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Combined exploitation of CO_2 and nutrient replenishment for increasing biomass and lipid productivity of the marine diatoms *Thalassiosira weissflogii* and *Cyclotella cryptica*

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Abstract Diatoms are promising candidates for sustainable production of biofuels but their use is restricted due to the difficulties of combining high-biomass productivity and lipid accumulation. Here, we report the effect of high levels of nutrients and supplementation of 10% CO_2 on biomass and lipid productivity of the marine diatoms *Thalassiosira weissflogii* and *Cyclotella cryptica*. Daily nutrient replenishment stimulated growth and increased the biomass but reduced lipid synthesis and dropped the level of triglycerides (TAG) close to zero. On the contrary, addition of 10% CO_2 (*v*/*v*) doubled or tripled lipid content in comparison with air-sparged cultures, but induced only a modest increase of biomass. Assessment of the content in carbohydrates, lipids, and proteins suggested that CO_2 stimulated lipogenesis from carbohydrates in both diatoms. In order to combine these effects, we also tested a two-stage cultivation that alternated nutrient replenishment together with addition of CO_2 during nutrient shortage. *T. weissflogii* and *C. cryptica* responded to these conditions by increasing dry biomass to 1.25 g L^{-1} without reduction of total lipid percentage. In both species, TAG became the main lipid component and accounted for more than 60% of total glycerolipids in *C. cryptica*. These results underline the metabolic plasticity of diatom cells and indicate a possible way to maximize the production of biomass and functional products by tuning culture conditions.

Keywords Biofuels · Nutrient supplementation · Carbon dioxide · Triglycerides · Diatoms

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Introduction

Diatoms are the dominant eukaryotic component of phytoplankton in marine and freshwater habitats (Malviya et al. 2016). They successfully cope with a wide range of environmental conditions which suggests sophisticated mechanisms to perceive and adapt to external changes (Falciatore et al. 2000; Depauw et al. 2012). The huge amount of organic compounds yearly synthesized by these photoautotrophic organisms forms the base of the marine food web and many authors believe that these products constitute a potential resource of added-value chemicals and biofuels (Leterme 2015; Rorrer et al. 2016; Yi et al. 2017). To date, the biotechnological potential of diatoms is underexploited, mainly because of technical issues related to large scale cultivation and limited information about their metabolism (Hildebrand et al. 2012; Levitan et al. 2014). Furthermore, optimization of culture parameters is mandatory to provide sustainable biomass production in cost-effective way (Brennan and Owende 2010; Lari et al. 2016).

Lipids account for a large fraction of diatom carbon. The first holographic reconstruction of the cells of the diatoms *Skeletonema marinoi* and the *Thalassiosira rotula* (Merola et al. 2017) revealed that up to one fifth the cell volume can be occupied by chloroplasts that are made of glycolipids, mostly monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG), and sulfoquinovosyldiacylglycerol (SQDG). These constituents together with phospholipids (PL) are the major diatom lipids under physiological conditions. On the other hand, triacylglycerols (TAG) represent the largest pool of energy storage and are

regarded as feedstock for commodity markets (e.g. biofuels, food) (Hu et al. 2008; Wijffels et al. 2010). Nutrient deprivation (especially silicate, nitrate, and phosphate) has been reported in model diatoms as a promising approach to increase lipid and TAG content, even if application to large scale cultures so far has not been attempted due to the unresolved problem of the concomitant reduction of biomass productivity (Roessler 1988; Yu et al. 2009; Sharma et al. 2012; Jeffryes et al. 2013; Minhas et al. 2016). The opposite regime of high nutrient supplementation to improve biomass productivity is less explored in diatoms and other microalgae (Valenzuela et al. 2013; Fields et al. 2014), and the majority of these studies have focused on photosynthetic efficiency rather than productivity (Tantanasarit et al. 2013).

Carbon dioxide (CO_2), usually at concentration from 1 to 15% in air, is recognized as an efficient tool for the enhancement of both algal growth and conversion of sugar to oil (Lam et al. 2012; Singh and Singh 2014). On the whole, very little is known about the effect of CO_2 on biomass and lipid productivity of diatoms, despite the significant efforts in deciphering the regulatory mechanism of CO_2 assimilation and ocean acidification on these microbial eukaryotes (Ishida et al. 2000; de Castro Araujo and Tavano Garcia 2005; Hopkinson et al. 2011; Picardo et al. 2013; Wang et al. 2014; Bailleul et al. 2015).

The diatoms *Thalassiosira weissflogii* and *Cyclotella cryptica* are promising candidates for lipid production (d'Ippolito et al. 2015). We showed that both species respond to different nutrient regimes by lipid pool remodelling and de novo synthesis of neutral lipids. Here, these diatom species were analysed for their response to high-nutrient regimes and CO_2 addition in terms of growth, biochemical composition, biomass and lipid productivity, TAG levels, and nutrient consumption. The final aim was to test a two-stage cultivation approach that combines the positive effects of nutrient supplementation and addition of 10% CO_2.

Materials and methods

Microorganisms and culture conditions

Thalassiosira weissflogii (P09) is a local strain, isolated from the Gulf of Naples, Italy (latitude 40° 43′ 90″ N; longitude 14° 10′ 167″ E). *Cyclotella cryptica* (CCMP 331) was purchased from the National Center of Marine Algae and Microbiota (NCMA, Bigelow Laboratory for Ocean Sciences, USA). Diatoms were cultured in 2.3-L polycarbonate flasks in 2-L f/2 medium (Guillard and Ryther 1962). Nutrients were added at the beginning of the experiments (control) or replenished every day (high-nutrient regime, HNR). Media were supplemented with 5 mL L^{-1} of 400 mM Tris (2-amino-2-hydroxymethyl-propane-1,3-diol) as buffer agent. pH was measured with a pH meter (MeterLab PHM210). Each culture was inoculated with axenic strains at a starting concentration of 10,000 cells mL^{-1} and kept at 21 ± 3 °C under gentle bubbling of sterile gas. Artificial light (200 μmol photons m^{-2} s^{-1}) was provided by daylight fluorescent tubes (OSRAM 965, Germany) with a 14:10-h light:dark photoperiod. Cell growth (cells mL^{-1}) was estimated using a Bürker counting chamber (depth 0.100 mm) under an inverted microscope. Where indicated, cultures were sparged with 10% (*v*/*v*) CO_2 in air for 8 h during light phase. Gas mixture was obtained by mixing pure CO_2 (SOL Spa, Caserta) with ambient air and percentage was measured on-line by an infrared carbon dioxide sensor (range 0–25%). Sparging data were acquired by Mamos II v. 8.9.3 software. pH was monitored at different points (supplementary material, Fig. S4).

Diatoms were harvested by centrifugation at 2300 ×*g* for 10 min. Cells were washed twice with 0.5-M ammonium formate to remove salts and immediately frozen in liquid nitrogen. The pellets were then lyophilized with a Micromodulyo 230 (USA) freeze drier, to estimate total biomass dry weight. Biomass and lipid productivities were calculated as in d'Ippolito et al. (2015).

Chemical analysis

Lipid extraction was performed by modified Folch method (Folch et al. 1957) and lipid content (mg L^{-1}) was determined gravimetrically. Composition and quantitation of microalgal lipids (free fatty acids, TAG, glycolipids and PL) were established by ERETIC ^{1}H-NMR (600 MHz) method according to Nuzzo et al. (2013), using 4-chlorophenyltrihexadecylsilane (CPHS) as standard for recovery (1 mg per 50-mg dry biomass). The protocol uses a reference electronic signal as external standard (ERETIC method) and allows assessment of total lipid content, saturation degree, and class distribution. ERETIC peak was calibrated on the NMR doublet at 7.40 ppm (J = 8.0 Hz) of 2.20 μmol of CPHS in 700 μL $CDCl_3/CD_3OD$ 1:1 (*v*/*v*). Crude organic extracts were dissolved in 700 μL $CDCl_3/CD_3OD$ 1:1 (*v*/*v*) and transferred to the 5-mm NMR tube. Chemical shift was referred to CHD_2OD signal at δ 3.34.

Concentrations of complex lipids were determined in microalgal extracts according to the following equation:

$$C = k\, A_S n^{-1}$$

where C is the concentration of the analyte in mole, n is the number of protons (one for CH; two for CH_2; three for CH_3), k is the ratio between the concentration and peak area of the ERETIC signal normalized to a single hydrogen, and A_S is the area of the NMR signal S diagnostic for the lipid class of interest. To minimize the effect due to signal saturation and relaxation delay, ^{1}H NMR experiments were recorded with one scan using a 90° pulse.

Protein content was determined by Lowry assay (RC DC protein assay, Bio-Rad) on cell pellets (Lowry et al. 1951).

Carbohydrate content was determined using the phenol-sulfuric acid method on cell pellets (Dubois et al. 1956).

Nutrient analysis

Ten millilitre of culture was centrifuged daily as described above. The supernatant was filtered through 0.22-μm syringe filter (Sterile Syringe Filter PES—Corning), aliquoted, and kept at −20 °C before carrying out nutrient assays. Spectrophotometric methods were used to measure dissolved silicates (Strickland and Parsons 1972), phosphates (Elardo 1997), and nitrates (Zhang and Fischer 2006).

Statistics

All values are expressed as mean ± standard deviation (SD). A Student's *t* test was used to evaluate differences between groups of discrete variables. Values of $p \leq 0.05$ were considered statistically significant.

Results

Effect of high-nutrient regime

Regardless of nutrient consumption (NO_3^-, SiO_3^{2-}, and $H_2PO_4^-$), *T. weissflogii* and *C. cryptica* were supplemented with nutrients contained in f/2 medium every day (HNR) or only at the beginning of the experiment (control). Cell density under HNR conditions was ten times higher than control experiments (Fig. 1a and b), thus yielding a net increase of the biomass (870 ± 48 mg L^{-1} for *T. weissflogii* and 775 ± 65 mg L^{-1} for *C. cryptica*) at the end of the stationary phase (Fig. 1c and d). Both diatoms also showed a corresponding increase in total lipid production (178 ± 14.7 mg L^{-1} and 156 ± 4.4 mg L^{-1} in *T. weissflogii* and *C. cryptica*, respectively) which was six times higher than in control cultures (Fig. 1c and d). In terms of percentage of total dry biomass, daily supplementation of nutrients also affected the relative composition of lipids, carbohydrates, and proteins. In particular, HNR promoted synthesis of proteins at the expense of carbohydrates (Fig. 1e and f). Although lipid percentage per biomass weight remained unvaried, nutrient availability induced a significant shift in the lipid composition. In fact, analysis of glycerolipids by ^{1}H-NMR revealed a massive drop of TAG and a proportional increase of lipids from plastidial membranes (Fig. 1g and h), mostly MGDG and SQDG. The overall response to HNR was (1) an increase of total lipid content and biomass production as result of the higher number of cells in stationary phase and (2) an evident shift from storage to structural components.

Analysis of nutrient consumption along the growth curve showed that daily silicate addition was sufficient to compensate the uptake of growing cells and to bring back silicon level at the initial concentration of 106 μM (supplementary material, Fig. S1). On the other hand, phosphates and nitrates were consumed more slowly and their daily addition led to an accumulation in the cultures (supplementary material, Fig. S1). This was particularly evident with nitrate whose concentration reached a cumulative level of 3.5 M (~four-fold the concentration of f/2 medium) at the end of the experiment.

Effect of CO_2 addition

Flue gases of combustion processes typically contain between 9.5 and 13% CO_2 in volume (Van Den Hende et al. 2012). With the aim of using flue gas in diatom cultures, we tested the effect of 10% CO_2 on growth performance indicators and composition in lipids, carbohydrates, and proteins of *T. weissflogii* and *C. cryptica*. The addition of 10% CO_2 did not induce observable changes in the growth curve and yielded only a minor increase of biomass (Fig. 2a and b). On the other hand, the algae cells produced a larger amount of lipids in comparison to air-sparged control cultures. The lipid percentage per dry biomass doubled in *T. weissflogii* (35% of biomass dry weight) and tripled in *C. cryptica* (45% of biomass dry weight) (Fig. 2c and d). This effect was counterbalanced by a decrease of carbohydrates, while protein level remained unchanged (Fig. 2e and f). In CO_2-sparged cultures, TAG accounted for the largest glycerolipid component (almost 70%) (Fig. 2g and h). These data indicated that diatoms responded to CO_2 by increasing lipid synthesis with a slight enhancement of storage lipids over structural lipids. Consequently, these conditions were responsible of accumulation of oil in both species (from 28.3 ± 0.2 to 64.6 ± 5.3-mg L^{-1} culture in *T. weissflogii*, and from 26.5 ± 2.0 to 78.6 ± 2.7-mg L^{-1} culture in *C. cryptica*) (Fig. 2c and d).

Combined effect of high-nutrient regime and CO_2

In order to achieve simultaneous increase of biomass and lipid production, *T. weissflogii* and *C. cryptica* were grown under HNR for 7 days and then were left to consume the excess nutrients for other 7 days with or without supplementation of 10% CO_2 (Fig. 3). At the end of the first phase, cultures of *T. weissflogii* reached a biomass dry weight of 318.1 ± 36.2 mg L^{-1} and those of *C. cryptica* reached 240.4 ± 10.2 mg L^{-1} (Fig. 3). The lipid content was 13 and 18% of total biomass, respectively, but, in agreement with the results reported above ("Effect of high-nutrient regime"), TAG was completely absent in the lipid extracts of both diatoms. During the following phase of nutrient consumption, cells continued growing and final yields in biomass and total

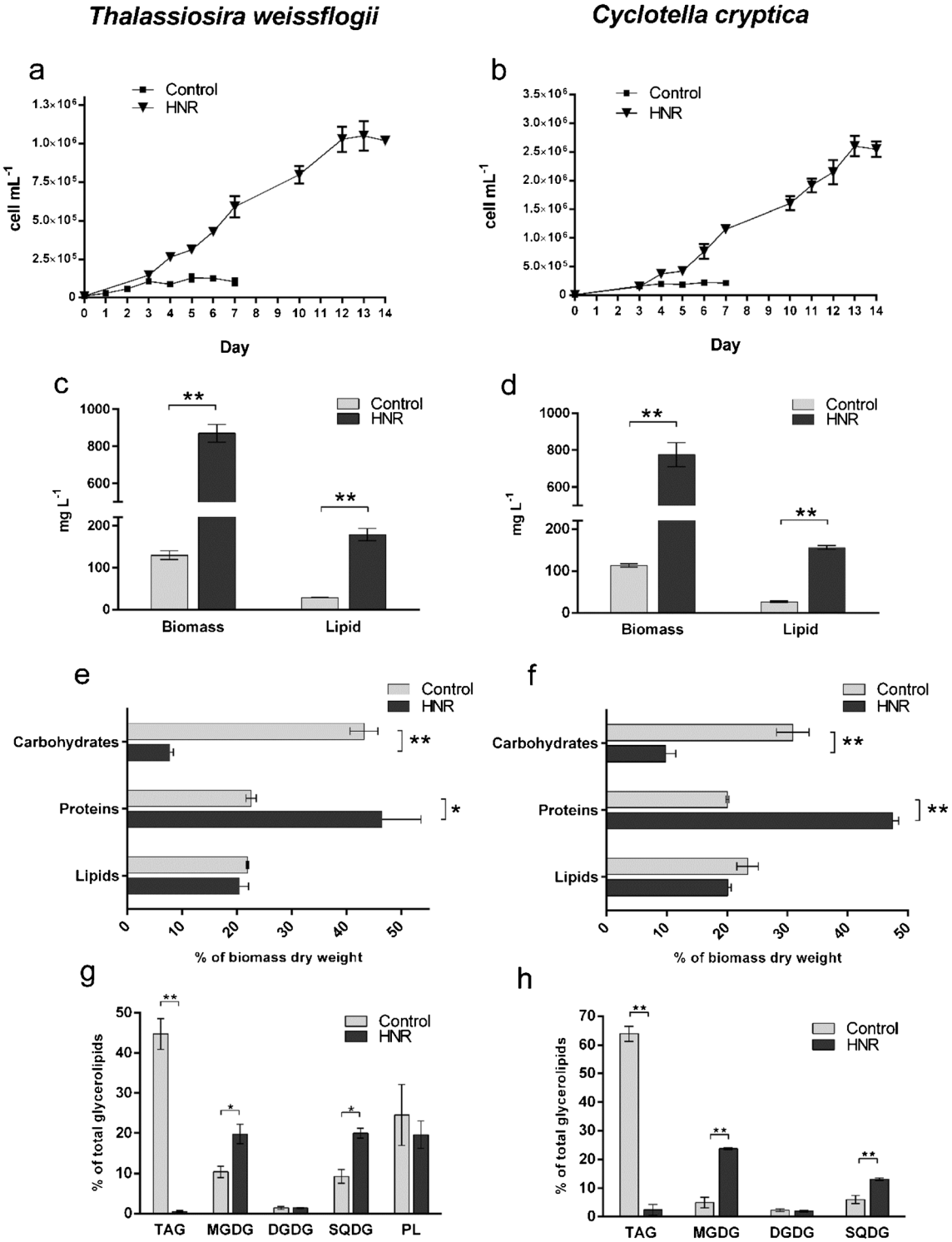

Fig. 1 Effect of high-nutrient regime (HNR) in *T. weissflogii* (**a**, **c**, **e**, **g**) and *C. cryptica* (**b**, **d**, **e**, **h**). Cell density (cells mL^{-1}) (**a**, **b**) (■ = control; ▼ = HNR); biomass and lipid production (mg L^{-1}) (**c**, **d**); lipid, protein, and carbohydrate content, expressed as percentage of biomass dry weight (**e**, **f**); glycerolipid distribution assessed by ^{1}H-NMR, expressed as percentage of total glycerolipids (**g**, **h**). *TAG* triacylglycerides, *MGDG* monogalactosyldiacylglycerol, *DGDG* digalactosyldiacylglycerol, *SQDG* sulfoquinovosyldiacylglycerol, *PL* phospholipids (* corresponds to $0.001 < p \leq 0.05$, ** corresponds to $p \leq 0.001$, Student's *t* test). Data are presented as means ± SD, $n = 3$ (*light grey bar* control, *dark grey bar* HNR)

lipids were only slightly lower than those obtained under one-stage HNR conditions ("Effect of high-nutrient regime"). The consumption of nutrients during the second stage induced the expected increase of TAG from 0.5 to 18% of total

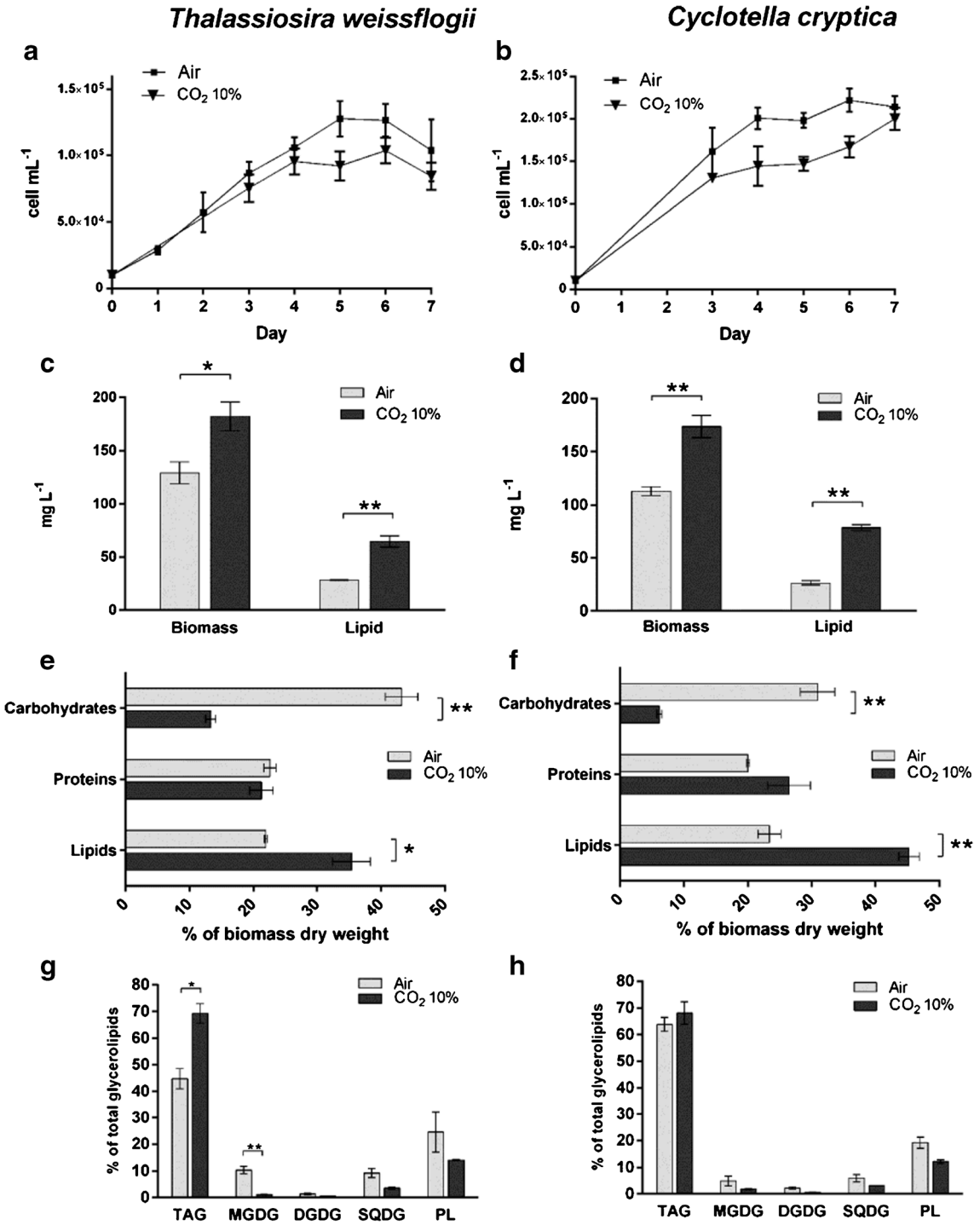

Fig. 2 Effect of 10% CO_2 addition in *T. weissflogii* (**a**, **c**, **e**, **g**) and *C. cryptica* (**b**, **d**, **e**, **h**). Cell density (cells mL^{-1}) (**a**, **b**); biomass and lipid production (mg L^{-1}) (**c**, **d**); lipid, protein, and carbohydrate content, expressed as percentage of biomass dry weight (**e**, **f**); glycerolipid distribution assessed by ^{1}H-NMR, expressed as percentage of total glycerolipids (**g**, **h**). *TAG* triacylglycerides, *MGDG* monogalactosyldiacylglycerol, *DGDG* digalactosyldiacylglycerol, *SQDG* sulfoquinovosyldiacylglycerol, *PL* phospholipids (* corresponds to $0.001 < p \leq 0.05$, ** corresponds to $p \leq 0.001$, Student's *t* test). Data are presented as means ± SD, $n = 3$ (*light grey bar* air-sparged culture, *dark grey bar* 10% CO_2-sparged cultures)

glycerolipids in *T. weissflogii* and from 2.3 to 33% of glycerolipids in *C. cryptica* (Fig. 3a and b).

Use of CO_2 in the second stage not only gave an additional increment of biomass production (about 1250 mg L^{-1} for both diatom species) but also stimulated lipid synthesis (Fig. 3). This latter response was especially evident in *C. cryptica* that showed a final lipid content of 474 mg L^{-1}, which was three times more than the lipids produced under one-stage HNR conditions

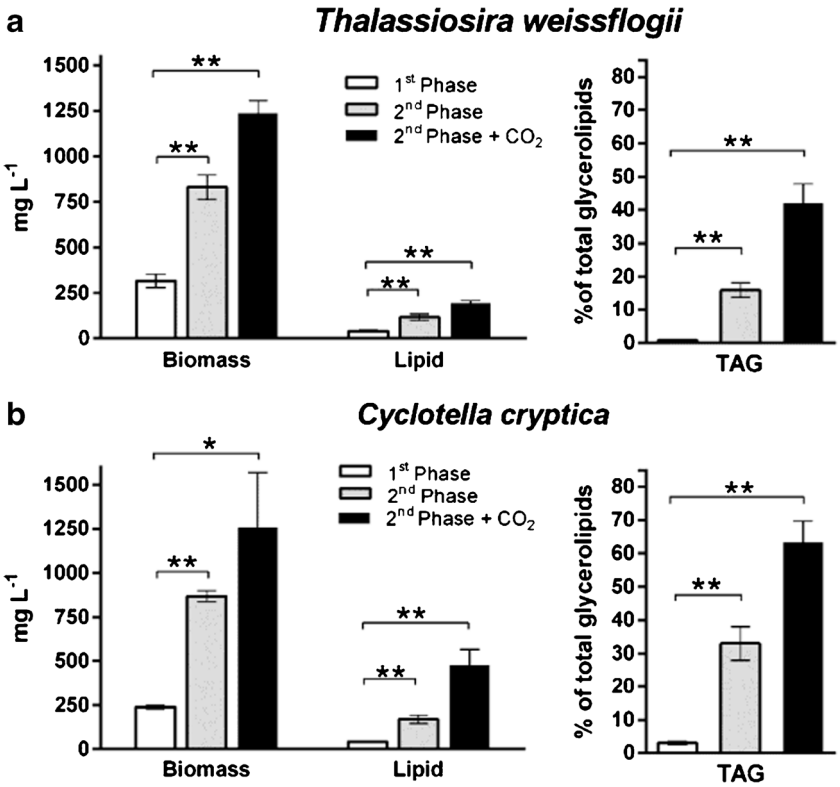

Fig. 3 Biomass and lipid production (mg L^{-1}) and % triglycerides with respect to total quantity of glycerolipids under two-stage cultivation in *T. weissflogii* (**a**) and *C. cryptica* (**b**). *I PHASE* cultivation by daily nutrient replenishment (*white*), *II PHASE* cultivation with no nutrient input without (*grey*) or with (*black*) addition of 10% CO_2 (* corresponds to $0.001 < p \leq 0.05$, ** corresponds to $p \leq 0.001$, Student's *t* test). Data are presented as means ± SD, $n = 3$

(Fig. 1) and six times more than under control conditions (Fig. 2). Furthermore, both diatom species responded to CO_2 addition with a massive accumulation of TAG that accounted for 63% of glycerolipids in *C. cryptica* and more than 40% in *T. weissflogii*. Increase of these lipids correlated with a comparable reduction of PL (supplementary material Table S1), thus suggesting that glycerolipid accumulation in both species was due to conversion of structural lipids into storage lipids.

The effect of two-stage cultivation on TAG content can be related to the nutrient depletion during the second stage of the experiment. Silicate and phosphate were fully depleted in *C. cryptica* by day 9 (Fig. 4), and their lack matched synthesis of TAG, as estimated by ^{1}H-NMR analysis of the lipid extracts (supplementary material, Fig. S2). On the contrary, *T. weissflogii* never completely consumed phosphate, which may explain the minor effect on TAG increase in this species (supplementary material, Fig. S3). In both diatoms, consumption rate of nitrate was not sufficient to uptake the daily addition, thus leading an accumulation in the medium. For this reason, nitrates are not a limiting factor in this experimental scheme.

Discussion

Nutrient input in microalgal culture is a critical factor to modulate biomass productivity and lipid composition. Although nutrient deprivation (especially silicate) is described as a promising approach to increase TAG production in marine diatoms (Roessler 1988; Gardner et al. 2012; Jeffryes et al. 2013; Valenzuela et al. 2013; Moll et al. 2014; Yang et al. 2014), the opposite regime with high-nutrient supplementation has been little studied except for the effect on growth and photosynthetic efficiency (Tantanasarit et al. 2013).

In this study, we studied the effect of HNR and high concentration of CO_2 (10% *v/v* in air) on the diatoms *T. weissflogii* and *C. cryptica*. Under HNR conditions, both diatoms yielded high-biomass production in comparison to control (Fig. 1a and b). The hypertrophic conditions led diatom cells to invest their metabolic energy in growth and protein synthesis rather than accumulate

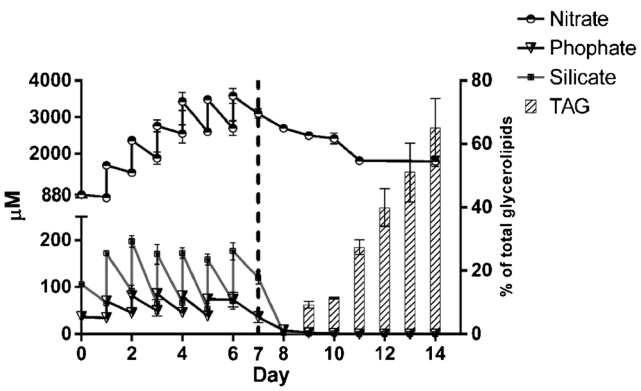

Fig. 4 Triglyceride accumulation along growth curve in the two-stage cultivation of the diatom *C. cryptica*, in relation to macronutrient concentration. *I PHASE* cultivation by daily nutrient replenishment (from day 0 to 7), *II PHASE* cultivation in nutrient shortage with addition of 10% CO_2 (from day 7 to 14)

Table 1 Biomass, lipid, and triglyceride productivities of *T. weissflogii* and *C. cryptica*. *Control* 7 days of cultivation with addition of nutrients (f/2 medium) only at the beginning of the experiment, *two-stage cultivation* (*TSC*): 14 days of cultivation divided into a first stage with daily supplementation of nutrients (f/2 medium) for 7 days and a second phase with CO_2 addition without nutrient supplementations for another 7 days. Data are presented as means ± SD, $N = 3$

		Biomass productivity (mg L^{-1} day^{-1})*	Lipid productivity (mg L^{-1} day^{-1})*	TAG (% of total glycerolipids)
Thalassiosira weissflogii	Control	18.4 ± 1.5	4.0 ± 0.04	44.7 ± 3.8
	TSC	88.4 ± 5	13.9 ± 1.0	41.8 ± 5.9
Cyclotella cryptica	Control	16.2 ± 0.6	3.8 ± 0.3	63.8 ± 2.6
	TSC	89.6 ± 7.6	33.9 ± 6.5	63.1 ± 6.5

* $p < 0.05$, Student's *t* test

reserve substances such as lipids and carbohydrates (Fig. 1c and d) (He et al. 2015). According to the literature (Kobayashi et al. 2013), the active cell division also imposed synthesis of structural lipids, mainly MGDG and SQDG directly involved in the functionality of photosynthetic machinery, to the detriment of TAG that were completely absent in the two species (Fig. 1g and h). On the other hand, 10% CO_2 stimulated lipid production and decreased carbohydrate level in comparison to the control cultures (Fig. 2e and f). Under these conditions, diatom cell apparently directed the extra-availability of carbon towards lipogenesis probably by increasing carbon flux throughout the glycolysis (Wang et al. 2014; Hennon et al. 2015). These results are in line with a recent report on *Chaetoceros muelleri* (Wang et al. 2014), even if independent studies have revealed that the CO_2 effect on the biochemical composition of diatom biomass is dependent on several factors including variation between species, CO_2 percentage in the sparging gas, mean gas residence time, gas sparging time, and culture conditions (Chrismadha and Borowitzka 1994; de Castro Araujo and Tavano Garcia 2005; Picardo et al. 2013; Giordano and Ratti 2013). However, CO_2 per se was not able to stimulate the growth rate, and there was no significant change in the cell density and biomass under ambient or high concentration of CO_2. (Fig. 2a–d). Thus, despite the increased lipid accumulation, the overall lipid content was lower than under nutrient replete conditions (Fig. 1c–d). It has been reported that CO_2 increases the growth of the diatom *Thalassiosira pseudonana* only at low or moderate intensity of light (Sakshaug 1977). Our experiments suggest that CO_2 sparging does not stimulate the diatom growth in presence of low levels of nutrients. Interestingly, a similar effect has been also predicted for phytoplankton blooms in natural environments (Verspagen et al. 2014).

The potential of this metabolic response of *T. weissflogii* and *C. cryptica* is well-exploited by a two-stage cultivation during which the diatoms were maintained in replete medium for 1 week before replacing nutrient addition with CO_2 sparging for another week. The overall outcome of the process was a massive growth in the first stage followed by a fast consumption of silicate and phosphate together with the reduction of nitrate in the second stage (Fig. 4).

As summarized in Table 1, biomass productivity of both *T. weissflogii* and *C. cryptica* increased to 88 mg L^{-1} day^{-1} which corresponded to an increase of more than five times in comparison to the productivity of the control. In addition, total lipid productivity reached values of 14 mg L^{-1} day^{-1} in *T. weissflogii* and 34 mg L^{-1} day^{-1} in *C. cryptica*, threefold and tenfold more than control, respectively. Particularly, noteworthy are the effects of the process on the increase of lipid and TAG productivity in *C. cryptica*, making this species potentially more suitable than *T. weissflogii* as candidate for oil production.

Mutual improvement of biomass and TAG productivity is the major target for the development of sustainable microalgae-based bioprocesses. Robust algal growth and high-lipid production are usually reciprocally exclusive (Sheehan et al. 1998; Tan and Lee 2016) and two-stage cultivation is a strategy proposed to overtake this limit. Generally, this approach is based on a first-cultivation stage (stage I) rich in nutrients followed by the harvesting of the biomass and the transfer of the cells in a medium (stage II) completely or partially deprived of essential macronutrients (nitrate, phosphate, and silicate) (Su et al. 2011; Mujtaba et al. 2012). To date, this strategy has been unsuitable for outdoor cultivation due to practical limitations imposed by exchange of media necessary between the stages I and II. Only recently Hu and co-workers have proposed a two-stage process that does not require change of the medium to improve lipid productivity of *Scenedesmus obtusus* (Xia et al. 2013). Two-stage cultivation strategy is less-documented in diatoms (Jeffryes et al. 2013; Ozkan and Rorrer 2017). Our cultivation system does not require the transfer from nutrient-rich to a nutrient-depleted medium, thus offering the potential to reduce the operational costs of the process. Moreover, supplementation of CO_2 seems to be very effective to increase lipid yields without reducing biomass quantity, which makes this approach suitable for large-scale application.

In conclusion, a simultaneous improvement of biomass and TAG productivity is the major target for the development of sustainable microalgae-based processes. In this study, we showed that use of CO_2 can have a cooperative effect with nutrient depletion (e.g. silicon starvation) and can be exploited in order to have an additional gain in the production of

biomass and bioproducts. The accumulation of lipids in the second stage is likely due to two distinct processes: (1) an increase in the proportion of newly assimilated carbon into lipids and (2) a slow conversion of previously assimilated carbon from polar lipids into TAG. These results are consistent with previous reports on oil accumulation of other diatoms in response to silicate and phosphate starvation (Reitan et al. 1994; Jeffryes et al. 2013). In this scenario, CO_2 enhances the plasticity of diatom metabolism in response to different nutrient regimes. It is to note that lipid accumulation induced by CO_2 during nutrient shortage suggests the presence of a nutrient-dependent signal transduction system to coordinate cellular growth versus lipid accumulation. Further work is required to tune nutrient requirements in these cultures and to make the process more efficient and sustainable at industrial scale.

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