Nitrogen Limitation Enhanced Calcification and Sinking Rate in the Coccolithophorid *Gephyrocapsa oceanica* Along With Its Growth Being Reduced

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Phytoplankton are exposed to different concentrations of nutrients in different waters along with changing light levels during diurnal and seasonal cycles. We grew the coccolithophorid *Gephyrocapsa oceanica* semi-continuously at different nitrate levels under indoor low and outdoor high light conditions, and found that reduced nitrate availability significantly increased its production of particulate inorganic carbon (PIC), with its growth being reduced. High light treatment suppressed the growth of nitrate-limited cells and their efficiency of N assimilation by up to 63% compared to low light treatment. The combination of high light and nitrate limitation increased contents of PIC per cell due to sustained photochemical energy transfer, resulting in faster sinking rates by up to 82% in comparison with nitrate-repleted cells. Additionally, the sinking rates were positively correlated with ratios of PIC to particulate organic carbon (POC). These results imply that coccolithophores distributed in oligotrophic waters could be more effective as the ballast in aggregates, facilitating particulate organic carbon flux to deeper waters.

Keywords: calcification, coccolithophore, growth, light, nitrate-limitation, photosynthesis, sinking rate

INTRODUCTION

Particulate organic carbon (POC) produced by phytoplankton and their grazers is sunk out the euphotic layer with particles and partly sequestered in the sediments, efficiently facilitating the absorption of atmospheric CO_2 by the oceans (Falkowski et al., 1998; Boyd and Trull, 2007). Recent findings highlight the ballast effect in particles sinking, e.g., opal and $CaCO_3$ produced by diatoms and coccolithophores can accelerate the sinking rate by increasing the specific gravity of particles during the sinking process (Armstrong et al., 2002; Klaas and Archer, 2002). For instance, when copepods were fed on coccolithophores or high-silica diatoms, the fecal pellets sank much faster than when they were fed on dinoflagellates or low-silica diatoms (Ploug et al., 2008; Liu and Wu, 2016). In addition, evidence on sediment traps has discovered that most POC fluxes are strongly correlated with amounts of $CaCO_3$ (Klaas and Archer, 2002; Lam et al., 2011). As the main calcifying phytoplankton, coccolithophores have contributed to 20–80% of biogenic carbonate

exported from the photic zone (Ziveri et al., 2007). Since the production of particulate inorganic carbon (PIC) by coccolithophores is susceptible to changes in pH, temperature, and nutrients (Feng et al., 2017; Zhang and Gao, 2021), the ballast effect is likely to be sensitive to ocean climate changes. It is known that the calcification of coccolithophores decreases under influence of ocean acidification, leading to a reduction of the $CaCO_3$ precipitation (Riebesell et al., 2000; Raven and Crawfurd, 2012), which decreased the sinking rates of particles (Riebesell et al., 2016).

Nitrogen (N) is one of the major limiting nutrients in contemporary pelagic oceans (Moore et al., 2013). Such limitation is suggested to be exacerbated with progressive stratification of the upper mixed layer caused by ocean warming since the upward transport of nutrients from deeper layers is hindered (Boyd et al., 2008). It is well-known that N-limitation restricts phytoplankton photosynthesis and growth (Behrenfeld et al., 2006) and pressures phytoplankton cells to be more susceptive to other environmental stresses (Beardall et al., 2014; Eberlein et al., 2016; Marañón et al., 2018). In coccolithophores grown under constant light conditions, some studies showed that N deficiency decreased POC but increased PIC production (Kaffes et al., 2010; Zhang and Gao, 2021), but other works indicated that it decreased or resulted in insignificant changes in PIC content per cell (Langer et al., 2012; Feng et al., 2017). Such discrepancy demands further studies to explore the relationship of their physiology and sinking rates with environmental changes.

It has been documented that phytoplankton species grown under natural fluctuating sunlight exhibited different physiological traits compared to their growth under artificial constant light (Litchman et al., 2004; Floder and Burns, 2005; Jin et al., 2013). Consequently, influences of nutrients limitation on the cells may differ between indoor low constant and outdoor high fluctuating light conditions. Therefore, we hypothesize that coccolithophores exhibit different photo-physiological responses under constant and fluctuating light conditions to nitrate limitation, influencing the PIC production and sinking rates of the cells. To test this, we grew *Gephyrocapsa oceanica*, one of the major coccolith-carbonate flux contributors in the sediment-trap survey (Ziveri et al., 2007), under a range of nitrate concentrations and different light conditions to determine how its growth would be affected by the interaction of nitrate availability and light. Furthermore, by directly measuring the sinking rates of the cells grown under different nitrate conditions, we established a relationship between PIC/POC and sinking rates.

MATERIALS AND METHODS

The coccolithophore *G. oceanica* (NIES-1318) was isolated from the East China Sea. Two types of experiments were carried out using PC bottles, and all cultures were in triplicate. Cells were grown semi-continuously by diluting the cultures every 24 or 48 h to maintain their exponential growth phase (Gao, 2021). The culture media were based on sterile seawater enriched with Aquil nutrients (Sunda et al., 2005) except that nitrate was supplied differently to obtain different concentrations.

The Indoor Cultures

Cells were grown in a plant growth chamber (Ruihua, Wuhan, China) at the photon flux densities [photosynthetically active radiation (PAR)] of 260 (HL), 115 (ML), or 50 μmol photons m^{-2} s^{-1} (LL), respectively, supplied by cool-white fluorescent tubes with a 12 light:12 dark cycle at 20°C. They were cultured under five levels of nitrate concentrations with initial values of 6, 12, 25, 50, and 100 μM in artificial seawater and diluted before the dark period every day down to 4.5×10^4 cells mL^{-1}. This experiment was designed to (1) investigate the responses of *G. oceanica* to different levels of light and nitrate, (2) determine the specific nitrate concentrations for the following outdoor experiment, and (3) investigate the sinking rates of the cells grown with different nitrate availabilities (6, 12, 25, and 50 μM).

The Outdoor Cultures Under Incident Sunlight

We conducted the outdoor experiment for 16 days from 18th December 2018 to 2nd January 2019. Cultures in PC bottles were incubated in a water bath under natural sunlight at 20°C, with temperature controlled by a cooling circulator (CAP-3000, Rikakikai, Japan). Two nitrate treatments (10 and 50 μM) with the addition of nitrate in the natural seawater (4.8 μM nitrate) were set up according to the previous indoor culture test. Cells were pre-cultured under corresponding nitrate concentrations for 3 days in the plant growth chamber (115 μmol photons m^{-2} s^{-1}, 20°C) before being transferred to and grown outdoor. Subsequently, they were acclimated to the solar PAR gradually according to Guan and Gao (2010). Briefly, the culture bottles were covered with three layers of neutral density screens for 2 days, then two layers for 4 days, and the bottles were subsequently exposed to full solar radiation. Since the PC bottles do not allow UV radiation (UVR) to penetrate and allow about 89% PAR to go through, the cells were exposed to about 89% incident sunlight. For the first 4 days, the cultures were diluted every 48 h. After that, dilutions were carried out every 24 h at about 17:30 with the renewed cell concentrations of about 4.5×10^4 cells mL^{-1}.

Measurements of Cell Size, Growth Rate, and Chlorophyll *a*

Cell size and concentrations were measured by using a Coulter Particle Count and Size Analyser (Z2, Beckman Coulter, Indianapolis, IN, United States). The growth rate (μ) was determined as follows: μ = $(lnC_2 - lnC_1)/(T_2 - T_1)$, where C_2 and C_1 represent the cell concentrations at T_2 and T_1 time, respectively. For Chlorophyll *a* (Chl *a*) measurements, at least 50 mL cultures were filtered on Whatman GF/F filters and extracted in absolute methanol at 4°C in the dark for 24 h. Then the absorption spectrum (400–800 nm) of the supernatant was measured using a scanning spectrophotometer (DU 800, Beckman Coulter, Indianapolis, IN, United States) after centrifuged at 6,000 × *g* for 10 min. Subsequently, the Chl *a* concentration was estimated according to Porra (Porra, 2002).

Determination of Coccosphere Size, Protoplast Size, and Coccosphere Thickness

Coccosphere and protoplast diameters were measured using the particle counter mentioned above. Protoplast size was obtained by dissolving the coccoliths with 0.1 mM HCl (Gerecht et al., 2015). In addition, the coccosphere thickness was calculated by subtracting protoplast diameters from coccosphere diameters and dividing by two.

Determination of Photochemical Parameters

All fluorescence parameters were measured using a pulse-amplitude-modulated fluorometer (Multi-color PAM, Walz, Germany). The indoor HL-cells were acclimated in the dark for 15 min to measure the maximal quantum yield of photosystem II (PSII) (F_v/F_m). The effective quantum yield of PSII (Yield) was measured at the actinic light of 250 µmol photons m^{-2} s^{-1}, which was similar to the growth high light level. The maximum relative electron transport rate (rETR$_{max}$) and apparent electron transfer efficiency (α) were obtained by analyzing the rapid light curves (RLCs) according to Ralph et al. (2005).

To test the responses of LN- and HN-cells grown under fluctuating sunlight to short-time high light, their effective quantum yields were measured at 0, 2, 5, 8, 13, 18, 28, 45, and 60 min using the Multi-color PAM after exposures to a PAR level of 600 µmol photons m^{-2} s^{-1} under a solar simulator. Samples were cultured in quartz tubes covered with 395-nm cutoff film (Ultraphan UV Opak, Digefra) which allows transmission of irradiances above 395 nm. The yield was monitored for 50 min after the cells were transferred to dim light (15 µmol photons m^{-2} s^{-1}) for photochemical recovery.

C and N Analysis

Particulate organic carbon (POC) and particulate organic nitrogen (PON) were determined using Elementar Vario EL cube (Vario EL cube, Elementar, Langenselbold, Germany). At least 50 mL cultures were filtered onto pre-combusted (450°C for 5 h) Whatman GF/F filters (25 mm) and frozen at −20°C until analysis. The filters for POC analysis were fumed with HCl to remove particulate inorganic carbon. And for total particulate carbon (TPC) analysis, samples were not treated with HCl. All samples were analyzed after drying. Cellular PIC content was calculated as PIC (pg cell^{-1}) = TPC (pg cell^{-1})–POC (pg cell^{-1}). The production rates of POC, PON and PIC (pg cell^{-1} d^{-1}) were calculated by multiplying cellular contents by the specific growth rates μ.

Determination of Sinking Rate and Cell Density

The sinking rate of *G. oceanica* cells was measured according to the SETCOL method (Bienfang, 1981) in an incubator at the constant temperature of 20°C. Briefly, settling tubes (about 385 mL, 0.59 m) were vertically fixed, and samples of all treatments were filled into settling tubes after shaking, which were sealed and placed in the chamber. Meanwhile, an equal volume of tested cultures was placed in the same chamber as the control to monitor changes in cell concentrations. The sinking rates were determined after the *G. oceanica* cells had acclimated to the treatments for at least 9 days under indoor and for 16 days under outdoor conditions. Cell concentrations were regarded as the biomass index for the sinking rate calculations.

Cell density was calculated according to Stokes' law: $v = 2gr^2$ (ρ'-ρ)/9η, where v the sinking rate (m s^{-1}), g the gravitational acceleration (m s^{-2}), r the radius of the coccosphere (m), ρ' the cell density (kg m^{-3}), ρ seawater's density (kg m^{-3}) and η is the dynamic viscosity of seawater (kg m^{-1} s^{-1}).
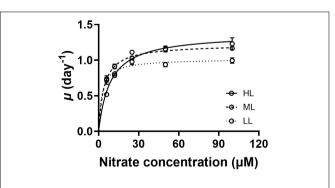
Data Analysis

The specific growth rates at different light levels were plotted against nitrate concentrations, and were fitted to the Michaelis-Menten function (Michaelis and Menten, 1913) to analyze the relationship of growth to nitrate availability. The relationships of sinking rate *vs.* coccosphere diameter, cell density and PIC/POC ratio were obtained by linear fitting.
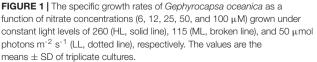
Results were presented as means ± SD for triplicate cultures for each treatment, and the data were analyzed with the software GraphPad Prism 8.0.2. The One-way ANOVA and Tukey *Post hoc* (Tukey HSD) test were used to test the differences between different treatments ($p < 0.05$).

RESULTS

The specific growth rate (μ) of the exponentially grown *G. oceanica* increased with increasing nitrate concentrations, regardless of light levels (**Figure 1**). The μ_{max} values of HL-cells were higher by 12 and 35% than that of ML- and LL-cells, respectively (**Table 1**, $p < 0.01$). Under the N-limited conditions, the specific growth rates were inhibited at the HL (260 µmol photons m^{-2} s^{-1}) by 28% at 6 and by 3% at 12 µM nitrate compared to that grown at LL, respectively.

In the outdoor experiment, μ of HN-grown cells was higher than LN-cells (**Figure 2B**). When the growth rates were normalized to daytime mean PAR intensity, μ values increased

FIGURE 1 | The specific growth rates of *Gephyrocapsa oceanica* as a function of nitrate concentrations (6, 12, 25, 50, and 100 µM) grown under constant light levels of 260 (HL, solid line), 115 (ML, broken line), and 50 µmol photons m^{-2} s^{-1} (LL, dotted line), respectively. The values are the means ± SD of triplicate cultures.

TABLE 1 | The μ_{max}, K_m, and efficiency of N assimilation in *Gephyrocapsa oceanica* cells grown under different levels of photosynthetically active radiation (PAR) (LL: 50 µmol photons m^{-2} s^{-1}, ML: 115 µmol photons m^{-2} s^{-1}, and HL: 260 µmol photons m^{-2} s^{-1}), which were derived from the relationship of growth vs. nitrate concentrations (**Figure 1**).

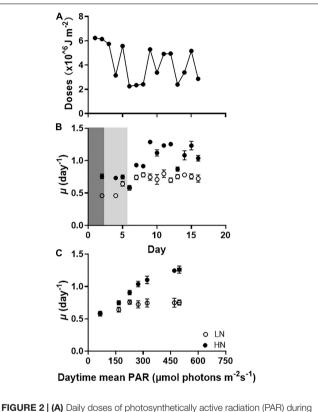|	µ $_{max}$	K_m	Efficiency of N assimilation	Goodness of fit R^2
LL	1.02 ± 0.05[c]	2.59 ± 1.00[b]	0.42 ± 0.12[a]	0.91
ML	1.22 ± 0.02[b]	4.05 ± 0.43[b]	0.30 ± 0.03[a]	0.99
HL	1.37 ± 0.02[a]	8.72 ± 0.34[a]	0.16 ± 0.004[b]	0.97

The efficiency of N assimilation was assessed as by μ_{max}/K_m. The superscripted letters by the values indicate significant differences among LL, ML, and HL treatments (p < 0.05).

by 75% ($p < 0.01$) in HN-cells and by 25% ($p = 0.02$) in LN-cells with daytime mean PAR increased till 270 µmol photons m^{-2} s^{-1} (**Figure 2C**). When the average daytime sunlight levels ranged between 270 and 500 µmol photons m^{-2} s^{-1}, µ of HN-cells still increased with increased light levels ($p = 0.01$). However, no significant changes in µ were observed in LN-cells ($p > 0.05$). By fitting, the μ_{max} and K_m for light of LN-cells was 51 and 84% lower than that of HN-cells, respectively (**Supplementary Figure 1**). In addition, by comparing growth rates at similar nitrate concentrations between indoor and outdoor cultures, we found that µ values under constant (indoor) light regimes were higher than that under fluctuating (outdoor) sunlight.

The photochemical yield in HN- and LN-cells, decreased within 28 min, then leveled off (**Supplementary Figure 2**). After 60 min-exposure, the yield decreased by 48 and 33% for LN- and HN-cells, respectively. In addition, the yield of HN-cells recovered to their initial values under the dim light in 50 min, while that of LN-cells did not, indicating less tolerance of high-light stress in LN-grown cells.

Higher nitrate concentrations resulted in larger coccosphere and protoplast size in *G. oceanica* (**Figure 3A** and **Supplementary Figure 4**). However, the coccosphere thickness increased with reduced nitrate availability (**Supplementary Figure 4**). The coccosphere thickness here referred to all the coccoliths outside the cell, including any possible stacked ones. This was consistent with cellular PIC quotas, which increased, respectively, by 24% under indoor light and by 35% under outdoor sunlight in the N-limited cells ($p > 0.05$, **Supplementary Table 1**). The PON, PIC, and POC production rates exhibited the same trend as cellular PON and POC quotas, with values significantly lower in the low nitrate treatments ($p < 0.01$). The PIC production rates were significantly reduced by lowered N availability under constant light conditions ($p < 0.01$). However, the production rates of PIC were relatively stable for LN- and HN-cells exposed to fluctuating sunlight. Additionally, the less decrease in calcification rates than organic carbon fixation rates in the low nitrate treatments resulted in higher PIC/POC ratios (**Supplementary Table 1** and **Figure 3C**).

The sinking rates of 6 µM nitrate-grown cells were significantly higher than that of the cells grown under other nitrate concentrations ($p < 0.01$, **Figure. 3D**). Outdoor LN-grown cells showed sinking rates of 2.19 ± 0.29 µm s^{-1}, which were significantly faster by 82% than HN-cells (1.20 ± 0.19 µm s^{-1}, $p < 0.01$). By plotting the relationships of sinking rates vs. coccosphere size and PIC/POC ratios irrespective of all the treatments, it revealed that the sinking
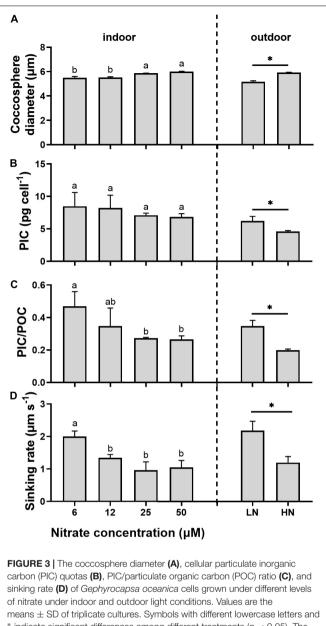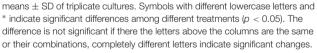

FIGURE 2 | (A) Daily doses of photosynthetically active radiation (PAR) during the outdoor culture periods (from 18th December 2018 to 2nd January 2019). **(B)** The specific growth rates of *Gephyrocapsa oceanica* cells grown under fluctuating light regimes and replete (HN) and limited (LN) conditions. Values are the means ± SD of triplicate cultures. The dark gray and light gray darkened areas indicate the periods of reduced levels of solar radiation for the cells to acclimate from 17 to 30% and then to 100% incident sunlight. **(C)** The specific growth rates of the cells grown at HN and LN as a function of daytime mean light levels. The values of daytime mean PAR exposed to the algal cells was 89% incident sunlight.

rate was positively correlated with cell density and the ratio of PIC to POC and negatively correlated with coccosphere size in *G. oceanica* (**Figure 4**).
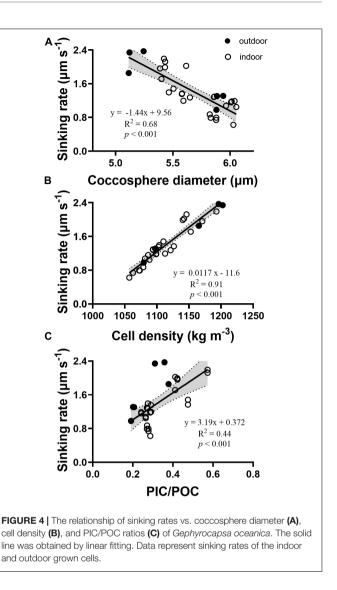
DISCUSSION

Our results indicated that *G. oceanica* cells grown under N-limited conditions increased their PIC quotas with thicker coccoliths, resulting in faster sinking rates, which were positively

FIGURE 3 | The coccosphere diameter **(A)**, cellular particulate inorganic carbon (PIC) quotas **(B)**, PIC/particulate organic carbon (POC) ratio **(C)**, and sinking rate **(D)** of *Gephyrocapsa oceanica* cells grown under different levels of nitrate under indoor and outdoor light conditions. Values are the means ± SD of triplicate cultures. Symbols with different lowercase letters and * indicate significant differences among different treatments ($p < 0.05$). The difference is not significant if there the letters above the columns are the same or their combinations, completely different letters indicate significant changes.

FIGURE 4 | The relationship of sinking rates vs. coccosphere diameter **(A)**, cell density **(B)**, and PIC/POC ratios **(C)** of *Gephyrocapsa oceanica*. The solid line was obtained by linear fitting. Data represent sinking rates of the indoor and outdoor grown cells.

correlated with PIC/POC ratios regardless of indoor constant light and outdoor fluctuating sunlight conditions. The microalgal cells sustained their photochemical performances with non-affected light use efficiency for photosynthetic electron transport even under nitrate deficient conditions (**Table 2**), so that energy required for PIC production was sufficiently provided.

With reduced availability of nitrate, the decrease in Chl *a* content (**Supplementary Figure 3**), and maximal relative electron transfer rate should hinder light harvesting and photosynthetic energy transfer (Kolber et al., 1988). However, our results showed that photochemical performances that are essential for energy transfer sustained even under the nitrate

limitation (**Table 2**), though the growth rates of *G. oceanica* were reduced (**Figures 1**, **2**). Excess light energy is suggested to harm the PSII of microalgae and lead to photoinactivation (Loebl et al., 2010; Campbell and Serôdio, 2020), which can be exacerbated due to reduced availability of macro-nutrients (Talmy et al., 2013; Li et al., 2015). However, proteins associated with mitigation of photo-oxidative stress as shown in *Emiliania huxleyi* could be prominently enhanced under N-limitation, allowing the cells to maintain relatively high F_v/F_m values (Rokitta et al., 2014). In this study, although N-limitation made the *G. oceanica* cells more susceptible to high-light stress (**Supplementary Figure 2**), the cells acclimated to LN sustained their light use efficiency (**Table 2**), supporting the energy supply for calcification and assimilation processes. Calcification of coccolithophores has been discussed to play roles as a sunshade or in photoprotection in coccolithophores against solar radiation or high light (Guan and Gao, 2010; Xu and Gao, 2012; Monteiro et al., 2016). Although the reduced levels of transmission of UVR and PAR by the

TABLE 2 | The maximum quantum yield (F_v/F_m), effective quantum yield (Yield), apparent electron transfer efficiency (α), and maximum relative electron transfer rate ($rETR_{max}$) of *Gephyrocapsa oceanica* cells grown under different nitrate concentrations.

N concentration (μ M)	F_v/F_m	Yield	α	$rETR_{max}$
6	0.55 ± 0.003^c	0.48 ± 0.01^b	0.21 ± 0.002^a	132.3 ± 5.7^b
12	0.57 ± 0.005^b	0.49 ± 0.003^{ab}	0.22 ± 0.004^a	142.4 ± 5.3^{ab}
	(0.56 ± 0.003)			
25	0.59 ± 0.005^a	0.50 ± 0.008^{ab}	0.22 ± 0.003^a	151.0 ± 5.1^a
50	0.59 ± 0.005^a	0.50 ± 0.005^a	0.21 ± 0.01^a	156.9 ± 2.6^a
	(0.58 ± 0.004)			

Values in brackets represent the data of the outdoor cultures. Others are the data of indoor HL-grown cultures. Values are the mean $\pm$ SD of triplicate cultures. Different superscripted letters indicate significant differences among treatments (p < 0.05).

coccosphere in *E. huxleyi* was quantified (Gao et al., 2009) and calcification increased with increased artificial or solar radiation levels in *E. huxleyi* (Guan and Gao, 2010; Zhang et al., 2019), enhanced PIC quotas with reduced nitrate availability in the present study (**Supplementary Table 1**) can only infer a photoprotective strategy associated with PIC-sheltering (Gao, 2017). From the energetic point of view, *G. oceanica* cells grown under LN could allocate relatively more energy to produce PIC in the sacrifice of growth and POC production. This was reflected in the model established by Rokitta and Rost (2012), showing that the light-depend energetics of the cell was further altered by secondary treatments. Moreover, the decreased cell size of LN-grown cells might be beneficial for coccoliths to be expelled to the outside of cells due to increased ratios of cell surface to volume. This is because coccoliths are formed in the intracellular coccolith vesicle (Paasche, 2001), and the smaller cell diameter, the shorter distance for coccolith transportation from the coccolith vesicle to the cell surface (Müller et al., 2012). Comparing the results of indoor and outdoor experiments, the effects of lowered nitrate availability on the physiological performance of *G. oceanica* did not appear to be altered by fluctuating sunlight (**Table 2**, **Figure 3**, and **Supplementary Figure 3**). Interestingly, the growth rates of HN- and LN-cells under constant light conditions were higher than that under fluctuating sunlight at low to mid daytime mean light levels, at which the growth of LN-cells was no longer stimulated with increased light availability. This implies that the cells demand more energy to cope with fluctuation of light in sacrifice of growth.

While the Stokes' law shows that the sinking rate of spherical particles is mainly dependent on size and density, here, we demonstrated that combination of N-limitation and high light gave rise to faster sinking rates that positively correlated with PIC content and PIC/POC ratios (**Figures 3**, **4**). In *E. huxleyi*, cell size was considered as the major determinant of sinking rate, though a significant increase of lipid in N-limited cells was detected (Pantorno et al., 2013). Contrastingly, our results point to the "ballast effect" of coccoliths on sinking, which was negatively correlated with cell size in *G. oceanica* (**Figure 4**). Additionally, the positive correlation of sinking rate with PIC/POC ratios has been demonstrated in *Coccolithus pelagicus* cells grown with different availabilities of phosphate (Gerecht et al., 2015). And it has also been reported in *E. huxleyi* under increased

temperature (Milner et al., 2016). Although N-limitation is known to facilitate the production of fatty acids and regulate the expression of related enzymes in coccolithophores (Rokitta et al., 2014; Bakku et al., 2017) and other microalgae (Kumar et al., 2019), specific gravities of microalgal species could be complicated by bio-mineralization, as reflected in *G. oceanica* in the present work.

Most POC exported to the deep ocean is in the form of aggregates such as fecal pellets and marine snow (Fowler and Knauer, 1986), and is partially re-mineralized to dissolved inorganic carbon (DIC) during sinking (Hedges, 1992; Iversen and Ploug, 2013). Faster sinking rates of aggregates can alleviate the POC loss caused by remineralization. Coccoliths in the form of calcium carbonate have been reported to be beneficial for aggregate formation and to protect the aggregates against disaggregation (Engel et al., 2009). Taken together with our results, we speculate that in the oligotrophic oceans, although the POC production is relatively slow, increased PIC content of coccolithophores as ballast in aggregates can enhance POC export efficiency. While, nutrient co-limitation matters more than nitrogen limitation alone in terms of phytoplankton growth in pelagic surface waters (Browning et al., 2021), coccolithophores in surface oceans under nutrient-co-limited conditions with high solar exposures may produce more PIC per POC. Consequently, the ballasting effect could be enhanced with progressive ocean climate changes due to less upward transport of nutrients associated with enhanced stratification of upper mixing layer (Gao et al., 2019). Nevertheless, the calcification-related genes for *E. huxleyi* were shown to be down-regulated under multiple stressors, including nutrients, warming, and CO_2 (Feng et al., 2020). Although omics-responses to multiple stressors have not yet well-documented in coccolithophores, future ocean climate changes can inevitably impact their photosynthesis and calcification (Bolton et al., 2016; Tong et al., 2019). This emphasizes the need to understand mechanistic responses of phytoplankton physiology and sinking to multiple environmental changes (Feng et al., 2021).

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

FUNDING

ACKNOWLEDGMENTS

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2022.834358/full#supplementary-material

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