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Harnessing coccolithophores for carbon capture and storage: exploring nutrient enrichment for future biotechnological applications

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A less studied yet promising microalgal group within the field of Carbon Capture Usage and Storage (CCUS) is the calcifying marine microalgae known as coccolithophores. They could have significant potential for carbon capture since they can capture CO_2, partitioning carbon into both their organic tissues and inorganic exoskeletons, composed of several micrometric plates of calcium carbonate ($CaCO_3$), called coccoliths. Moreover, the complex coccolith architecture offers valuable potential for nanotechnological applications, promoting also their reuse within a circular economy. However, comprehensive knowledge of their biotechnological potential and preliminary strain screening for quality assessment remain limited. In this study, a screening aimed at identifying the most promising strains for potential industrial applications was carried out by testing their response and yield under increasing nutrient and carbon supplies: dry weight (DW) and nutrient consumption efficiency were measured for the species *Gephyrocapsa huxleyi* (formerly *Emiliania huxleyi*) and two strains of the species *Chrysotila roscoffensis,* to identify the most promising strain for industrial applications. We documented a positive effect of nutrient enrichment and an even stronger response to carbon supplementation in the form of sodium bicarbonate ($NaHCO_3$) on the growth of *C. roscoffensis* and on $CaCO_3$ production in *G. huxleyi*. One *C. roscoffensis* strain proved to be the most promising, exhibiting the highest DW (1,172.7 ± 42.2 mg/L) and CO_2 absorption (1,210.7 ± 3.1 mg/L) compared to *G. huxleyi* (569.4 ± 20.5 mg/L; 329.9 ± 11.9 mg/L), as well as a stable ratio between Particulate Inorganic Carbon (PIC) and Particulate Organic Carbon (POC) during cultivation. Our experiments also highlighted the ability of *G. huxleyi* to produce significant amounts of carbonate (2.1 ± 1.1 PIC:POC) compared with the less calcified *C. roscoffensis* (0.34 ± 0.01 PIC:POC) under enhanced carbon supply. This study emphasizes the importance of preliminary screening to identify the most suitable strain for industrial exploitation, particularly among understudied microalgae such as coccolithophores.

KEYWORDS

biomass production, calcite production, *Chrysotila rocosffensis*, *Gephyrocapsa huxleyi*, nutrient enrichment, strain screening

1 Introduction

The constant increase in greenhouse gases resulting from anthropogenic activities, which significantly contribute to global warming, represents one of the main challenges of this century, requiring close collaboration between researchers and industrial stakeholders to develop effective solutions. Over the years, several strategies have been proposed for capturing and removing carbon dioxide (CO_2), such as chemical or physical absorption, membrane separation, and CO_2 bio-fixation (e.g., Daneshvar et al., 2022; Moheimani, 2005). To date, amine-based alkaline solvent absorption remains the dominant CO_2 capture method because it is technologically more mature and less expensive than most alternative technologies, although several disadvantages persist, including high energy consumption for solvent regeneration, low CO_2 absorption capacity, poor thermal stability, and high corrosivity (Liang et al., 2016). For this reason, current CO_2 removal efforts are increasingly shifting toward nature-based solutions, such as CO_2 bio-fixation through microalgae cultivation, which could offer a greener and more sustainable alternative to conventional methodologies.

Indeed, photosynthetic unicellular microorganisms are expected to have 10–50 times higher CO_2 bio-fixation rate than terrestrial plants, owing to their much faster growth rates, up to 400 times higher (Falkowski, 2012; Ighalo et al., 2022). Combined with the advantages of yielding high-value products, requiring limited land area, and enabling efficient biomass production and harvesting in photobioreactors, microalgae cultivation represents a strategic alternative to traditional CO_2 mitigation approaches (Cheng et al., 2025; Lam et al., 2012).

Since the 1990s, microalgae such as green and red algae or diatoms have been extensively studied for Carbon Capture Usage and Storage (CCUS) applications (e.g., Cheng et al., 2025; Hoque et al., 2025). However, even though coccolithophores are well studied in fundamental marine carbon-cycle research, they remain comparatively less explored in applied CCUS cultivation systems. These golden-brown marine microalgae possess the distinctive ability to cover their cells with calcite scales–called coccoliths–ranging from 1 to 20 μm in size, which together form an exoskeleton known as the coccosphere (Brownlee et al., 2021; Young, 1997; Monteiro et al., 2016). Thus, for coccolithophores, the total mass comprises both the organic fraction and the inorganic $CaCO_3$. Since $CaCO_3$ makes up the main component of the coccolithophore exoskeleton, based on the stoichiometric composition of $CaCO_3$, the carbon contained in the mineral phase corresponds to ~12% of its mass. Therefore, these organisms have the potential to sequester more carbon than other microalgae lacking this life strategy (Villiot et al., 2021). In most microalgal systems, the CO_2 captured during growth is eventually released back to the atmosphere when the biomass or derived bioproducts are degraded or consumed. By contrast, in coccolithophores a substantial fraction of the carbon precipitates as $CaCO_3$ in coccoliths, and it is stored over long timescales in the deep-sea sediments, providing a unique form of carbon sequestration among microalgae (Brownlee et al., 2021; Moore et al., 2021). In nature, coccolithophores account for approximately 20% of oceanic primary productivity and contribute up to 50% of the ~1.6 Pg y^{-1} of calcite produced in the pelagic zone (Brownlee et al., 2021; Monteiro et al., 2016; Taylor et al., 2017). They are thus key players in global carbon biogeochemical cycles (e.g., Rost and Riebesell, 2004; Ziveri et al., 2007). Their natural ability to permanently remove inorganic carbon from seawater and the atmosphere and to store it within their $CaCO_3$ exoskeleton has led to their identification as promising candidates for new technologies in CCUS (Brownlee et al., 2021; Moore et al., 2021).

Coccolithophore carbon production varies greatly among species. For instance, *Gephyrocapsa huxleyi* produces less than 6 pg C $cell^{-1}$, whereas larger species like *Scyphosphaera apsteinii* contain up to 200 times more carbon. The PIC:POC ratio also varies significantly between and within species. For example, *Chrysotila carterae* exhibits ratios ranging from 0.17 (Gafar et al., 2019) to 0.8 (Zou et al., 2017), whereas *Calcidiscus quadriperforatus* reaches 2.08 (Gafar et al., 2019). The ecological preference of coccolithophores for oligotrophic regions (Winter et al., 1994; Young et al., 2005) may have limited research on their response to nutrient-rich conditions and their potential applications in bioremediation or biotechnology. However, some opportunistic taxa–such as *Chrysotila roscoffensis* and *G. huxleyi*–are well adapted to turbulent, eutrophic environments (Dimiza et al., 2020; Reifel et al., 2001) and are known to seasonally form large oceanic blooms (Brownlee et al., 2021; Reifel et al., 2001).

This theoretically high potential has been explored in only a few studies so far, particularly from a biotechnological perspective. Only a limited number of papers have investigated the intensive cultivation of coccolithophores in photobioreactors (Jakob et al., 2018; Moheimani et al., 2011; Moheimani and Borowitzka, 2011), and just one has examined cultivation in raceway ponds (Moheimani and Borowitzka, 2006). These studies reported that *C. carterae* exhibited the highest overall performance in plate photobioreactors, reaching dry weight (DW), lipid, and $CaCO_3$ productivities of 0.54, 0.12, and 0.06 g L^{-1} d^{-1}, respectively. Regardless of reactor configuration, *C. carterae* achieved the highest productivity, followed by *G. huxleyi* and *Gephyrocapsa oceanica.* Moreover, *C. carterae* contained the greatest proportions of both lipids (20%–25% of DW) and $CaCO_3$ (11%–12%). When cultured in outdoor raceway ponds, *C. carterae* maintained a total productivity of approximately 0.19 g L^{-1} d^{-1}, with $CaCO_3$ contributing up to 10% of DW. This corresponds to an annual areal productivity of about 60 t ha^{-1} y^{-1} of total mass and roughly 5.5 t ha^{-1} y^{-1} of $CaCO_3$ (Moheimani and Borowitzka, 2006). Despite these promising results, no further research has been conducted on this topic.

A crucial aspect of evaluating coccolithophores for CCUS applications is strain selection. Key traits to consider include: i) the amount of $CaCO_3$ produced relative to the organic fraction, ii) the capacity to reach high cellular densities, iii) growth rate, iv) response to medium composition, v) tolerance to stressors such as culture mixing, and vi) specific coccolith morphology for potential product reuse. We selected three strains: one of *G. huxleyi* and two belonging to the species *C. roscoffensis*. *G. huxleyi* is a non-motile species (4–10 μm) carrying 10–50 coccoliths per coccosphere, each 2–5 μm in size (Young et al., 2022) and displaying a highly variable PIC:POC ratio (0.24–1.38; Gafar et al., 2019). *Chrysotila* spp. are flagellated, motile species (12–15 μm) bearing smaller coccoliths (~1–2 μm)

(Young et al., 2022) and showing lower PIC:POC ratios (~0.17; Gafar et al., 2019) than *G. huxleyi*. The three coccolithophore strains were chosen based on (i) published evidence of their successful cultivation in bioreactors and raceway ponds and their potential for biotechnological exploitation (e.g., *C. carterae*; Moheimani and Borowitzka, 2006), (ii) reports of intense natural blooms in high-nutrient environments (e.g., *C. roscoffensis*; Reifel et al., 2001), and (iii) the high calcification capacity and bloom-forming behaviour of *G. huxleyi*, which is known to produce multiple layers of coccoliths. Despite these individual studies, a targeted comparison of their performance under the high-nutrient regimes required to sustain calcified biomass production for CO_2 capture is still lacking; the present screening was specifically designed to address this gap.

Since nitrogen (N) and phosphorus (P) are fundamental building blocks for the organic fraction and for the efficient utilization of supplied CO_2 (Daneshvar et al., 2022; Jakob et al., 2018; Zuccaro et al., 2020), and since coccolithophores typically thrive in oligotrophic oceanic waters (Han et al., 2025; Winter et al., 1994), it is essential to investigate their tolerance to higher nutrient concentrations as a preliminary step toward maximizing yield. The amount of carbon supplied is also a key factor sustaining biomass production, especially for coccolithophores, which require CO_2 for photosynthesis as well as bicarbonate ions (HCO_3^-) for calcification processes (Brownlee et al., 2015; Takano et al., 1995). Therefore, we tested the response of the above-mentioned strains under increasing macronutrient levels–namely, N, P, and carbon in the form of nitrates (NO_3^-), phosphates (PO_4^{3-}), and sodium bicarbonate ($NaHCO_3$), respectively–evaluating also their potential for CO_2 sequestration within both the organic fraction and $CaCO_3$ exoskeleton. For the *C. roscoffensis* strains, the effects of elevated N and P concentrations relative to the standard maintenance medium, along with additional carbon supply, were examined here for the first time.

The ultimate aim of this study is to identify the strain with the highest yield under non-limiting conditions, enhancing both organic and inorganic production, and to provide a preliminary evaluation of the feasibility of large-scale CCUS applications for coccolithophores.

2 Materials and methods

2.1 Selected coccolithophore strains

For this study, we selected one strain of *G. huxleyi* from the Roscoff Culture Collection (RCC, France), isolated in the Pacific Ocean, and two strains of the species *Chrysotila roscoffensis*: strain 1 (CS1) from the Collection of Sea Microorganisms (CoSMi) at the National Institute of Oceanography and Applied Geophysics (OGS, Trieste, Italy), isolated in the Gulf of Trieste (northern Adriatic Sea, Italy); and strain 2 (CS2) from RCC, isolated from the Pacific Ocean.

The strains are maintained at CoSMi in a climatic chamber in 100 mL flasks, without mixing, at a constant temperature of 20 °C, with a light intensity of 100 μmol m^{-2} s^{-1} (4000 K), and a 12:12 h light: dark (L:D) cycle.

The medium is prepared using natural seawater collected in the Gulf of Trieste at 35‰ salinity, filtered with a 0.22 μm pore-size Durapore membrane filter (Millipore). The filtered seawater is autoclaved, let cool down for 24 h, and then enriched with macronutrients, trace elements, and vitamins to obtain the final concentrations of the f/2 medium, slightly modified from the standard f/2 medium (mod-f/2; see Supplementary Material, Supplementary Table S1 for details on the medium recipe).

2.2 Experimental set-up

All experiments were run in a climatic chamber, under constant temperature, light intensity, and L:D cycle. To favor gas exchanges and to ensure culture suspension and homogeneity, all strains were stirred: CS1 and CS2 were mixed by orbital shaking (orbital diameter 20 mm, speed 80 rpm), while *G. huxleyi* was stirred magnetically at double speed (160 rpm).

The different mixing strategies were chosen because *G. huxleyi*, being a non-motile species compared to *Chrysotila*, which is flagellated (Young et al., 2003), aggregates at the bottom center of the flask under the same culturing condition of *C. roscoffensis*, which can compromise the health and homogeneity of the culture (Supplementary Figure S1). Indeed, when cultivating coccolithophores, species-specific sensitivity must be considered to determine the most efficient mixing approach (Moheimani et al., 2011; Moheimani and Borowitzka, 2007). Three experimental settings were tested by modifying macronutrient supplies while keeping micronutrient and vitamin concentrations constant, as in the mod-f/2 medium.

1. mod-f/2: nutrient concentrations of mod-f/2 (Supplementary Table S1);
2. N70: enhanced macronutrient supply where N and P were increased to 70 mg/L and 8 mg/L, respectively. These levels were selected based on the highest values tested for *G. huxleyi* in the literature that showed no negative effects on cell density (Jakob et al., 2018);
3. N70 + C: enhanced macronutrients as for N70, plus additional carbon supply. A solution of $NaHCO_3$ 1 M was prepared after salt sterilization under UV radiation for 30 min and used to prepare a filtered stock solution. This stock was added to each flask as daily spikes (~200 μL per flask; adjusted for the remaining culture volume) to achieve a target amendment rate of approximately 13 mg C L^{-1} d^{-1}, corresponding to a cumulative addition of ~180 mg C L^{-1} over the entire incubation. This procedure ensured adequate dissolved inorganic carbon (DIC) availability throughout the experiment, supporting culture carbon demand while avoiding large fluctuations in carbonate chemistry that might occur with a single addition of $NaHCO_3$ at inoculation.

Before each of the above-mentioned settings, the strains were acclimated for 1 week. Each culture was inoculated at an initial optical density (OD) of approximately 0.1 at 750 nm in autoclaved 500 mL flasks, filled to 200 mL to ensure sufficient exchanges between the headspace and the cultures. Treatments were run in triplicate for statistical robustness and monitored for 14 days from inoculation.

2.3 Analytical methods

2.3.1 Cell growth and dry weight

Cell growth was monitored by measuring OD at 750 nm (Griffiths et al., 2011; Bradley and Laws, 2024), and DW. The OD of the strains was measured daily using a Jenway 6,300 spectrophotometer by collecting 1.5 mL subsamples of culture. The absorbance of the medium was measured at the same wavelength and subtracted from the total sample absorbance. All OD measurements were performed within the linear range (OD ≤ 1.0), considering the linearity limits of the Beer–Lambert law (e.g., Schagerl et al., 2022). Whenever the OD of the cultures exceeded this range, samples were appropriately diluted so to fit the linearity range, and the OD value was recalculated, multiplying the dilution factor accordingly. Since *G. huxleyi* has been recorded to retain from three to five layers of coccoliths and to shed them during its life cycle (e.g., Balch et al., 1992; Poulton et al., 2013), here we assessed any interference of shed coccoliths in the *G. huxleyi* culture using decalcification. In detail, a 10 mL culture sample (N70 + C condition) was decalcified by adding 0.5 M HCl, ensuring that the pH was lowered to 3 and immediately raised to 8 using 0.5 M NaOH. This treatment allowed measurement of the OD of naked cells (without coccoliths) while keeping cells intact (Supplementary Figure S2). For comparison, OD was measured before and after this treatment.

To measure DW, cellulose acetate filters (Sartorius, Ø 47 mm, pore size 0.45 µm) were first weighed to obtain the tare using an analytical balance (Mettler-Toledo XPR205DU, resolution 0.01 mg), after being dried at 105 °C for 15 min to remove residual moisture. Then, a known volume of culture was filtered through a vacuum filtration system with the vacuum applied to avoid diffusion of liquid and salts to the filter margin (following Zhu and Lee, 1997). The retentate on the filters was washed with deionized water at a 1:2 culture-to-water ratio to ensure salt removal. The filters were then dried at 105 °C for 2 h and immediately weighed (Diotto et al., 2022).

The collected DW data were used to construct OD–DW calibration lines for GH, CS1, and CS2. Details on the OD–DW calibration are provided in Supplementary Figure S3 of the Supplementary Material.

2.3.2 PIC:POC ratio

The PIC:POC ratio was calculated to distinguish between organic and inorganic fractions in coccolithophores. The cultures were sampled at the stationary phase to determine the concentration of particulate total carbon (PTC) and POC. PIC was then determined as the difference between PTC and POC. The aliquots of culture were filtered onto a Whatman GF/F filter (nominal pore size 0.7 µm, Ø 25 mm) pre-combusted at 450 °C for 4 h, and then stored frozen (−80 °C) until analysis. Before analysis, filters were oven-dried (60 °C, ~1 h) and packed into tin capsules. For POC determination, the filters were previously treated with HCl 1 N to remove carbonates (Nieuwenhuize et al., 1994). The analyses of PTC and POC were performed using a CHNS-O elemental analyzer (Costech) ECS 4010 as described in Quero et al. (2020). PIC and POC data are expressed as µmol C L^{-1} (Supplementary Table S2).

2.3.3 Macronutrients in the medium

To measure the concentrations of N and P in the medium, a culture aliquot was collected during the stationary phase and filtered through a Whatman GF/F filter (nominal pore size 0.7 µm, ø 25 mm) in sterile tubes pre-rinsed with the sample, and then immediately frozen (−20 °C) for subsequent analysis. The concentrations of NO_3^- and PO_4^{3-} were measured via colorimetric approach using a Cary 60 Shimazu Spectrophotometer. For NO_3^- concentration, the intensity of the yellow-brown color produced by the reaction of nitrate to nitrite by the addition of 5-hydroxysalicylic acid to the sample was determined by using the Reasol kit Hydrochek (code 6,223) (Trentin et al., 2023). A calibration curve was prepared in the linear range 5–100 mg/L NO_3^-. Two replicates of each sample were analyzed after 1 min vortexing and 10 min for color development by measuring the absorbance at 514 nm in 1.5 mL plastic cuvettes. Culture samples were prepared with two different dilutions up to 1/4 and 1/5 of the initial sample to avoid calcium and carbonate interference, and analyzed as previously described, using the equation of the calibration line (Equation 1). The final values were averaged among the replicates to obtain the final N residual amount in the medium.

$$NO_3^-\,[mg/L] = 327.06 \cdot OD_{514} - 8.3689 \tag{1}$$

The concentration of PO_4^{3-} was determined using the method of Murphy and Riley (1962). This method is based on the colorimetric reaction between PO_4^{3-} and molybdenum under reducing conditions, resulting in the formation of a blue phosphomolybdic complex. A calibration curve was prepared in the range 0.two to five mg/L PO_4^{3-}. The detailed composition of the reagent is provided in Supplementary Table S3 (Supplementary Material). Two replicates of each sample were analyzed after 1 min of vortexing and 10 min of color development, by measuring the absorbance at 705 nm in 1.5 mL plastic cuvettes. Culture samples were diluted to 1:5 of the initial concentration and analyzed as previously described, using the equation of the calibration curve (Equation 2). The final values were averaged across the replicates to obtain the residual P concentration in the medium.

$$PO_4^{3-}\,[mg/L] = 7.8728 \cdot OD_{705} - 0.4784 \tag{2}$$

2.4 Total mass and CO_2 capture estimates

Given the PIC:POC ratio (R), the organic fraction (m_{bio}) in the total mass (m_{tot}), measured as DW, was calculated from the POC fraction according to Equations 3,4 considering that the POC is around 50% of the m_{bio} (Zuccaro et al., 2020) and the PIC is stoichiometrically the 12% of $CaCO_3$ exoskeleton (m_{carb}).

$$m_{tot} = m_{bio} + m_{carb} = \frac{POC}{0.5} + \frac{PIC}{0.12} \tag{3}$$

$$m_{bio}\,[\%] = \left(\frac{m_{tot}}{1 + R \cdot \frac{0.5}{0.12}}\right) \cdot 100 \tag{4}$$

To evaluate the most suitable strain for future CCS applications, the CO_2 capture potential was calculated for m_{bio} (Equation 5) and m_{carb} (Equation 6). Assuming carbon content of 50% of m_{bio} and

12% of m_{carb}, the carbon mass is converted into the corresponding CO_2 mass using the molar mass ratio of C to CO_2 (i.e., ~27%):

$$CO_{2,mbio}\ [g/L] = \frac{0.5 \cdot m_{bio}\ [g/L]}{0.27} \quad (5)$$

$$CO_{2,mcarb}\ [g/L] = \frac{0.12 \cdot m_{carb}/L]}{0.27} \quad (6)$$

2.5 Statistical analysis

All response variables (N consumption, P consumption, m_{bio}, PIC:POC) were analyzed in R (version 4.4.2; R Core Team) by two-way ANOVA with strain (three levels) and nutrient condition (two levels) as fixed factors. For each model, ANOVA assumptions were checked by visual inspection of residuals (Q–Q plots, residuals vs. fitted and scale–location plots) and formally tested using the Shapiro–Wilk test for normality and Levene's test for homogeneity of variance. When significant main effects or interactions were detected, pairwise comparisons among strain × condition combinations were performed using Tukey's honestly significant difference (HSD) test based on estimated marginal means. A significance level of $p < 0.05$ was adopted for all analyses.

3 Results and discussion

To date, the scientific community studying coccolithophores has focused primarily on their physiology and ecology, with only a few articles exploring strategies to increase monospecific coccolithophore culture densities and enhance yields. Only in a few studies, *C. carterae* has been successfully cultivated in both closed photobioreactors and raceway ponds, achieving promising areal productivity using f/2 medium; thus at the same macronutrient levels of our mod-f/2 experiments (i.e., ~75 mg/L $NaNO_3$ and 5 mg/L $NaH_2PO_4{\cdot}H_2O$). Only three previous studies have modified macronutrient and carbon supply (mainly in *G. huxleyi*) compared to the standard medium composition with the objective of improving coccolithophore production for biotechnological applications (Takano et al., 1995; Jakob et al., 2018; Moreira et al., 2023). In our calculations, we used a typical microalgal biomass composition derived from the Redfield molar ratio (C:N:P = 106:16:1). When converted to a mass basis, the ratio is usually rounded to 50:10:1, i.e., ~50 wt% C, 10 wt% N and 1 wt% P, a commonly used approximation for microalgal biomass in mass-balance calculations (Zuccaro et al., 2020). Under this assumption, if N accounts for ~10 wt% of m_{bio}, a supply of 12 mg/L N (total N in the mod-f/2; total P: 1.43 mg/L) can support at most ~120 mg/L m_{bio}. Therefore, we designed experiments to test tolerance to six-fold higher nutrients (total N: 70 mg/L; total P: 8.11 mg/L, hereafter N70) and to evaluate whether additional inorganic carbon (daily $NaHCO_3$ additions; N70 + C) could further increase growth.

For all the three strains screened, nutrient enrichment up to 70 mg/L N and 8 mg/L P (N70 condition) did not lead to an increase in m_{tot}. Therefore, the main differences are observed with carbon addition compared to the other two conditions, showing a general increase in OD and DW (Figure 1; Table 1). This experimental outcome confirms that carbon availability remained a pivotal limiting factor for further increase in m_{tot}.

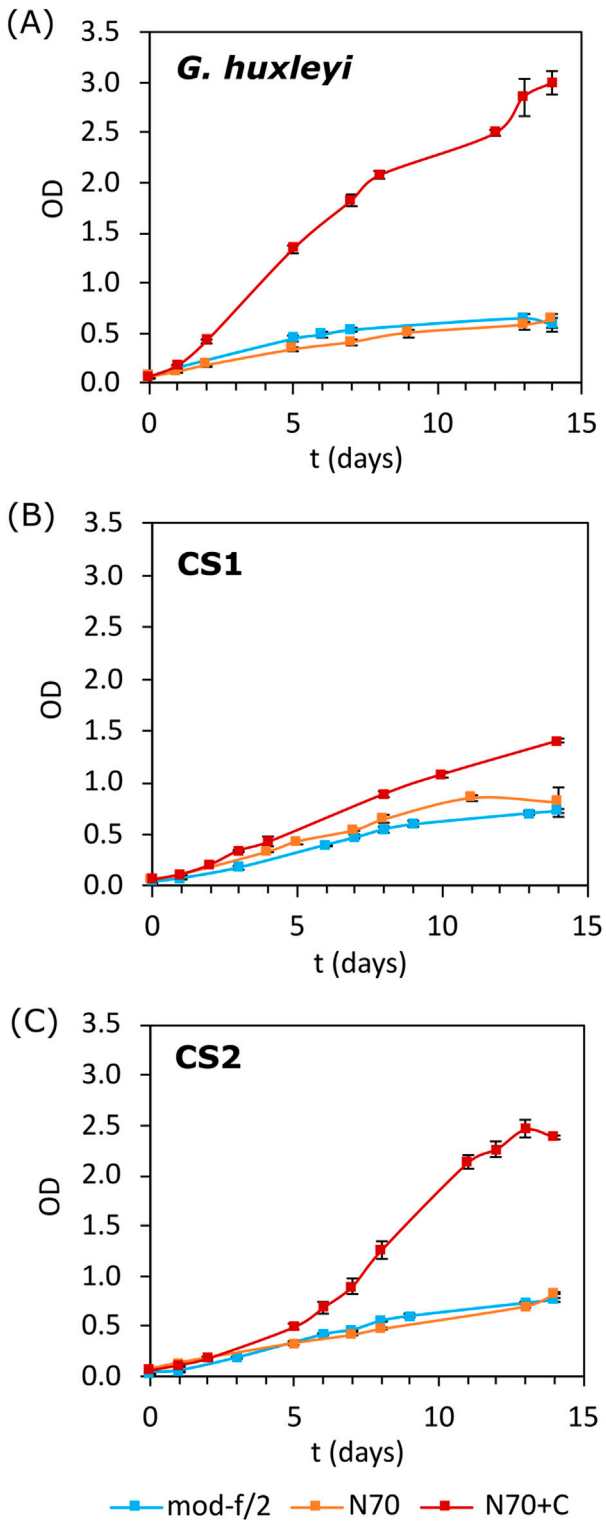

FIGURE 1
Growth curves of **(A)** *G. huxleyi*, **(B)** *C. roscoffensis*–strain 1 (CS1) and **(C)** *C. roscoffensis*–strain 2 (CS2) cultivated in f/2 mod (blue) or control (CTR), N70 (orange) and N70 + C (red) conditions.

Based on a qualitative comparison of the OD trend in the growth curves, *G. huxleyi* enters the stationary phase faster than CS1 and CS2 under all tested conditions, stabilizing at around an OD of ~0.5 in

TABLE 1 Total mass (m_{tot}) and PIC:POC ratio in the stationary phase, together with the calculated CO_2 permanently incorporated in the inorganic fraction (m_{carb}) and the total CO_2 uptake in m_{tot} for *G. huxleyi* and *C. roscoffensis* (CS1 and CS2) in the tested conditions (N70 and N70 + C). Values are presented as the mean calculated between two replicates; ± standard deviation.

Strain	Condition	m_{tot} [mg/L]	PIC:POC	$CO_{2,mcarb}$ [mg/L]	$CO_{2,mtot}$ [mg/L]
G. huxleyi	N70	111.1 ± 7.5	0.2 ± 0.05	22 ± 1.5	134.1 ± 9.1
	N70 + C	569.4 ± 20.5	2.1 ± 1.1	225.5 ± 8.1	329.9 ± 11.9
CS1	N70	217.5 ± 5.5	0.3	50	234.3
	N70 + C	454.8 ± 16.1	0.2 ± 0.04	94.1 ± 3.3	536.1 ± 18.9
CS2	N70	166.5 ± 5.9	0.2 ± 0.04	35.9 ± 1.3	191.6 ± 6.7
	N70 + C	1,172.7 ± 42.2	0.3 ± 0.01	310.8 ± 0.8	1,210.7 ± 3.1

mod-f/2 and ~0.4 in N70 at t7, corresponding to a DW of around 0.17 g/L and 0.13 g/L, respectively (Figure 1; Supplementary Figures S2, S3). In *G. huxleyi*, the addition of carbon leads to more than a 5-fold increase in OD at t7 (0.59 g/L), which can be related to increased coccolith production since the recorded average PIC:POC ratio equals 2.1 ± 1.1 (Table 1). This pronounced rise in PIC:POC under N70 + C is unusually high compared with values reported in literature for *G. huxleyi* (the highest previous value reported is 1.38; Gafar et al., 2019), and might reflect species-specific calcification responses to elevated HCO_3^-. However, the intrinsic variability among biological replicates must be considered when making this assumption. Considering this PIC:POC ratio, the calculated accumulation of m_{bio} for *G. huxleyi* is 0.06 ± 0.02 g/L, which corresponds to about 10% of m_{tot}. The same amount of m_{bio} is obtained under the N70 condition, where it accounts for 55% of DW since the PIC:POC ratio decreases to 0.2 ± 0.05 (Figures 2B,C). Thus, although the OD increases markedly with $NaHCO_3$ additions, m_{bio} is not scaling proportionally because a large fraction of the signal and m_{tot} increase derives from coccolith production. The low m_{bio} production in *G. huxleyi* is also confirmed by the nutrient consumption data reported in Table 2. The N consumed at t7 is the lowest measured among the studied strains, reaching up to 7.4 ± 2.2 mg/L in N70 + C compared to 32.4 ± 0.5 and 47.4 ± 1.7 mg/L for CS1 and CS2, respectively, under the same treatment. The same trend is recorded for P, with values of 1.9 ± 0.01 mg/L (N70) and 3.3 ± 0.08 mg/L (N70 + C), representing the lowest P consumption among the selected strains (Table 2). These uptake patterns reinforce the interpretation that increased OD in *G. huxleyi* under high HCO_3^- primarily reflects enhanced coccolith production rather than increased m_{bio}.

Similarly to *G. huxleyi*, the strains of the species *C. roscoffensis* show similar OD trends in both mod-f/2 and N70 conditions. As described above for *G. huxleyi*, the addition of carbon in N70 + C increases OD up to 1.08 (DW 0.48 g/L) in CS1 and even higher for CS2, which reaches an OD of 2.46 in the stationary phase, corresponding to a DW of 1.2 g/L–the highest measured DW among the strains and tested conditions (Figure 1; Table 1). For *C. roscoffensis*, the PIC:POC ratio remains mostly stable, especially for CS1 with an average value of 0.3 in N70 and 0.2 ± 0.04 in N70 + C; only CS2 shows a slight increase in the PIC:POC ratio, up to 0.3 ± 0.01, under the carbon-replete condition (Table 1; Figure 2C). Consequently, for *C. roscoffensis* strains, OD increases reflect substantial gains in m_{bio} as well as m_{carb}.

For the *C. roscoffensis* strains, macronutrient consumption is usually higher than for *G. huxleyi*, reaching N up to 32.4 ± 0.5 mg/L and 47.4 ± 1.7 mg/L for CS1 and CS2, respectively, in N70 + C (Table 2; Figure 2A). Even though the percentage of N removal is higher, in N70 + C condition P is almost completely depleted (~100%) for both CS1 and CS2 (Table 2). This pattern is consistent with the onset of P limitation for further growth; however, given the potential for P luxury uptake, as well as the possible influence of other limiting factors (e.g., light, inorganic carbon or trace nutrients), the observed P depletion could be interpreted as an indication of P-limited growth, requiring to balance N and P when aiming to maximize growth. Notably, for CS2 in particular, the substantial enhancement of nutrient uptake under carbon-replete conditions is consistent with the five-fold increase in m_{bio} (Figure 2B), the highest among the strains tested here (Table 2). Indeed, CS2 records the highest accumulation in m_{bio}, corresponding to 480.8 ± 17.3 mg/L (Table 2).

Two-way ANOVA showed that m_{bio} was strongly affected by strain, nutrient condition and their interaction (all $p < 0.001$; Figure 2B). Under N70, *G. huxleyi* had lower biomass than CS1 and CS2 (*G. huxleyi* vs. CS1: $p = 0.0001$, *G. huxleyi* vs. CS2: $p = 0.011$), and only strains CS1 and CS2 exhibited a marked increase in biomass under N70C (CS1 and CS2: $p < 0.0001$; *G. huxleyi*, $p = 0.55$). N and P consumption displayed similar patterns (Figure 2A). N70C strongly enhanced N removal in strains CS1 and CS2 (both $p < 0.0001$) compared to N70 but had little effect on *G. huxleyi* ($p = 0.44$), while only CS1 consumed significantly more N than *G. huxleyi* ($p = 0.013$) under N70. In contrast, under N70C all three strains differed from one another, with CS2 showing the highest N consumption (all pairwise comparisons $p < 0.0001$; different letters in Figure 2A). For all strains, P removal was significantly higher under N70C than under N70 (all $p < 0.0001$). Under N70, P consumption increased from *G. huxleyi* to CS1, with all pairwise differences being highly significant (all $p < 0.0001$). Under N70C, *G. huxleyi* still showed the lowest P removal, whereas strains CS1 and CS2 reached similarly high P consumption ($p = 0.99$), as reflected by the compact letter display in Figure 2A. P consumption was always different from N70 ($p < 0.0001$).

The PIC:POC ratio, analyzed on log-transformed data, was also significantly affected by strain, condition and their interaction (all $p < 0.05$), with generally higher PIC:POC under N70C and strain-specific responses as indicated by the compact letter display in Figure 2C. This points to a particularly strong shift towards calcification in *G. huxleyi* under the N70C regime.

From the collected data, the amount of CO_2 captured per liter of culture was calculated for the conditions N70 and N70 + C (Table 1). In this initial screening, CO_2 in m_{carb} was estimated from the inorganic carbon associated with the harvested biomass.

Detached coccoliths remaining in the culture medium were not explicitly quantified, and our values should therefore be considered conservative, particularly for species that actively shed coccoliths such as *G. huxleyi*. In the carbon-replete condition, CO_2 capture is greater in CS2 compared to the others, and approximately one-quarter (~311 mg/L CO_2) is stored in m_{carb}. This amount is even higher than that calculated for *G. huxleyi* (~225 mg/L CO_2) despite its higher PIC:POC ratio, and thus greater coccolith production (Table 1). Therefore, in our experiments, CS2 combined higher yield in m_{bio} and stable inorganic carbon storage in m_{carb}, producing the greatest overall CO_2 capture among the tested strains under N70 + C.

We used daily additions of $NaHCO_3$ to supply DIC rather than continuous CO_2 bubbling, because coccolithophores are more sensitive to bubbling and bicarbonate addition offers a more stable DIC pool and avoids rapid CO_2 outgassing. At the pH values observed (pH > 8 after daily $NaHCO_3$ additions), HCO_3^- is the dominant inorganic carbon species in the medium. Shifts in carbonate chemistry (higher HCO_3^-/CO_2) can change the relative availability of the proximal substrates and thereby differentially constrain photosynthesis and calcification. Experimental work in coccolithophores (Bach et al., 2013; Bach et al., 2015; Brownlee et al., 2015) shows that photosynthesis/growth can be sensitive to CO_2, whereas calcification is closely linked to HCO_3^- supply, consistent with compartmentation/transport constraints and acclimatory regulation. Therefore, this chemical shift towards HCO_3^- in our experiments could have favored calcification (PIC formation) over photosynthetic POC production in species that preferentially use HCO_3^- for calcification while relying on CO_2 for photosynthesis. The discrepancy we observed between strong OD increases and limited POC-based biomass in *G. huxleyi* suggested interference from detached coccoliths inflating optical readings. We verified this by decalcifying *G. huxleyi* cultures grown in N70 + C at t7: the measured OD dropped from 1.75 to 0.45 after decalcification (Supplementary Figure S2), confirming that detached coccoliths biased optical-density–based m_{bio} estimates for *G. huxleyi* but not (to the same degree) for *C. roscoffensis*, which has smaller coccoliths and has not the same tendency to shed large numbers of plates.

Taken together, these results emphasize two practical points for coccolithophore cultivation: (1) raising macronutrients alone (N70) is insufficient to substantially increase m_{tot} without concurrent increases in bioavailable carbon, and (2) while we did not independently manipulate individual inorganic carbon species, the shift in the carbonate chemistry toward HCO_3^- in N70 + C could have species- and even strain-specific impacts on the balance between photosynthesis (POC) and calcification (PIC), consistently with prior work showing differential sensitivities of photosynthesis and calcification to CO_2 versus HCO_3^- in *G. huxleyi*. More broadly, our results motivate targeted control of carbonate chemistry and phosphate availability, together with other growth-relevant constituents (e.g., major ions involved in calcification such as Ca^{2+}), when designing cultivation strategies that aim simultaneously to maximize m_{bio} production and stable inorganic carbon sequestration in m_{carb}. Finally, and importantly for biotechnological scaling, these experiments illustrate the value of strain selection within a species: CS2 seems to be a promising strain for forthcoming experiments, since it showed higher nutrient conversion efficiency into m_{bio} compared to *G. huxleyi* (Figure 2B) and higher stability of the PIC:POC ratio across different conditions (Figure 2C), which are attractive traits for envisioning a future large-scale application. Coupled with literature reports showing promising results for *C. carterae* in photobioreactors and raceway ponds, our findings corroborate that the genus *Chrysotila*, and specifically the species *C. roscoffensis,* can be further studied as a candidate for future large-scale applications where permanent inorganic carbon sequestration is a desired outcome.

In addition to modulating the nutrient supply, light intensity and wavelength are also fundamental for increasing growth efficiency and pigments production in microalgae (Daneshvar et al., 2022 and references therein). Indeed, it has been highlighted that photosynthesis, calcification, as well as growth rate in coccolithophores can be affected by light availability (Zondervan et al., 2002; Ramos et al., 2012; Rokitta and Rost, 2012). To date, among the coccolithophore group, only a few species, such as *G. huxleyi, C. carterae* and *G. oceanica*, have been investigated in their response to different light intensities (e.g., Zhang et al., 2023; Zhang et al., 2015; Zondervan, 2007; Zondervan et al., 2002; Heinle, 2014). In coccolithophores, calcification seems to be less light-dependent than photosynthesis (e.g., Balch et al., 1992; Zondervan, 2007 and references therein), with the PIC:POC ratio not significatively or clearly affected by light (Heinle, 2014). The species that shows a higher adaptation to light is *G. huxleyi*, recording any sign of photoinhibition up to 2,500 μmol m^{-2} s^{-1} (with an optimum at 900 μmol m^{-2} s^{-1}), whereas *C. carterae* records its optimum at 500 μmol m^{-2} s^{-1} (Heinle, 2014). Recently, Zhang et al. (2023) studied the wavelength effects on *G. huxleyi*, documenting a key role of blue and red lights on cell proliferation and fucoxanthin production. It must be noted that all these studies tested the effects of light through dilute batch experiments, neglecting a biotechnologically oriented approach. In fact, further experiments testing light in high cell density cultures would be useful to better understand coccolithophore response to this crucial parameter.

Besides permanent sequestration, coccolithophores offer diverse potential applications not available with other microalgal groups, especially those without a biosynthesized shell. Unfortunately, to date, many of those uses remain largely theoretical and only partially explored. Indeed, their dual organic–inorganic composition and distinctive morphology make coccolithophores attractive for various technological applications, including biomedicine, construction, nanotechnology, and sustainable materials (Green et al., 2014; Jakob et al., 2018; Moheimani, 2005; Moore et al., 2021; Moreira et al., 2023; Santomauro et al., 2020; Skeffington and Scheffel, 2018; Walsh et al., 2018). Optimizing their productivity is therefore essential to fully exploit their potential, and further effort is needed in this direction for future studies.

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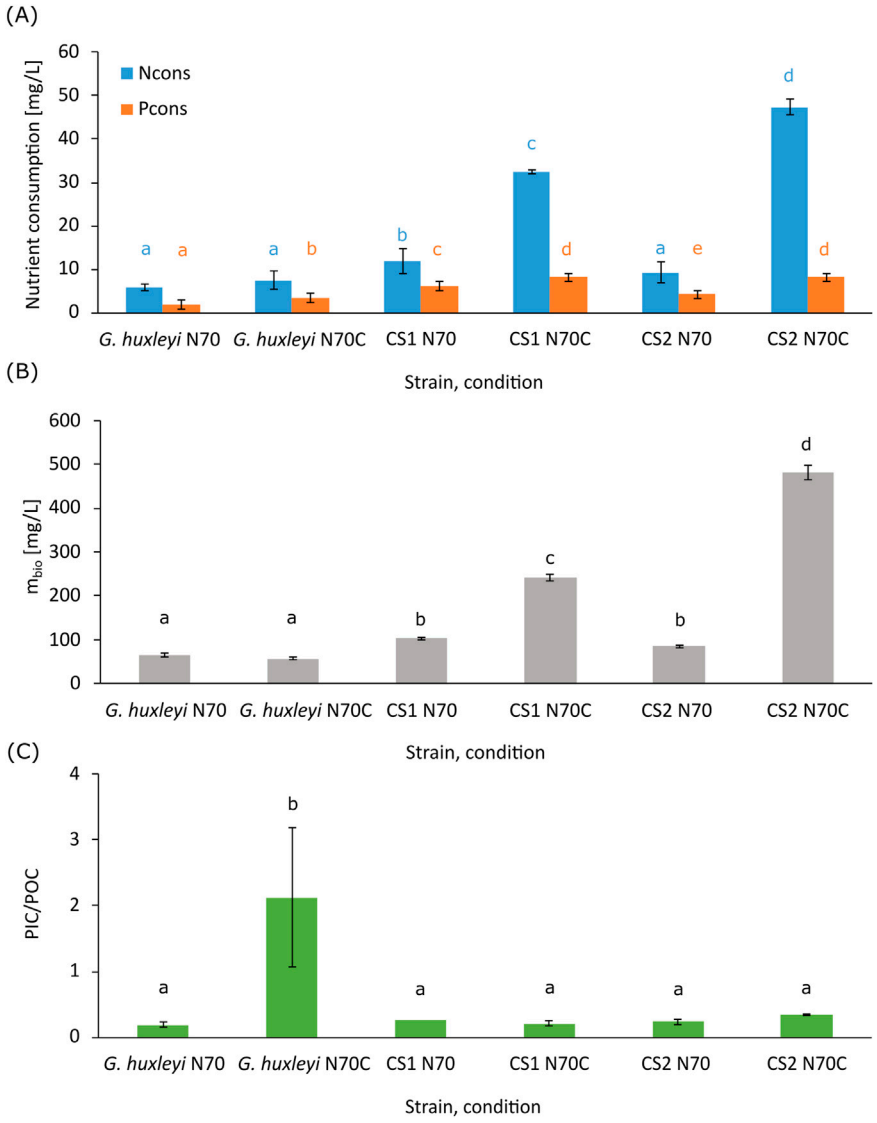

FIGURE 2
(A) Nutrient consumption, **(B)** organic fraction (m_{bio}) in the dry weight and **(C)** PIC:POC ratio in the stationary phase for *G. huxleyi*, *C. roscoffensis*–strain 1 (CS1), and *C. roscoffensis*–strain 2 (CS2) cultivated in N70 and N70 + C conditions. Data are represented as mean ± standard deviation. Different letters indicate significant differences among treatments (two-way ANOVA followed by Tukey's HSD test, $p < 0.05$).

TABLE 2 Organic fraction (m_{bio}) and nutrient consumption (N_{cons}, P_{cons}) in the stationary phase for *G. huxleyi* and *C. roscoffensis* (CS1 and CS2) in the tested conditions (N70 and N70 + C). Values are presented as the mean calculated between two replicates; ± standard deviation.

Strain	Condition	m_{bio} [mg/L]	N_{cons} [mg/L]	P_{cons} [mg/L]
G. huxleyi	N70	61.1 ± 4.1	5.8 ± 0.7	1.9 ± 0.01
	N70 + C	56.9 ± 2.1	7.4 ± 2.2	3.3 ± 0.08
CS1	N70	102.2 ± 2.6	11.9 ± 2.9	6 ± 0.1
	N70 + C	241 ± 8.5	32.4 ± 0.5	8.1 ± 0.003
CS2	N70	84.9 ± 3	9.1 ± 2.4	4.1 ± 0.2
	N70 + C	480.8 ± 17.3	47.4 ± 1.7	8.1 ± 0.009

4 Conclusion

For all the three screened strains, nutrient enrichment up to 70 mg/L N and 8 mg/L P (N70 condition) did not lead to an increase in m_{tot}. Instead, the main differences were observed with carbon addition (N70 + C) compared to the other two conditions, showing a general increase in both OD and DW. Under N70 + C the *C. roscoffensis* strains—particularly CS2—displayed increased m_{bio} and overall CO_2 capture potential, whereas *G. huxleyi* preferentially increased calcification (high PIC:POC) and released abundant coccoliths that biased optical-density measurements. These outcomes indicate that balanced provision of bioavailable carbon together with N and P, as well as careful control of carbonate chemistry and pH, are essential to maximize both POC production and stable inorganic carbon sequestration in scaled coccolithophore cultivation.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

Author contributions

EP: Data curation, Investigation, Formal Analysis, Visualization, Writing – original draft. ES: Writing – review and editing, Resources, Conceptualization, Supervision. SAA: Writing – review and editing, Investigation. FR: Writing – review and editing, Resources. CD: Writing – review and editing, Resources. AC: Funding acquisition, Writing – review and editing. MB: Funding acquisition, Writing – review and editing, Project administration, Resources, Conceptualization, Supervision.

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Conflict of interest

Author AC was employed by A2A S.p.A.

The remaining author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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