School of Marine Sciences, University of Haifa, Haifa, Israel; [5]Co-Innovation Center of Jiangsu Marine Bio-industry Technology, Jiangsu Ocean University, Lianyungang, China

Scientific Significance Statement

Phytoplankton species have been shown to produce methane (CH_4) during photosynthesis, which contributes to the accumulation of CH_4 in oxic environments. However, the mechanistic relationship between phytoplankton-derived CH_4 production and primary production, especially in situ, is poorly understood. To our knowledge, this is the first study to simultaneously measure these two important processes in natural phytoplankton assemblages, allowing us to reveal positive correlations between CH_4 production and primary production in highly productive coastal waters in the southern East China Sea. Our findings suggest that CH_4 produced by the coastal phytoplankton community could be another source of atmospheric CH_4 and that changes in the phytoplankton community structure may not only influence CO_2 sequestration but also the global CH_4 budget.

Abstract

Oxic methane (CH_4) production (OMP) occurs in diverse oxygenated surface waters worldwide. However, phytoplankton-driven OMP in natural marine environments remains poorly documented. During a research cruise in the highly productive southern East China Sea, we measured OMP by incubating phytoplankton-rich surface waters and found that CH_4 production was positively correlated with chlorophyll *a* concentration and primary production, and that natural phytoplankton communities predominated by diatoms led to higher CH_4 production. Oxic methane production ranged between 0.9 and 2.1 mg CH_4 g Chl a^{-1} h^{-1}, indicating that 0.02–0.06% of the photosynthetically fixed CO_2 could be released as CH_4. Measurements of the phytoplankton-free filtrate demonstrated a negligible contribution to OMP by heterotrophs, substantiating that phytoplankton are contributing to the CH_4 oversaturation in the coastal oxic layer of this region. Moreover, high OMP in the photic zone partially counterbalances photosynthetic CO_2 sequestration by phytoplankton and should be accounted for in assessing fluxes of greenhouse gases.

*Correspondence: ksgao@xmu.edu.cn

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Methane (CH_4) is an important greenhouse gas with a global warming potential 20–80 times greater than carbon dioxide (CO_2), depending on the calculation period (100 or 25 years, IPCC 2021). Traditionally, it was believed that CH_4 is produced exclusively in anoxic environments by obligate anaerobes (Ferry and Lessner 2008). Yet, evidence demonstrates CH_4 oversaturation in oxygenated surface layers across freshwater and marine ecosystems—a phenomenon known as the "methane paradox" (Gucinski 1991).

Persistent CH_4 oversaturation in oxic layers requires in situ production or transport to compensate for the CH_4 oxidation by methanotrophs and CH_4 flux from the aquatic environments to the atmosphere (León-Palmero et al. 2020). In shallow lakes, CH_4 oversaturation in the oxic layers may partly originate from anoxic sediments (Peeters et al. 2019; Peeters and Hofmann 2021) or in situ production during stratification (Bartosiewicz et al. 2023; Ordóñez et al. 2023; Thottathil et al. 2022). In deep and thermally stratified systems, the thermocline acts as a physical barrier which, combined with CH_4 oxidation, prevents the CH_4 upward transportation to the surface waters (Tang et al. 2016; Thalasso et al. 2020; Vachon et al. 2019). Therefore, different CH_4 production in the oxic layers may better explain the methane paradox (Damm et al. 2015; Donis et al. 2017; Günthel et al. 2020, 2021; Holmes et al. 2000; Karl et al. 2008; Scranton and Brewer 1977).

In situ studies demonstrated a consistent link between dissolved CH_4 concentration and phytoplankton-related parameters (e.g., chlorophyll *a*, cell density, Bogard et al. 2014; Günthel et al. 2020; Hartmann et al. 2020; León-Palmero et al. 2020). These associations likely arise from co-occurring yet different metabolic pathways associated with phytoplankton activity (Günthel et al. 2021). One pathway is CH_4 released as a by-product when microorganisms, including cyanobacteria, use methylphosphonate (MPn) under phosphate limitation (Beversdorf et al. 2010; Karl et al. 2008; Repeta et al. 2016; Teikari et al. 2018). Demethylation of methylamines and dimethyl sulfide (DMS) may also contribute to CH_4 production in oxic waters (Bizic-Ionescu et al. 2018; Wang et al. 2021). Alternatively, phytoplankton may release CH_4 during photosynthesis, as shown by the release of ^{13}C-CH_4 when incubated with ^{13}C-bicarbonate under light (Bizic et al. 2020; Hartmann et al. 2020; Klintzsch et al. 2019, 2020; Lenhart et al. 2016). This process results in a isotopic signature (−33.7 ‰, Klintzsch et al. 2023) distinguishable from CH_4 produced by methanogenic archaea and relies on photosynthetic electron transport and carbon fixation (Rao et al. 2024). Yet, the link between CO_2 fixation and CH_4 production by phytoplankton communities has not been tested in the marine environment.

Coastal waters may contribute ~ 50% of the total global ocean CH_4 flux, where most CH_4 produced in the sediments is released into the atmosphere before being oxidized (Resplandy et al. 2024; Weber et al. 2019). However, the limited availability of global and regional data generates high uncertainty in the quantitative estimates of CH_4 emission from these areas (Saunois et al. 2025).

We chose four stations in Dongshan Bay, southern East China Sea representing varying nutrient levels and phytoplankton communities (Zhong et al. 2020) to assess the phytoplankton's capacity to produce CH_4. We measured photosynthetic carbon fixation and CH_4 production simultaneously and found positive correlations between CH_4 production, photosynthetic carbon fixation, and phytoplankton biomass. Sequencing analyses indicated that the *Thalassiosirales*' predominance in these stations was associated with CH_4 production, suggesting that diatoms may contribute to oxic CH_4 production in this region.

Materials and methods

Field studies and experimental setup

Four different shallow (< 21 m depth) stations in Dongshan Bay, East China Sea were investigated on April 30th, 2024 (Fig. 1). Chlorophyll *a* concentrations, dissolved oxygen, salinity, and temperature of each station (except DS09) were measured in situ with a CTD (CastAway, SonTek, USA) equipped with algae (EXO sensor, YSI, USA) and dissolved oxygen sensors (Oxix sensor, mjk, Australia). Surface water (< 1 m) was filtered through a 20 μm sieve mesh to exclude copepod nauplii and large organic particles. The filtrate containing phytoplankton cells were then immediately transferred into 1.1 L borosilicate bottles sealed with lids (GL 45, 3 ports, Rao et al. 2024), leaving 200 mL headspace. Three independent replicates were prepared per station and incubated at 24°C in a plant growth chamber (Ruihua, China) with cool white light at ~ 180 μmol photons m^{-2} s^{-1} and a 12: 12 light: dark cycle matching the natural light cycle.

Photosynthetic CO_2 fixation and CH_4 production were simultaneously measured to establish CH_4 production quotients (MPQs, mole CH_4 released per mole fixed CO_2). CH_4 production quotient values for the 5 h incubations were obtained on May 1st (10:00–15:00) and for the 12 h incubations were obtained on May 2nd (sunrise to sunset).

Measurement of major nutrients and chlorophyll *a* concentration

Samples for nutrient analyses ($NO_2^- + NO_3^-$ and PO_4^{3-}) were filtered through 0.45 μm cellulose acetate membranes and stored at −20°C prior to measurements using a four-channel auto-analyzer (AA3, Seal, Germany). $NO_2^- + NO_3^-$ were analyzed using the copper-cadmium reduction method. PO_4^{3-} was measured using the typical spectrophotometric method (Knap et al. 1994). The calibration curves were shown in Supporting Information Fig. S1.

Samples for chlorophyll *a* measurements were filtered onto GF/F membranes (Whatman, USA) at < 0.02 MPa and extracted overnight in pure methanol at 4°C in the dark. The extracts were centrifuged (8000 × *g*, 10 min), and the supernatant's absorption at 750 nm, 665 nm, and 652 nm was measured using a UV–VIS spectrophotometer (TU1810, Persee,

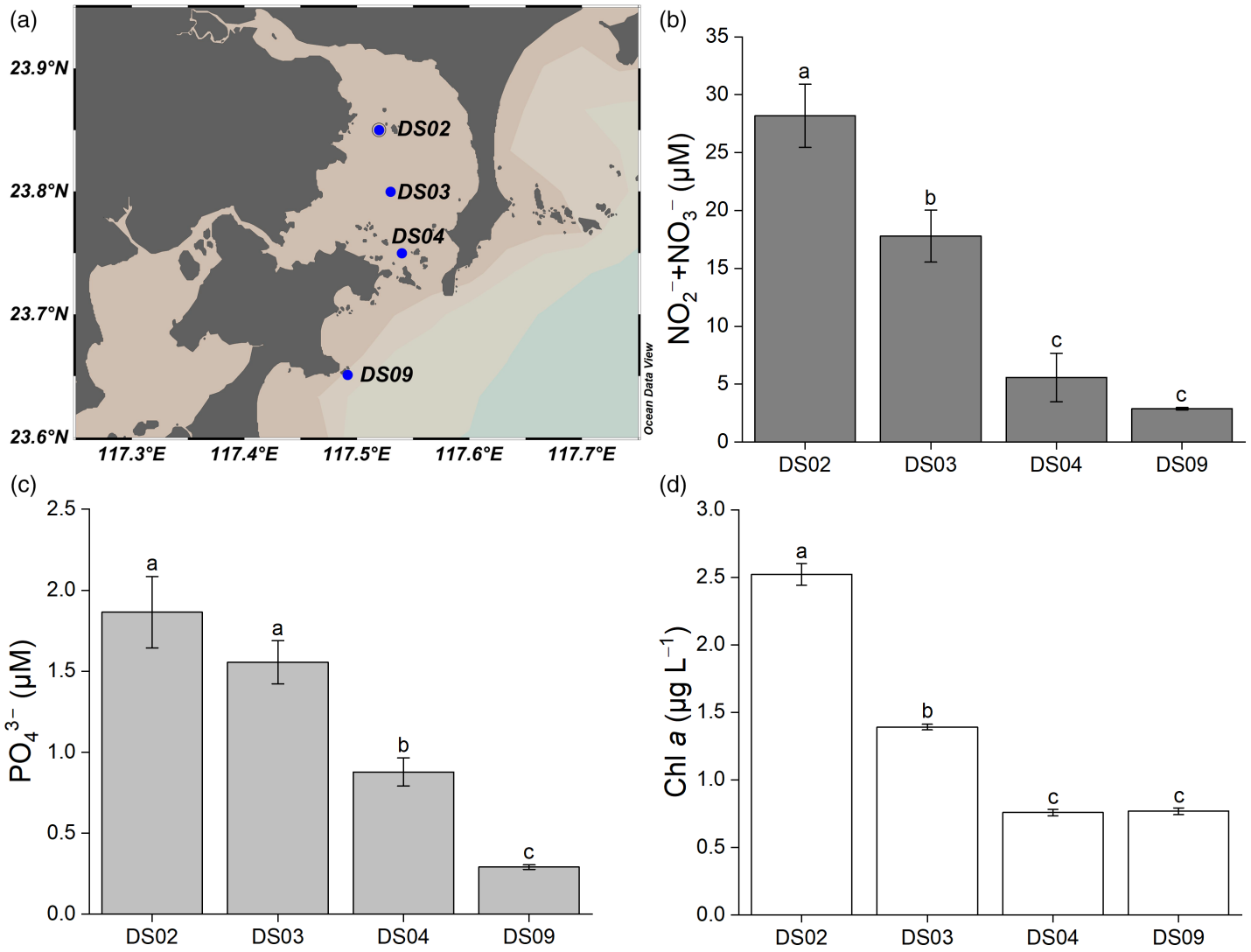

Fig. 1. Sampling sites (blue dots) in the East China Sea near the western Taiwan Strait from which surface water was sampled for on deck experiments on April 30th, 2024. (**a**), nutrient concentrations of $NO_2^- + NO_3^-$ (**b**), PO_4^{3-} (**c**), and Chl *a* (**d**) of the surface water prior to the incubation experiments. Data are means ± SD of triplicates for each station; different letters mark significant differences between samples from different stations ($p < 0.05$).

China), and chlorophyll *a* concentrations were calculated accordingly (Ritchie 2006).

Determination of CH_4 production

The CH_4 mixing ratio in the headspace was measured with a methane/nitrous oxide analyzer (Picarro G2308, Picarro Inc., CA, USA). Gas samples (200 mL) were prepared to maintain a stable reading with a sensitivity < 2 ppbv. During sampling, the same volume of sterilized air (ambient air filtered through 0.22 μm filter) was introduced into the headspace to maintain air pressure and gently mixed with the headspace. Calculations accounted for the CH_4 mixing ratio of the sterilized air (Zou et al. 2024). The CH_4 concentration in the water phase was calculated according to the Bunsen solubility coefficient (Wiesenberg and Guinasso 1979) and the following equation (Johnson et al. 1990):

$$CH_4(\text{nmol L}^{-1}) = (C_{g2} - C_{g1}) \cdot ((\beta/V_m) \cdot R \cdot T + V_g/V_l)$$

where C_{g2} and C_{g1} represent the CH_4 concentrations at time t_2 and t_1 in nmol L^{-1}, respectively. β is the CH_4 Bunsen solubility coefficient in the seawater in L L^{-1} atm^{-1}, V_m the molar volume of CH_4 in L mol^{-1}, T temperature in K, R the ideal gas constant in L atm mol^{-1} K^{-1}; V_g and V_l indicate the volumes of gas phase and water phase in L, respectively.

Measurement of photosynthetic carbon fixation

Subsamples (50 mL) from each incubation were transferred into quartz tubes and incubated for 5 h or 12 h with 100 μL

5 μCi $NaH^{14}CO_3$ (ICN Radio chemicals, USA). After incubation, cells were filtered onto GF/F membrane under low light and stored at −20°C. The filters were fumed with HCl overnight and dried at room temperature to remove unincorporated inorganic ^{14}C, then placed with 5 mL scintillation cocktail (Hisafe 3, Perkin-Elmer, USA) to count the incorporated ^{14}C using a liquid scintillation counter (Beckman, LS6500, Germany), and the photosynthetic C fixation was calculated as previously reported (Gao et al. 2007).

Identification of phytoplankton and microbial assemblage

Subsamples of 500 mL from each incubation were filtered on 0.22 μm polycarbonate membranes (GTTP, 47 mm, Millipore, USA) and transferred to liquid nitrogen immediately. The filters were stored at −80°C until 16S/18S rRNA gene extraction.

Microbial DNA was extracted using the HiPure Soil DNA Kits (Magen, Guangzhou, China). The target region of the ribosomal RNA gene was amplified by PCR (95°C for 5 min, followed by 30 cycles at 95°C for 1 min, 60°C for 1 min, 72°C for 1 min and a 7 min final extension at 72°C) using the primers described in Supporting Information Text S1. The PCR reaction system (New England Biolabs (USA)) is a 50 μL mixture containing 5× Q5@ Reaction Buffer (10 μL), 5 × Q5@ High GC Enhancer (10 μL), 2.5 mM dNTPs (1.5 μL), 1.5 μL of each primer (10 μM), Q5@ High-Fidelity DNA Polymerase (0.2 μL), and template DNA (50 ng).

Amplicons were extracted from 2% agarose gels and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, USA), then quantified using a real-time PCR system (ABI StepOnePlus, Life Technologies, USA). The purified amplicons were pooled in equimolar concentrations and paired-end sequenced (PE250) on an Illumina platform (Novaseq 6000).

Excluding the effects of heterotrophs on CH_4 concentration changes

1.2 μm PC membranes were used to remove phytoplankton cells from the in situ water, and the filtrate containing potential heterotrophs and methanotrophs was incubated as the bacterial control. Meanwhile, 2-bromoethanesulfonic acid (2-BES) was added to the incubations (final concentration of 5 mM) to exclude the potential influence of methanogenic archaea (Logroño et al. 2022).

Statistical analyses

The data are expressed in raw form or presented as means ± standard deviation (SD) with $n = 3$ (independent triplicate cultures). We used one-way ANOVA to assess significant differences among different stations at the 95% confidence level. The detailed methods for bioinformatics analyses are described in Supporting Information Text S1.

Results

Initial seawater characterization at the sampling stations

The dissolved oxygen (DO) and Chl *a* concentrations in station DS02 peaked at ~1.8 m depth, while in DS03 and DS04, DO concentrations did not significantly change with increased depth (Supporting Information Fig. S2).

$NO_2^- + NO_3^-$ and PO_4^{3-} concentrations varied significantly between stations ($F_{(3,8)} = 96.97$, $p < 0.001$, $\eta^2 = 0.97$; $F_{(3,8)} = 80.26$, $p < 0.001$, $\eta^2 = 0.968$, respectively), with concentrations decreasing from the estuary to the lowest at DS09 (Fig. 1b,c). Post hoc analyses showed no significant difference in $NO_2^- + NO_3^-$ concentrations between DS04 and DS09 ($p = 0.145$), or in PO_4^{3-} concentrations between DS02 and DS03 ($p = 0.537$). Chl *a* concentration showed a similar trend ($F_{(3,8)} = 1012.19$, $p < 0.001$, $\eta^2 = 0.997$) with the highest Chl *a* measured near the estuary and the lowest concentrations at the offshore stations; no significant difference was observed between DS04 and DS09 ($p = 0.803$, Fig. 1d).

Photosynthetic carbon fixation and CH_4 production

Excluding CH_4 production in DS09, similar trends were observed between carbon fixation, phytoplankton biomass (indicated by Chl *a* concentrations), and CH_4 production (5 h incubation) (Figs. 1d and 2a,b). Primary production showed significant spatial variations ($F_{(3,8)} = 49.63$, $p < 0.001$, $\eta^2 = 0.949$). The highest primary production was measured at DS02, gradually declining to minimal values at DS09, with no significant difference between DS04 and DS09 (post hoc test, $p = 0.754$). Although the spatial variation in carbon fixation rate was not significant ($F_{(3,8)} = 2.73$, $p = 0.114$, $\eta^2 = 0.505$), the highest rate in DS02 (0.31 ± 0.05 μmol C μg Chl a^{-1} h^{-1}) was significantly higher by 50% than the lowest rate in DS04 (0.19 ± 0.05 μmol C μg Chl a^{-1} h^{-1}, $p = 0.028$, post hoc test, Fig. 2c). CH_4 production showed no significant spatial variation ($F_{(3,8)} = 3.15$, $p = 0.087$, $\eta^2 = 0.541$), being highest at DS02 and lowest at DS04. Post hoc tests showed no significant difference between DS02 and DS03, or between DS04 and DS09 ($p = 0.676$ and 0.259, Fig. 2b). However, CH_4 production rate significantly varied between stations ($F_{(3,8)} = 4.21$, $p = 0.046$, $\eta^2 = 0.612$), the highest rate in DS09 was 105% higher than the lowest rate in DS02, with no significant differences among DS03, DS04, and DS09 (post hoc tests, $p = 0.584$, 0.058, and 0.14, Fig. 2 d). The photosynthetic C fixation and CH_4 production after 12 h incubations were similar to those measured after 5 h incubations (Fig. S4a,b), with comparable carbon fixation rates across stations (Fig. S4c). The highest CH_4 production rate was at DS04 (Fig. S4d).

Relationships between CH_4 production, primary production, and nutrient concentrations

Primary production (μmol C L^{-1} h^{-1}) showed a clear linear correlation with phytoplankton biomass for both 5 h and 12 h incubations (Fig. 3a and Supporting Information

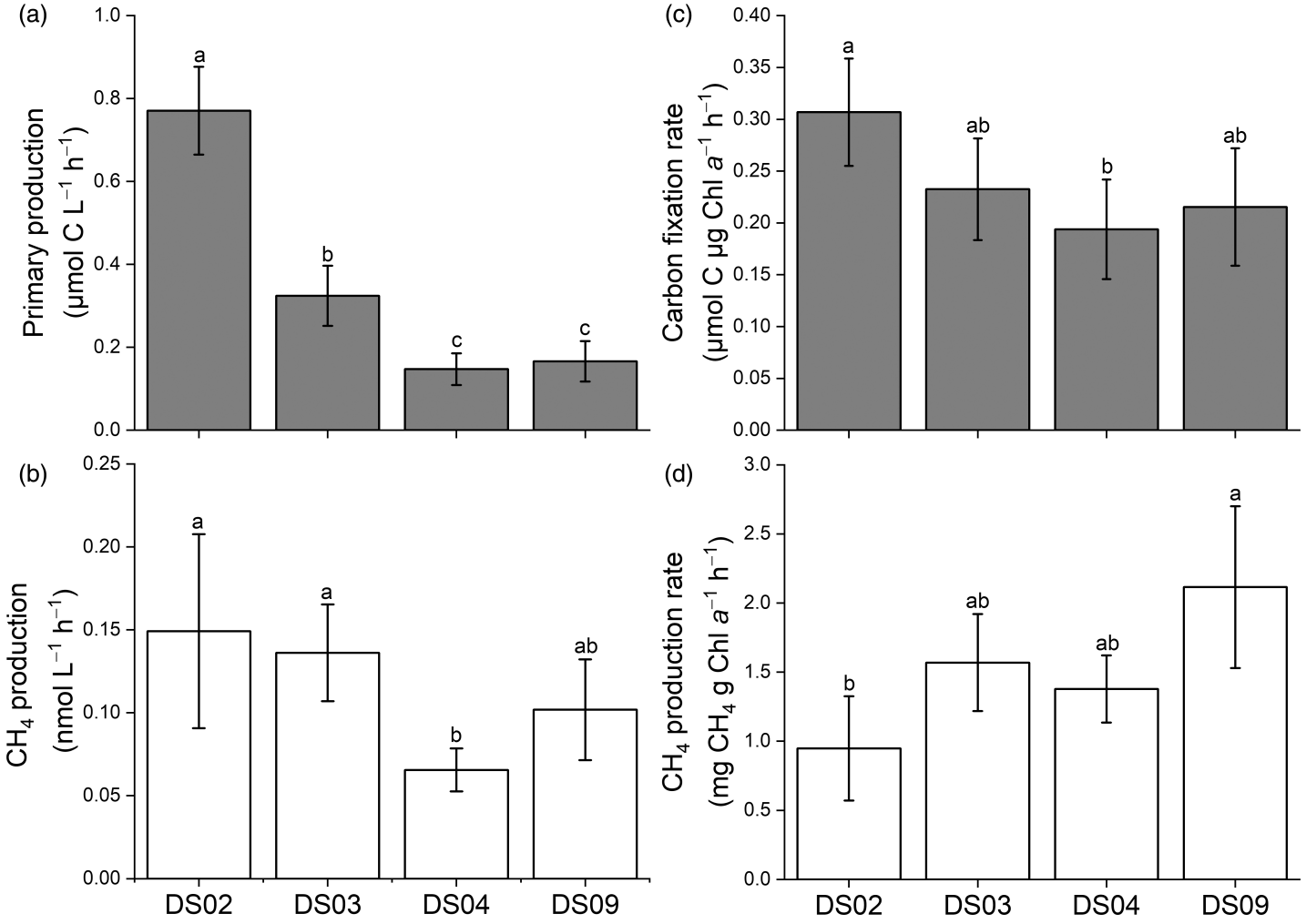

Fig. 2. Primary production (**a**), changes in CH_4 concentration (**b**), rates of carbon fixation (**c**) and CH_4 production (**d**) per gram Chl *a* based on 5 h incubation under light (180 μmol photons m^{-2} s^{-1}). Different letters mark significant differences between sampling sites ($p < 0.05$), and error bars mark the standard deviation for triplicate cultures.

Fig. S5a, Pearson's $R = 0.963$ and 0.862, $p < 0.001$). CH_4 production was positively correlated with Chl *a* concentration and primary production (Fig. 3b,c; Pearson's $R = 0.614$ and 0.578, $p = 0.033$ and 0.048; Spearman's $\rho = 0.713$ and 0.755, $p = 0.009$ and 0.005; Fig. S5b,c; Pearson's $R = 0.731$ and 0.733, $p = 0.007$ and 0.007).

The CH_4 production quotients ranged between 1×10^{-4} and 6×10^{-4} and showed significant spatial variations ($F_{(3,8)} = 4.62$, $p = 0.037$, $\eta^2 = 0.634$) among stations. MPQ increased by 200% when comparing the estuarine station (2×10^{-4}) to the open water station (6×10^{-4}) after 5 h incubation. The highest MPQ after 12 h incubation was measured at DS04 (Fig. 3d and Supporting Information Fig. S5d). Both primary production and CH_4 production were positively correlated with the major nutrients concentrations (Fig. 4 and Supporting Information Fig. S6).

Microbial community structure

The 18S rRNA gene sequence analyses (124,757 clean reads) identified 17 phyla, 34 classes, 47 orders, 52 families, 54 genera and 48 eukaryotic species. Alveolata, Bacillariophyta, Chlorophyta, and Ciliophora were the most abundant phyla in each station; the phylum Cnidaria was also abundant at DS09 (11.69%, Fig. 5a). The predominant orders comprising the phytoplankton communities were Thalassiosirales, Cymatosirales, Gymnodiniales, and an unnamed order in the class Trebouxiophyceae (Fig. 5b). Alpha diversity analyses showed no significant difference between communities for all stations (Fig. S8a).

The 16S RNA gene sequence analyses (123,789 clean reads) identified 9 phyla, 12 classes, 33 orders, 43 families, 73 genera, and 39 species of prokaryotes. Pseudomonadota, Bacteroidota, Actinobacteriota, and Planctomycetota were the most

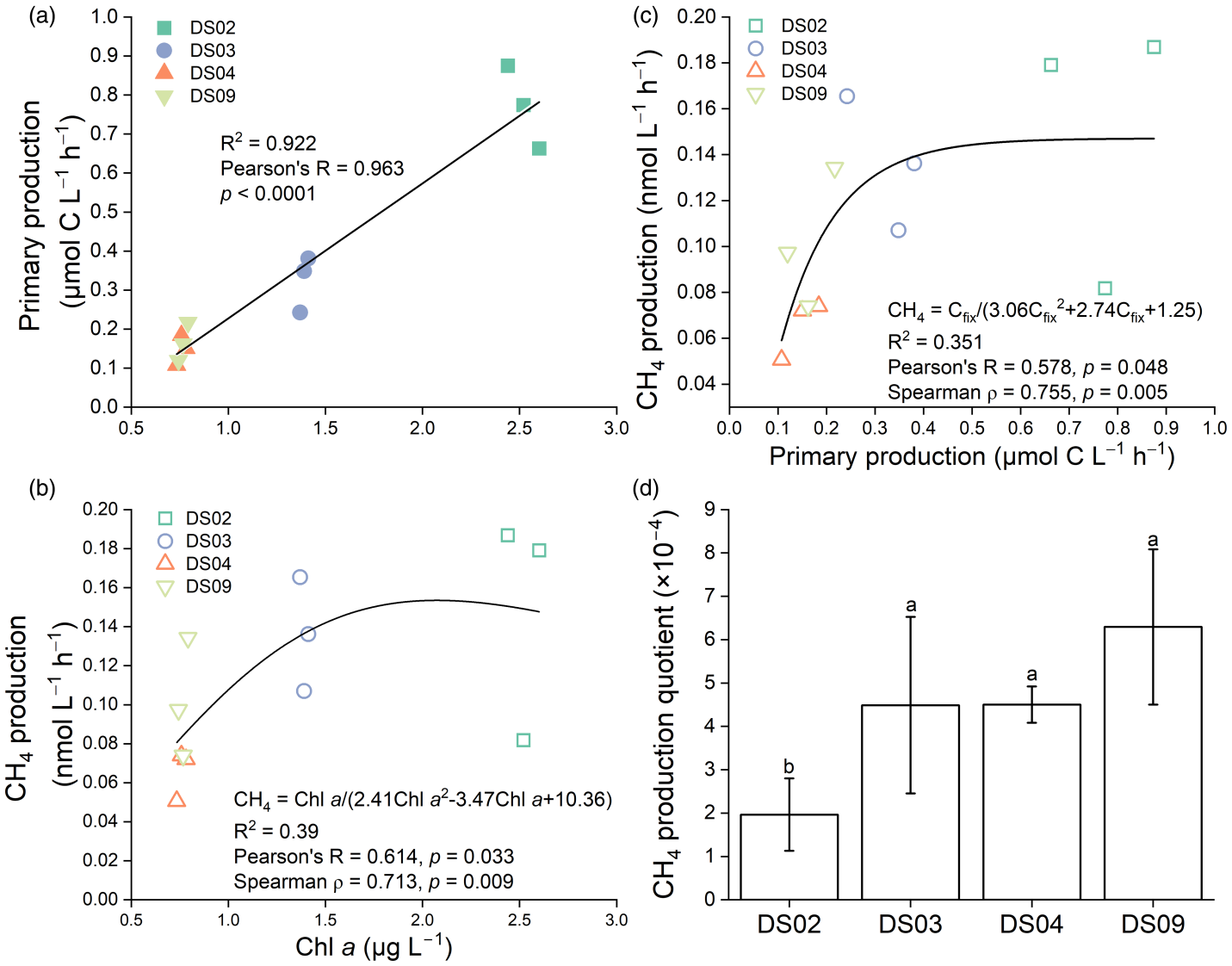

Fig. 3. Correlation of primary production (**a**) and CH_4 production (**b**) vs. Chl *a* concentration in each station, the relationship of CH_4 production and primary production (**c**), and CH_4 production quotients (CH_4 released vs. CO_2 fixed) based on 5 h incubations of the samples taken from each station (**d**). In panels a, b and c, the values are raw data of independent triplicate cultures, symbols with different colors represent different stations. The values in panel (d) represent means, and error bars indicate the standard deviations of the triplicate cultures. Different letters above the bars mark significant differences between samples from different stations ($p < 0.05$).

abundant taxa at the phylum level of all stations (Fig. 5c). The predominant groups comprising the bacterioplankton community were from the classes Alphaproteobacteria, Bacteroidia, Gammaproteobacteria, and Acidimicrobiia (Fig. 5d). Cyanobacteria comprised < 3% of the community at all stations. The alpha diversity was significantly higher in DS02 than in DS04. No significant difference in community composition was observed between DS02 and DS03 or DS09 (Fig. S8b).

Discussion

In this study, we investigated the relationship between photosynthetic carbon fixation and oxic methane production (OMP) utilizing on-deck microcosm incubations of surface waters from 4 stations in the southern East China Sea. 2-BES inhibited potential archaeal methanogenesis (Grossart et al. 2011), and phytoplankton-free filtrates showed negligible heterotrophic CH_4 production (Fig. S7). Subsequently, our results showed that CH_4 production was positively correlated with Chl *a*, primary production, and dissolved inorganic nutrient at each station.

Alphaproteobacteria, Gammaproteobacteria, and Cyanobacteria may produce CH_4 from MPn or methylamine under phosphate or nitrogen limitation (Carini et al. 2014; Repeta et al. 2016; Wang et al. 2017; Bizic-Ionescu et al. 2018; Ye

et al. 2020; Wang et al. 2021). These pathways may not be responsible for the CH_4 production in our study, as the positive correlations between CH_4 production and major nutrients (Fig. 4 and Supporting Information Fig. S6) contradict previous studies demonstrating that increasing phosphate concentrations inhibit CH_4 production via MPn demethylation (Beversdorf et al. 2010). In addition, ammonium availability in our study area (Zhang et al. 2025) may inhibit methylamine demethylation, a process currently demonstrated only in freshwater (Wang et al. 2021).

Synechococcus and *Prochlorococcus* are present in the study area (Zhong et al. 2020) and may exist in the bacterial control, could also contribute to photosynthesis-associated CH_4 production (Bizic et al. 2020), but their contribution was likely negligible in our incubations (Fig. S7). In contrast, positive correlations were found between CH_4 production and the relative abundance of *Thalassiosirales* (diatom) and *Trebouxiophyceae* (Chlorophyta) (Supporting Information Table S1). Previous studies have shown that diatoms and green algae (Günthel et al. 2020; Hartmann et al. 2020) can produce CH_4. Thus, it is most likely that diatoms and green microalgae contributed to the observed CH_4 production in our incubations (Fig. 5a,b).

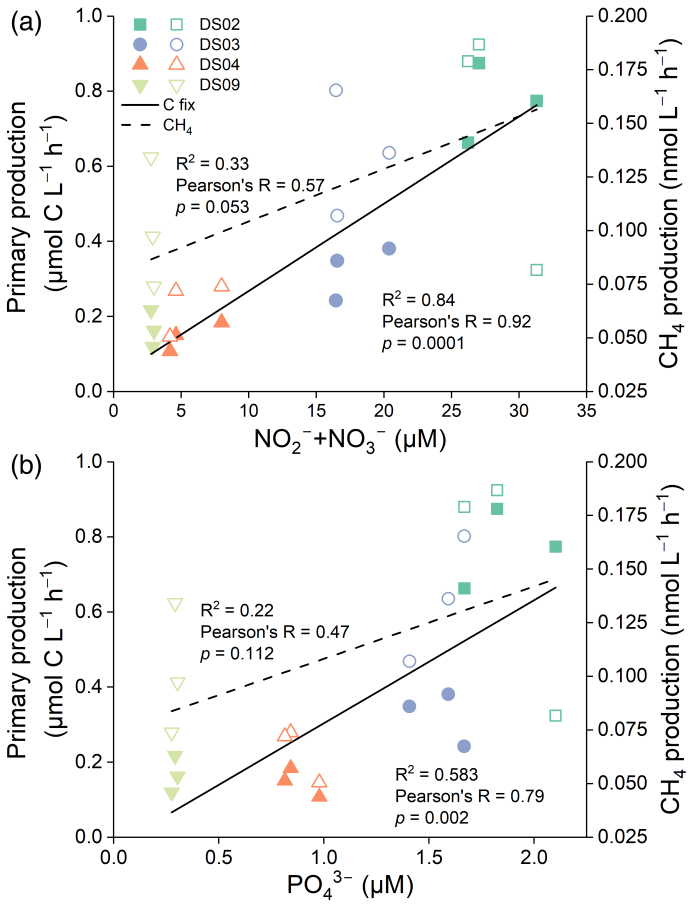

Fig. 4. Linear relationships of primary production (solid) and CH_4 production (hollow) vs. $NO_2^- + NO_3^-$ (**a**) and PO_4^{3-} (**b**) concentrations on 5 h incubation. The values are raw data of independent triplicate cultures, symbols with different colors represent different sampling stations.

To demonstrate a direct link between photosynthesis and OMP, light is required to convert bicarbonate to CH_4 and CH_4 production is thus light-induced (Bizic et al. 2020; Günthel et al. 2020; Hartmann et al. 2020; Klintzsch et al. 2020; Rao et al. 2024). Our results clearly showed that CH_4 production was correlated with primary production and phytoplankton biomass; both CH_4 and primary production positively correlated with macronutrients (Figs. 3 and 4; Supporting Information Figs. S5 and S6), suggesting nutrient levels may indirectly affect CH_4 production via influencing photosynthesis. Nutrient influences photosynthesis by affecting the synthesis of Rubisco, Chl *a*, ATP, NADPH and other photosynthetic components (Hipkin et al. 1983; Raven et al. 2016). Thus, higher nutrient levels in the estuary stations could have resulted in larger photosynthetic unit with lower ratio of reaction centers: Chl *a* (Perry et al. 1981; Malerba et al. 2018), resulting relatively lower photosynthetic rates per Chl *a* (Supporting Information Table S2). Consequently, CH_4 production rate normalized to Chl *a* did not increase with increased Chl *a* concentration (Figs. 2 and 3; Supporting Information Fig. S4).

The CH_4 production quotients (MPQ) varied between the stations and different incubation times. While CH_4 production decreased from the estuary to the offshore with decreasing nutrient availability, higher MPQ values were obtained at DS04 and 09. This may be due to species specific differences in the CH_4 production capacity (Bizic 2021) or the shift of predominant species from diatoms in estuary to heterotrophic (mixotrophic) dinoflagellates in open water (Fig. 5b), leading to lower primary production (Bjørnsen and Kuparinen 1991; Huang et al. 2021). Alternatively, the presence of methanotrophs, although in relatively low abundance (*Methlyoparacoccus* in DS02, 0.003% and *Methylocystis* in DS03, 0.006%), might have affected the MPQ. As our incubations did not account for CH_4 oxidation, either by using inhibitors (Frenzel and Bosse 1996) or by isotopic approaches (Hartmann et al. 2020), our data point to net CH_4 production. The longer light exposure and higher phytoplankton biomass resulted in increased CH_4 production (Fig. 2b and Supporting Information Fig. S4b; Klintzsch et al. 2020), and the MPQ was further modified by higher CH_4 oxidation during longer incubation, resulting in lower MPQ values than shorter incubation (Fig. 3d and Supporting Information Fig. S5d). Therefore, CH_4 consumption by oxidation should be considered in future studies for more precise estimates of OMP in marine environments.

This study suggested that phytoplankton inhabiting the coastal oxygenated photic layer likely contribute to CH_4 oversaturation and subsequent sea-to-air flux. Although photosynthesis-related CH_4 production was relatively lower than that reported in lake systems (Günthel et al. 2020, 2021; Hartmann et al. 2020), it remains an overlooked pathway in

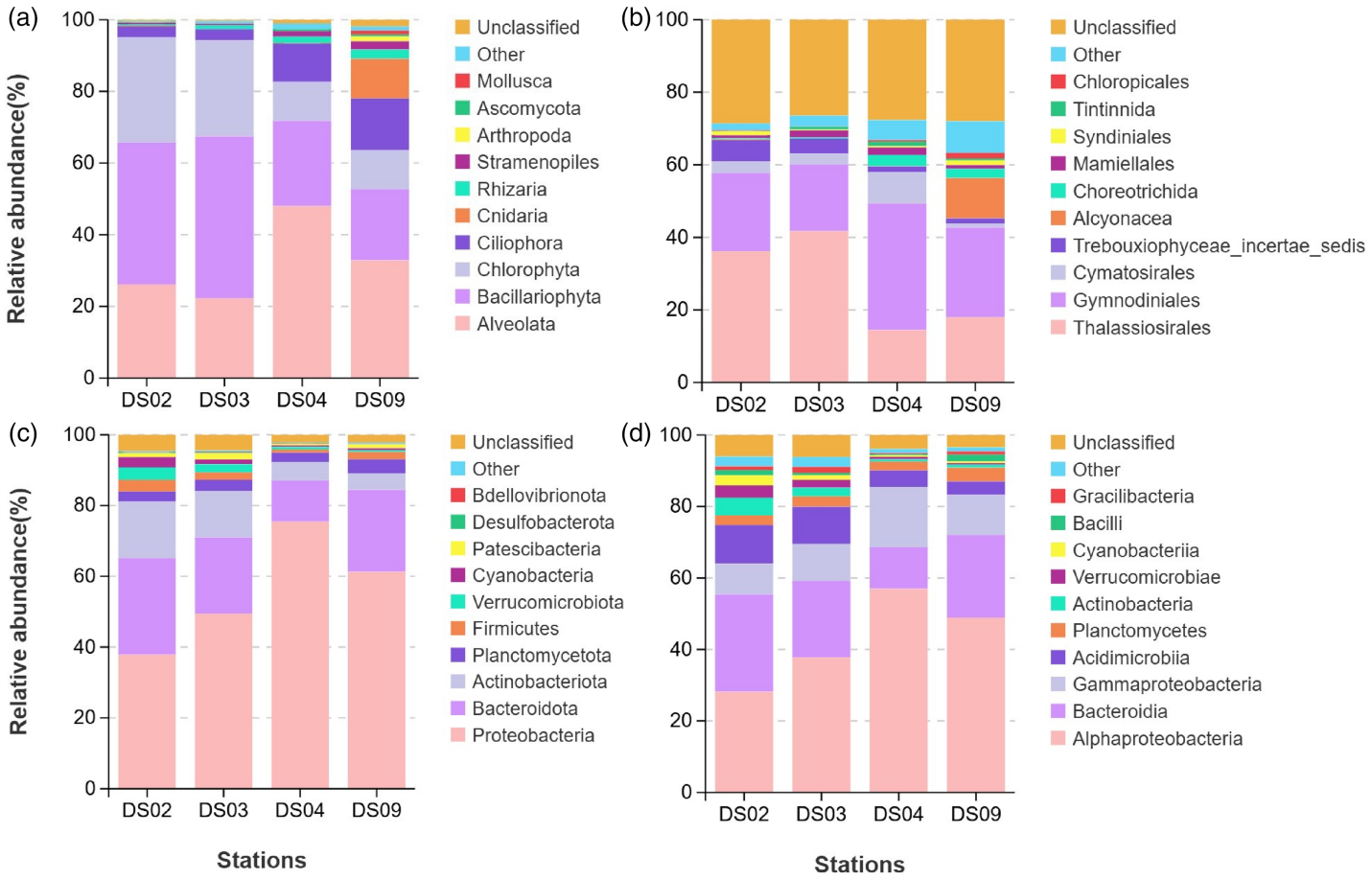

Fig. 5. Community structure of eukaryotic plankton (**a**: phylum, **b**: order) and bacteria (**c**: phylum, **d**: class) from the surface water sampled from each station and used for the microcosm experiments (analyses are from in-situ water prior to incubation). The x-axis represents the sample station, and the y-axis represents the relative abundance of different groups of plankton.

marine ecosystems and may partially counteract the role of phytoplankton in mitigating the greenhouse effect through the biological carbon pump (Rao et al. 2024). In general, eukaryotic phytoplankton produce less CH_4 than cyanobacteria (Bizic 2021); however, since marine primary production is dominated by cyanobacteria (Aguilo-Ferretjans et al. 2021; Flombaum et al. 2013), photosynthesis-related CH_4 production cannot be neglected and should be further investigated for reliable ocean–atmosphere CH_4 flux estimates.

Our results substantiate previous studies showing that CH_4 production is regulated by temperature (Klintzsch et al. 2020), nutrients (Fig. 4 and Supporting Information Fig. S6; Ordóñez et al. 2023), and light (Klintzsch et al. 2020; Rao et al. 2024), and that seasonal variations altering photosynthesis may further modify the CH_4 production and MPQ. Notably, although some large phytoplankton may have been excluded by filtration from our incubations (Nogueira et al. 2014), our data (Supporting Information Table S1) still suggests that diatoms may be a CH_4 source in coastal waters. Only a few diatoms have been tested for CH_4 production (Günthel et al. 2020; Hartmann et al. 2020). Given their frequent occurrence in coastal waters and key role in primary production (Kiene 2008), the global climate feedback of diatoms should be re-evaluated for their ability to produce CH_4. Evaluation of MPQ across different phytoplankton groups and oceanic regions is therefore required to assess the warming potential associated with CO_2 and CH_4 in a changing ocean.

Author Contributions

Yuming Rao and Kunshan Gao designed the experiment. Yuming Rao conducted the experiment and wrote the original manuscript. All of the authors contributed to the analyses, interpretation of data, and manuscript writing.

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Conflicts of Interest

None declared.

Data Availability Statement

The original data that supports the findings in this work is available at Figshare (https://figshare.com/s/67876f32fab99863c894) (Rao et al. 2025), the raw sequence data reported in this work have been deposited in the Genome Sequence Archive (GSA; Chen et al. 2021) in National Genomics Data Center, China National Center for Bioinformation/Beijing Institute of Genomics, Chinese Academy of Sciences (GSA: CRA028480 and GSA: CAR028479) and available at https://ngdc.cncb.ac.cn/gsa/s/Pw946F7o and https://ngdc.cncb.ac.cn/gsa/s/97yg8R9j.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

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