A complete energy balance from photons to new biomass reveals a light- and nutrient-dependent variability in the metabolic costs of carbon assimilation

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Abstract

The energy balance of Phaeodactylum tricornutum cells from photon to biomass have been analysed under nutrient-replete and N-limiting conditions in combination with fluctuating (FL) and non-fluctuating (SL) dynamic light. For this purpose, the amount of photons absorbed has been related to electrons transported by photosystem II, to gas exchange rates, and to the newly formed biomass differentially resolved into carbohydrates, proteins, and lipids measured by means of Fourier transform infrared (FTIR) spectroscopy. Under high nutrient conditions, the quantum efficiency of carbon-related biomass production \( \Phi_C \) and the metabolic costs of carbon \( C \) production were found to be strongly controlled by the light climate. Under N-limited conditions, the light climate was less important for the efficiency of primary production. Thus, the largest range of \( \Phi_C \) dependent on the nutrient status of the cells was observed under non-fluctuating light conditions which are comparable with stratified conditions in the natural environment. It is evident that N limitation induced pronounced changes in the composition of macromolecular compounds and, thus, influenced the degree of reduction of the biomass as well as the metabolic costs of \( C \) production. However, \( \Phi_C \) and the metabolic costs are not predictable from the photosynthesis rates. In consequence, the results clearly show that bio-optical methods as well as gas exchange measurements during the light phase can severely mismatch the true energy storage in the biomass especially under high nutrient in combination with non-fluctuating light conditions.

Key words: Alternative electron pathways, diatom, dynamic light, energy balance, FTIR, global warming, nitrate, photosynthesis.

Introduction

The upper mixed layer in the aquatic environment can be a highly dynamic system where phytoplankton is exposed to tremendous changes in irradiance, nutrient availability, and temperature on a spatial and temporal scale (Raven and Geider, 2003). The resulting variability in photosynthesis can hamper the estimation of global phytoplankton primary production by upscaling from discrete carbon-based \textit{in situ} measurements. To overcome this problem, satellite-based ocean colour sensors have been developed to estimate the chlorophyll (Chl) concentration in the upper ocean (Behrenfeld et al., 2005). To yield an integrated biomass from ocean colour, a carbon to chlorophyll relationship (C:Chl) has to be assumed (Carr et al., 2006). The estimation of a column integrated primary production rate has to account for the photosynthetically available radiation (PAR), the temperature (measured as sea surface temperature), the amount of radiation absorbed by the phytoplankton, nutrient availability, and the quantum yield of photosynthesis (Morel et al., 2006). The last is usually obtained from \( ^{14} \text{C} \)-based production–irradiance curves. Although the radio-carbon method is used as a standard method in marine
ecosystems, it requires the artificial incubation of discrete water samples and there are still some uncertainties about the correlation of $^{14}$C fixation rates to net or gross photosynthesis rates (Geider et al., 1998). Furthermore, this procedure assumes a certain quantum efficiency of carbon production ($\Phi_C$) dependent on the abiotic factors listed above. Nevertheless, Wagner et al. (2006) have shown that light fluctuations, for example, due to water movement or clouds, and species-specific physiological characteristics can drastically influence $\Phi_C$.

Several models have been developed to estimate primary production in the ocean (Behrenfeld and Falkowski, 1997) which can be used to convert ocean colour-based estimates of Chl concentrations and photosynthetic parameters into primary production rates. A basic source of deviations from the correlation of primary production models to measured photosynthesis rates is the acclimation of phytoplankton to changing environmental conditions (Siegel et al., 2005). Carr et al. (2006) suggested that the deviations between those models can be overcome by improved data about the vertical Chl distribution, the light field, and the quantum yield of photosynthesis ($\Phi_{PSII}$, reviewed in Wilhelm et al., 2004). To derive $\Phi_{PSII}$, the chlorophyll fluorescence techniques, such as the pulse amplitude-modulated (PAM) fluorescence technique (Schreiber et al., 1986) and fast repetition rate (FRR) fluorometry (Kolber et al., 1998), could be a supplemental possibility (Behrenfeld et al., 2006).

Fluorescence techniques have been developed to characterize qualitatively basic photosynthetic reactions and, for example, to use them as a diagnostic tool for stress in photosynthetic organisms (DeEli and Toivonen, 2003). The fast and non-invasive character of the method always attracted researchers to try to convert fluorescence-based electron transport rates (ETRs) into oxygen evolution or carbon assimilation rates (Geel et al., 1998; Gilbert et al., 2000b; Franklin and Badger, 2001; Glud et al., 2002). However, bearing in mind that the fluorescence-based ETR displays the total amount of electrons released at photosystem (PS) II, it is not surprising that a correlation of ETR and oxygen evolution/carbon fixation exists under optimally physiological conditions only (Genty et al., 1989). Particularly at high irradiance, a deviation from this correlation can be observed due to redirecting of photosynthetic electrons into alternative electron sinks, e.g. cyclic electron transport around PSII (Prasil et al., 1996), the Mehler reaction (Asada, 1999), or nitrite reduction (Lomas and Gilbert, 1999).

Recently, Kroon and Thoms (2006) have presented a generalized model of phytoplankton steady-state growth which is linked to Chl fluorescence. The model integrates the main photosynthetic mechanisms and processes. A new aspect is the introduction of a parameter which characterizes the general degree of reduction for biomass and represents the number of electrons needed to synthesize 1 mol of C biomass. This parameter is believed to allow the conversion of fluorescence-based electron transport rates into primary production and growth rates. The intention to introduce such a parameter is reasonable as it is known that especially nutrient limitation can drastically change the elemental composition of the biomass (Burkhart and Riebesell, 1997; Leonardos and Lukas, 2000; Uriarte et al., 2006). Nitrate limitation is known to induce a decrease in cellular carbon content (Reynolds et al., 1997; Stramski et al., 2002). Furthermore, changes in the macromolecular composition, such as an increase in cellular carbohydrates at the expense of proteins, have been observed (Rhee, 1978; Plumley and Darley, 1985). As a consequence, the decreased Chl and protein content affects the light-harvesting efficiency and the carbon acquisition by Rubisco (Kolber et al., 1988; Geider et al., 1993, 1998).

Whereas the primary physiological effects caused by nutrient limitation are well known, there has been no study to date to quantify the effects of N limitation on $\Phi_C$ under dynamic light conditions with respect to changes in the biomass composition and alternative electron consumption. $\Phi_C$ is defined as the number of absorbed quanta required to form one unit of carbon biomass. This efficiency is influenced by several metabolic processes, for example, the efficiency of light harvesting, photochemistry, and electron usage. The last is controlled by the activity of alternative electron sinks and by changes in the degree of reduction of the biomass. N limitation and dynamic light conditions are expected to change these processes drastically. Therefore, in the present study, the aim was to quantify the energy flow from photons absorbed to electrons delivered to the energy stored in the newly formed biomass in the diatom Phaeodactylum tricornutum under N limitation and in combination with simulated natural light conditions. This approach is complemented by Fourier transform infrared (FTIR) microspectroscopy which allows the quantification of the macromolecular composition. The determination of the amount of alternative electrons is based on the comparison of the PSII-related electron transport rates (fluorescence, $P_F$) with the oxygen evolution rates (Clark-type electrode, $P_O$). $P_F$ displays the total amount of electrons released at PSII, whereas $P_O$ is the gross photosynthesis rate which is biased by alternative, light-dependent oxygen-consuming reactions, such as the PSII cycle and the Mehler reaction. Therefore, the ratio of fluorescence- to oxygen-based photosynthesis rates describes the energy losses at the level of alternative electron pathways, whereas the macromolecular composition of the new biomass reflects the degree of its reduction.
Materials and methods

Culture conditions

Experiments were performed with the diatom *P. tricornutum* (SAG 1090-1a, Göttingen, Germany) in a continuous culturing system (Kroon et al., 1992). To simulate natural light conditions, algae were illuminated with two dynamic light climates (Fig. 1) according to Wagner et al. (2006): (i) a sine light climate [SL, non-fluctuating; daily photosynthetic photon fluence rate (PPFR) 14.5 mol m\(^{-2}\) s\(^{-1}\)]; and (ii) a sine light climate superimposed by exponential fluctuations (FL, fluctuating; PPFR 3.1 mol m\(^{-2}\) s\(^{-1}\)). As a modification of the growth conditions in Wagner et al. (2006), illumination was provided with a daylength of 10 h and by an HQI-T 1000 W/D (Osram, Germany). In many studies where the effects of different dynamic light conditions on algal growth have been investigated, the daily PPFR was kept constant to allow a direct comparison of the growth rates. In the present study, it was preferred to compare light conditions with the same maximum photon fluence rate of 980 \(\mu\)mol m\(^{-2}\) s\(^{-1}\). In this way additional effects of algal adaptation to different maximum light levels are avoided. However, this procedure requires a careful estimation of the amount of light absorbed by the algae.

Cells were grown in modified artificial seawater (ASP 2; Provasoli et al., 1975) under either nitrate-replete conditions (turbidostat-grown) or nitrate-limited conditions (chemostat-grown). To achieve conditions of N limitation, the concentration of nitrate has been decreased to 0.59 mM and to 0.47 mM under SL and FL conditions, respectively, and the dilution rates have been decreased to approximately 50% compared with the respective nutrient-replete control. Thus, depending on the dilution rate, the total N supply was 109 \(\mu\)mol l\(^{-1}\) and 54 \(\mu\)mol l\(^{-1}\) per day under SL and FL conditions, respectively.

To avoid self-shading of algal cells, the Chl content was kept constant at 2 mg l\(^{-1}\) except for N-limited cells under SL conditions. Here, the Chl content was lowered to 1 mg l\(^{-1}\) to compensate for the higher absorption efficiency of the cells (see Chl-specific absorption \(a^*\) in Table 1) and to achieve similar amounts of absorbed radiation in both nutrient conditions. Under each growth condition, the measurements for the determination of photosynthesis rates and biomass production (see below) started after the cell culture reached a constant Chl content (at a given dilution rate) and a constant Chl per cell ratio for at least 3 d.

**Measurement of photosynthesis rates and carbon-related biomass production**

The measurement of growth rates (\(\mu\)), of fluorescence- and oxygen-based photosynthesis rates (\(P_F\) and \(P_o\), respectively), the calculation of photosynthetically absorbed radiation (\(Q_{phar}\)) and theoretical biomass production were performed as described in Wagner et al. (2006). Essentially, it is assumed that the fluorescence-based photosynthesis rate (\(P_F\)) is the maximum amount of electrons (expressed as oxygen evolution) transported through the electron transport chain. \(P_F\) (pmol O\(_2\) cell\(^{-1}\) d\(^{-1}\)) can be calculated as:

\[
P_F = \Phi_{PSII} \times Q_{phar} \times 0.5 \times 0.25 / (\text{d} \times \text{Chl}) / \text{Chl}_{cell}
\]

where \(\Phi_{PSII}\) is the effective fluorescence quantum yield of PSII (Genty et al., 1989), \(Q_{phar}\) (mmol quanta Chl\(^{-1}\) d\(^{-1}\)) is the daily amount of absorbed radiation in the culture vessel (Gilbert et al., 2000a), d is the optical pathlength of the culture vessel, Chl is the a*, Chla-specific absorption coefficient; \(Q_{phar}\), photosynthetically absorbed radiation; C, carbon; \(P_F\), fluorescence-based photosynthesis rate; \(P_o\), oxygen-based photosynthesis rates; \(R\), respiration; \(PQ\), photosynthetic quotient. Data are given as mean values and standard deviations (in parentheses) of at least three independent replicates.

<table>
<thead>
<tr>
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<tr>
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<td>N-replete</td>
<td>N-limited</td>
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</tr>
<tr>
<td>Chl (fg cell(^{-1}))</td>
<td>620 (60)</td>
<td>329 (30)</td>
<td>424 (20)</td>
<td>87 (3)</td>
</tr>
<tr>
<td>(a^*) (m(^2) (\text{g Chl})^{-1})</td>
<td>10.6 (0.1)</td>
<td>12.1 (0.19)</td>
<td>12.0 (0.08)</td>
<td>19.1 (0.9)</td>
</tr>
<tr>
<td>(Q_{phar}) (mmol Chl(^{-1}) d(^{-1}))</td>
<td>25 (0.5)</td>
<td>26.2 (0.4)</td>
<td>121 (3)</td>
<td>197 (11)</td>
</tr>
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<td>(Q_{phar}) (pmol cell(^{-1}) d(^{-1}))</td>
<td>15.6 (1.5)</td>
<td>8.6 (0.8)</td>
<td>51 (2.4)</td>
<td>17 (0.9)</td>
</tr>
<tr>
<td>C:N (mol mol(^{-1}))</td>
<td>7.9 (0.2)</td>
<td>10.8 (0.5)</td>
<td>7.7 (0.3)</td>
<td>14.5 (0.6)</td>
</tr>
<tr>
<td>C (pg cell(^{-1}))</td>
<td>9.9 (0.1)</td>
<td>8.2 (&lt;0.1)</td>
<td>9.1 (0.3)</td>
<td>7.6 (0.3)</td>
</tr>
<tr>
<td>(\mu) (d(^{-1}))</td>
<td>0.22 (0.02)</td>
<td>0.12 (0.02)</td>
<td>0.35 (0.02)</td>
<td>0.18 (0.01)</td>
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<tr>
<td>(P_F/P_o)</td>
<td>1.27 (0.01)</td>
<td>1.19 (0.02)</td>
<td>1.44 (0.05)</td>
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<td>(R) (pmol O(_2) cell(^{-1}) d(^{-1}))</td>
<td>0.23 (0.03)</td>
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<td>0.38 (0.01)</td>
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<td>(PQ)</td>
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The growth conditions in Wagner et al. (2006) are given as control. Essentially, it is assumed that the fluorescence-based photosynthesis rate (\(P_F\)) is the maximum amount of electrons (expressed as oxygen evolution) transported through the electron transport chain. \(P_F\) (pmol O\(_2\) cell\(^{-1}\) d\(^{-1}\)) can be calculated as:

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P_F = \Phi_{PSII} \times Q_{phar} \times 0.5 \times 0.25 / (d \times \text{Chl}) / \text{Chl}_{cell}
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where \(\Phi_{PSII}\) is the effective fluorescence quantum yield of PSII (Genty et al., 1989), \(Q_{phar}\) (mmol quanta Chl\(^{-1}\) d\(^{-1}\)) is the daily amount of absorbed radiation in the culture vessel (Gilbert et al., 2000a), d is the optical pathlength of the culture vessel, Chl is the a*, Chla-specific absorption coefficient; \(Q_{phar}\), photosynthetically absorbed radiation; C, carbon; \(P_F\), fluorescence-based photosynthesis rate; \(P_o\), oxygen-based photosynthesis rates; \(R\), respiration; \(PQ\), photosynthetic quotient. Data are given as mean values and standard deviations (in parentheses) of at least three independent replicates.

**Table 1. Physiological parameters of Phaeodactylum tricornutum in the comparison of fluctuating (FL) and non-fluctuating dynamic (SL) light climates under nutrient-replete and N-limited conditions**

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Chl concentration, and Chl\textsubscript{eq} is the Chl concentration per cell. The factors 0.5 and 0.25 are based on the assumption that the linear transport of one electron requires two quanta and that four electrons are required for the evolution of one molecule of oxygen, respectively. For the determination of \(\Phi_{\text{PSII}}\), a PAM fluorometer (PAM 101, Walz, Effeltrich, Germany) was used.

Oxygen-based photosynthesis rates (\(P_O\), pmol O\textsubscript{2} cell\textsuperscript{-1} d\textsuperscript{-1}) were measured using a Clark-type electrode (MI 730, Micro-electrodes Inc., NH, USA). Gross oxygen production was derived by correcting net oxygen evolution rates for the corresponding dark respiration. It is assumed that the oxygen-based photosynthesis rate represents the electron transport rate at PSII which is biased by light-dependent, alternative electron pathways, such as the oxygen-consuming Mehler reaction or the electron cycle around PSII. Thus, the ratio \(P_O/P_O\) describes the activity of alternative electron-consuming reactions.

The carbon-related biomass production (\(B_C\), pg C cell\textsuperscript{-1} d\textsuperscript{-1}) was derived from the following equation:

\[
B_C = C_{\text{cell}} \times \mu
\]  

(2)

where \(C_{\text{cell}}\) is the cellular carbon content (pg C cell\textsuperscript{-1}) obtained from the measurement of the elemental composition (see below) and \(\mu\) is the daily growth rate.

The quantum yield of oxygen evolution (\(\Phi_O\), mol oxygen mol\textsuperscript{-1} quanta) and of carbon-related biomass production (\(\Phi_C\), g C mol\textsuperscript{-1} quanta) were calculated from:

\[
\Phi_O = P_O/Q_{\text{phar}}
\]  

(3)

\[
\Phi_C = B_C/Q_{\text{phar}}
\]  

(4)

where \(P_O\) is the integrated oxygen-based photosynthesis rate per day, \(B_C\) is the carbon-related biomass production, and \(Q_{\text{phar}}\) is the molar amount of absorbed quanta per day.

From \(P_O\) a theoretical carbon production rate (\(B_O\), pg C cell\textsuperscript{-1} d\textsuperscript{-1}) can be calculated:

\[
B_O = \left[\frac{(P_O/P_Q) - (R \times R_Q)}{12}\right]
\]  

(5)

where \(R\) is the integrated respiration rate (pmol O\textsubscript{2} cell\textsuperscript{-1} d\textsuperscript{-1}), and \(P_Q\) and \(R_Q\) are the photosynthetic and respiratory quotients, respectively.

**Photosynthetic and respiratory quotient**

All measurements of gas exchange to calculate the photosynthetic and the respiratory quotients were performed using a respirometer (Biometric Systems, Weiterstadt, Germany) as described in Wagner et al. (2006). The photosynthetic (\(P_Q\)) and the respiratory quotient (\(R_Q\)) were calculated with the following equations:

\[
P_Q = |\text{OER}|/|\text{CER}|
\]  

(6)

\[
R_Q = |\text{CER}|/|\text{OER}|
\]  

(7)

where \(|\text{OER}|\) is the absolute O\textsubscript{2} exchange rate (\(\mu l\) h\textsuperscript{-1}) and \(|\text{CER}|\) is the absolute CO\textsubscript{2} exchange rate (\(\mu l\) h\textsuperscript{-1}).

**Elemental and biomass composition**

The elemental composition was measured according to Wagner et al. (2006) using a CHNSO analyser (vario EL, Analytik Jena GmbH, Germany). To follow the changes in the main biochemical cell compounds, the FTIR microspectroscopy method was used. The correlation of the quantitative determination of the biomass composition in microbial cells by FTIR spectroscopy and chemical analyses was demonstrated in Grube et al. (1999). In the present study, an adapted method according to Stehfest et al. (2005) was used. During the light periods, samples were taken hourly from the culture vessel. From the washed and concentrated cell suspension, samples of 1 \(\mu l\) (with \(\sim 5 \times 10^5\) cells) were placed on a microtiter plate and dried at 40 °C for 5 min. IR spectra were recorded in the range of 4000–700 cm\textsuperscript{-1} without further sample treatment in transmission mode with 32 scans oversampling to enhance the signal-to-noise ratio (Vector 22 laser unit, HTS-XT microtitre module, OPUS and OPUSLab v5.0 software, Bruker Optics, Germany). The spectral quota of proteins, carbohydrates, lipids, and nucleic acids were quantified by recalculating baseline-corrected spectra with reference spectra of bovine serum albumin, laminarin, palmitic acid triglyceride, and DNA, respectively. Apart from nucleic acids (Invitrogen), all substances were obtained from Sigma. Spectra of reference substances were recorded at a concentration range of 0.5–30 \(\mu g\) under the conditions described above.

For this purpose, substances were dissolved in distilled water (bovine serum albumin, laminarin, and nucleotides), or in trichloromethane (palmitic acid triglyceride). A calibration curve was determined for each substance, which allowed a correlation of spectral quota with the amount of the substance at a characteristic absorption frequency (bovine serum albumin, 1545 cm\textsuperscript{-1}; laminarin 1150 cm\textsuperscript{-1}; palmitic acid triglyceride, 2849 cm\textsuperscript{-1}; DNA 1243 cm\textsuperscript{-1}).

The biomass composition was calculated by analysing IR spectra in the frequency range of 3100–700 cm\textsuperscript{-1}. IR spectra of cells and reference substances were analysed using a linear combination algorithm. Spectral quota of proteins, carbohydrates, lipids, and nucleic acids were yielded as factors and multiplied by the respective reference spectra, resulting in defined absorption intensities. By applying absorption intensities of each reference spectrum at its characteristic frequency, the amount of cellular substances was calculated from aligned calibration curves. Calculated data were then quoted as weight per cent per dry weight. The method is validated by conventional biochemical procedures (Stehfest, 2006).

**Results**

**Chlorophyll content, absorbed radiation, and elemental composition**

The cellular content of Chla in *P. tricornutum* was dependent on both light and nutrient availability. The highest cellular Chla concentration was observed under fluctuating light (FL) and nutrient-replete conditions (Table 1). Under non-fluctuating light conditions (sine light climate, SL) the cellular Chla content decreased by 32% relative to FL. This was due to the higher mean amount of PAR in SL (440 \(\mu mol\) photons m\textsuperscript{-2} s\textsuperscript{-1}) compared with FL conditions (95 \(\mu mol\) photons m\textsuperscript{-2} s\textsuperscript{-1}). Nitrate limitation induced a decrease in the content of Chla per cell by 47% in FL and by 80% in SL conditions in comparison with nutrient-replete conditions. The changes in the cellular Chla content were accompanied by an increase in the Chla-specific absorption (\(a^*\)) by 20% in FL- and by 60% in SL-grown cells compared with nitrate-replete conditions (Table 1). As a result, in FL conditions, the amount of absorbed radiation (\(Q_{\text{phar}}\) per cell decreased by 45%, whereas the total \(Q_{\text{phar}}\) within the culture vessel did not change depending on the nutrient status (Table 1). However, the increase of \(Q_{\text{phar}}\) per Chla...
in SL-grown cells under N limitation required a decrease of the Chl content in the culture vessel from 2 mg l\(^{-1}\) in nitrate-replete conditions to 1 mg l\(^{-1}\) in N-limited conditions. With this adjustment, a total amount of absorbed radiation of 243 mmol photons l\(^{-1}\) d\(^{-1}\) compared with 197 mmol l\(^{-1}\) d\(^{-1}\) was achieved in nutrient-replete and N-limited conditions, respectively.

As expected, pronounced changes in the elemental composition under N limitation were observed in the C:N ratios, which increased by a factor of 1.4 and 1.9 in comparison with N-replete conditions under FL and SL, respectively (Table 1). In response to N limitation, the cellular carbon content decreased slightly under both light conditions (Table 1).

**Growth rates, photosynthetic capacity, carbon-related biomass production, and respiration**

Nutrient-replete turbidostat cultures of *P. tricornutum* exhibited growth rates of 0.22 d\(^{-1}\) and 0.35 d\(^{-1}\) in FL and SL conditions, respectively (Table 1). To induce N limitation, nitrate concentrations in the growth medium and dilution rates of the chemostat cultures were adjusted to yield a decrease in growth rates of approximately 50% (Table 1). Under N-limited FL conditions, the fluorescence- and oxygen-based photosynthesis rates decreased by 50% in comparison with replete growth conditions (Fig. 2A, B). This result corresponds to the decrease of the carbon-related biomass production \(B_C\) under N-limited FL conditions by 52% (Fig. 2c).

However, in SL under N limitation compared with replete conditions, the fluorescence- and oxygen-based photosynthesis rates were found to be lowered by approximately 75% (Fig. 2). On the other hand, in accordance with the decrease in the growth rates under N limitation, \(B_C\) in N-limited SL-grown cells was lowered by 57% in comparison with nutrient-replete conditions (Fig. 2C). This implies that in N-limited SL-grown cells, fewer electrons are required to form one unit of C-related biomass as compared with nutrient-replete conditions (see below). Interestingly, only in combination with the SL condition was the activity of alternative electron pathways increased under N limitation (expressed as the ratio of fluorescence- to oxygen-based photosynthesis rates, \(P_F/P_O\)), whereas in FL conditions the reduction in nitrate supply decreased the alternative electron consumption slightly (Table 1).

The photosynthetic reactions were more strongly affected by the nutrient status in SL conditions than under FL, in contrast to the respiration rates. Here, in response to N limitation, the respiration rate \(R\) decreased in SL conditions by 76% (which is consistent with the changes in the photosynthesis rates) but remained constant in FL conditions (Table 1). Thus, the quotient photosynthesis per respiration remained constant under non-fluctuating conditions (Table 1).
light conditions but strongly increased under fluctuating light conditions in the comparison of nutrient-replete and N-limited conditions, respectively.

**Composition and degree of reduction of the biomass**

The biosynthetic pathways of cellular macromolecules determine the electron requirement per unit biomass formed. For example, lipids and proteins are more reduced and require more electrons per unit carbon assimilated than do carbohydrates. This can be expressed in relative terms with the $PQ$ (ratio of $O_2$ evolution to $CO_2$ consumption). Thus, the production of biomass compounds reduced more than glucose ($PQ=1$) will increase the $PQ$. With sufficient nutrient supply in both light conditions, cells of *P. tricornutum* were characterized by a $PQ$ of 1.6 (Table 1). These observations indicate a high degree of reduction in the produced biomass. This is supported by the FTIR analysis which revealed that the biomass of N-replete cells of *P. tricornutum* was composed of approximately 36% carbohydrates, 47% proteins, and 11% lipids (percentages per dry weight) in both FL and SL conditions (Fig. 3).

As expected, N limitation caused an increase in the content of carbohydrates at the expense of proteins in both light conditions (Fig. 3). Here, the content of carbohydrates increased to 48% and 59% in FL- and SL-grown cells, respectively, whereas the protein fraction decreased to 37% and 24% in FL- and SL-grown cells, respectively. Thus, the increase in the carbohydrate/protein ratio in response to N limitation was larger in cells from SL-compared with FL-grown cells. The changes in the biomass composition were accompanied by a strong decrease in $PQ$ (Table 1). Therefore, the degree of reduction of the biomass and the requirement of electrons for biomass production decreased in response to N limitation.

**Efficiency of carbon-related biomass production**

The efficiency of carbon-related biomass production ($\Phi_C$, g C mol$^{-1}$ photons) is determined by the photosynthetic efficiency of oxygen evolution ($\Phi_O$, mol$^{-1}$ O$_2$ mol photons), by respiratory losses, and by the electron requirement per carbon fixed in the biomass. However, $\Phi_O$ is directly correlated to $\Phi_C$, and increases in respiration or in the degree of reduction of the biomass will lower $\Phi_C$.

Under nutrient-replete conditions, $\Phi_C$ is mainly affected by the photosynthetic efficiency (Fig. 4A). Due to the higher daily PPFR under non-fluctuating light conditions, $\Phi_O$ and concurrently $\Phi_C$ (Fig. 4B) were much lower compared with cells grown under fluctuating light conditions.

Under N-limited conditions $\Phi_C$ in cells grown under fluctuating light was slightly reduced, although no changes in $\Phi_O$ have been observed. As the lower degree of reduction in the biomass under N limitation (see above)
would rather increase $\Phi_C$, it can be assumed that the observed decrease in $\Phi_C$ was due to the strongly enhanced respiration in relation to the photosynthesis rates (see above).

Unexpectedly, $\Phi_C$ in cells from N-limited and non-fluctuating light conditions was slightly higher, whereas $\Phi_O$ was decreased compared with nutrient-replete conditions, respectively (Fig. 4B). The decrease in $\Phi_O$ is due to the enhanced activity of alternative electron-consuming reactions (as indicated by the higher ratio $P_F/P_O$) and to a higher non-photochemical quenching (data not shown). Here, the strong decrease in the degree of reduction of the biomass (see above) compensated for the lower $\Phi_O$ in comparison with nutrient-replete conditions.

The metabolic costs of carbon production have been expressed as the ratio of the photosynthesis rate per carbon-related biomass production. Hereby, the ratio $P_F/B_C$ reflects the conversion efficiency of all electrons released at PSII into carbon found in the biomass. In Fig. 5A it is shown that the fluorescence-based photosynthesis rates exceeded the carbon production rate at least by a factor of 4. This high ratio can be due to losses of photosynthetic electrons by respiration, alternative electron consumption and is also determined by the degree of reduction in the biomass. By far the highest $P_F/B_C$ ratio was observed under nutrient-replete conditions. To analyse the reasons for this observation, it is worth comparing $P_F/B_C$ with the ratio of net oxygen evolution per carbon production $P_{\text{Onet}}/B_C$ (Fig. 5B). Here, under N limitation, $P_{\text{Onet}}$ matched $B_C$ in both FL and SL conditions. Under nutrient-replete conditions, $P_{\text{Onet}}$ exceeded $B_C$ by a factor of 1.4 in FL and by a factor of 2.2 in SL. Thus, again, the highest metabolic costs have

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**Fig. 4.** Quantum efficiency of oxygen evolution (A) and carbon-related biomass production (B) in *Phaeodactylum tricornutum* in the comparison of fluctuating (FL) and non-fluctuating dynamic (SL) light climates under nutrient-replete (grey bars) and N-limited (white bars) conditions. $\Phi_O$ and $\Phi_C$ were calculated from data presented in Table 1 and Fig. 2.

**Fig. 5.** Metabolic costs of carbon production expressed as the ratio of photosynthesis rates per carbon-related biomass production. The photosynthesis rates are given as (A) $P_F$ (fluorescence-based photosynthesis rate) and (B) $P_{\text{Onet}}$ (net photosynthesis rate). Data were obtained in the comparison of fluctuating (FL) and non-fluctuating dynamic (SL) light climates under nutrient-replete (grey bars) and N-limited (white bars) conditions. Ratios were calculated from data presented in Table 1 and Fig. 2.
been observed under nutrient-replete SL conditions. It should be emphasized that net oxygen evolution rates are not biased by respiration or alternative electron-consuming processes. Therefore, only the degree of reduction in the biomass and the excretion of carbon are supposed to influence $P_{\text{Onet}}/B_C$. From the highly comparable pattern of $P_{\text{E}}/B_C$ and $P_{\text{Onet}}/B_C$ it can be assumed that the higher metabolic costs under nutrient-replete SL conditions in comparison with the three other growth conditions are due to the biomass composition of the cells.

In principle, higher metabolic costs could also be attributed to an excretion of carbon from the cells. However, in the present study, significant carbon losses by excretion can be excluded due to the strong correlation of the measured ($B_C$) to the calculated oxygen-based carbon production ($B_O$, Fig. 6). The excretion of carbon should have resulted in an overestimation of carbon production calculated from oxygen-based photosynthesis rates.

**Discussion**

The observed changes in the cellular Chl content and in the elemental composition clearly reveal the effects of N limitation on the physiology of *P. tricornutum* cells compared with replete culture conditions. In numerous studies regarding nitrate limitation or starvation in algae, decreasing growth rates, lower Chl per cell ratios, and an increase in C:N have been observed (Thomas and Dodson, 1972; Rhee, 1978; Geider *et al.* 1993; Berges and Harrison, 1995).

In general, N limitation lowers the nutrient content of an autotrophic cell (Geider *et al.*, 1998) which, usually, is accompanied by an increase in the carbohydrate/protein ratio (Plumley and Darley, 1985). As this change in the biomass composition decreases its degree of reduction, a readjustment of the photosynthetic energy supply to the new cellular energy demand is required under N limitation. Thus, the aim of this study was to quantify the energy flow from light absorption to energy stored in the biomass in adapting to N limitation under dynamic light conditions. These investigations yield an estimation of the quantum efficiency of carbon-based biomass production under simulated natural light conditions in the comparison of nutrient-replete and N-limited conditions. The results obtained confirm that the extent of nitrogen limitation depends on the light conditions applied (Coleman *et al.*, 1988). Thus, the changes in basic cellular parameters (e.g. C:N ratio, Chl per cell) in response to N limitation were much more pronounced in the less dynamic non-fluctuating light climate than in the highly dynamic fluctuating light conditions. Furthermore, different responses of the cells to N limitation depending on the light conditions were also observed at the physiological level, which is discussed in detail below.

**Fluctuating light conditions**

In combination with the observed increase in the C:N ratio, the FTIR analysis confirmed the increase in the carbohydrate/protein ratio under N-limited FL conditions in comparison with nutrient-replete cells. This change in the macromolecular composition of the cells induced a decrease in the degree of reduction of the biomass. As a consequence, the electron requirement per carbon fixed in the biomass was lower under N limitation compared with replete conditions. To meet the lowered energy demand of N-limited cells, the photosynthetic energy supply had to be decreased. Under FL this was achieved by the decrease in the amount of cellular light absorption by 45% due to the lowering of the Chla content per cell. The strong correlation of the decrease in growth rates to the lowering of the photosynthesis rates and of the carbon-related biomass production indicates that the cells under N limitation were still in a balanced state of cellular energy supply and demand. This result is in accordance with the study of Geider *et al.* (1998) on *Dunaliella tertiolecta*, where a balanced growth was observed in chemostat cultures under N limitation. In *D. tertiolecta* the balance was achieved by a decrease in light absorption and by changes in the quantum efficiency of photosynthesis.

In the present study, the quantum efficiency of oxygen evolution did not change in response to N limitation. Obviously, the decreased light absorption was already sufficient to keep a balanced level of energy supply and demand under FL in combination with N-limited
conditions. Furthermore, no additional energy-dissipating mechanisms were required as there were no changes in non-photochemical quenching and in the amount of alternative electrons (measured by the ratio \( P_{\text{N}}/P_{\text{O}} \) under N limitation in comparison with nutrient-replete conditions. These observations are somewhat in contrast to the expectations in consideration of the suspected role of nitrate in diatoms. In comparison with other groups of phytoplankton, diatoms are known for their higher nitrate uptake rates than other phytoplankton groups at comparable substrate concentrations (Eppley et al., 1969). The ability of diatoms to release nitrite and ammonium into the medium (Lomas et al., 2000) supports the hypothesis that diatoms can assimilate and reduce nitrate in excess of the nutritional demand (Lomas and Glibert, 1999). Thus, the energy flow from light harvesting to carbon fixation can be balanced, especially under conditions of transient excess energy supply (Lomas and Glibert, 1999). In this case, the reduction of nitrite accounts for an extra electron sink in cells with a high energy status. Under N limitation and in combination with transient periods of strong illumination, it would then be necessary to guide electrons into sinks different from nitrite reduction. Obviously, this was not the case under fluctuating light conditions in combination with N limitation as applied in the present study.

However, N limitation caused an increased respiratory activity (relative to the photosynthesis rates) in cells grown under FL conditions. Although no data on nitrate uptake rates are available, it can be assumed that the energy from respiration supports the additional uptake and assimilation of nitrate during the short dark periods in between the fluctuating light pulses. Dark nitrogen uptake has been observed in several groups of phytoplankton and is known to be induced under nitrate limitation (Syrett, 1981). It is assumed that this mechanism is ecologically important for vertically distributed cells, such as diatoms (Clark et al., 2002; Flynn et al., 2002). Vanlerberghe et al. (1992) showed a clear correlation of dark nitrate assimilation and the activity of dark respiration in green algae, which supplies the reductants, ATP and carbon skeletons for assimilation of nitrate.

The increases in the respiratory losses of cellular energy could be expected to lower the quantum efficiency of carbon-related biomass production. Nevertheless, \( \Phi_C \) was only slightly decreased under N-limited conditions, which can be attributed to the changes in the degree of reduction in the biomass. Here, the increase in the content of carbohydrates at the expense of proteins led to a less reduced biomass as indicated by the lower \( PQ \). This means that fewer electrons are required to produce one unit of carbon biomass which, in consequence, should increase \( \Phi_C \). Apparently, the changes in the biomass composition compensated for the enhanced respiratory losses to a large extent and \( \Phi_C \) decreased only slightly.

Dynamic but non-fluctuating light conditions

The growth of \( P. \) tricornutum in SL strongly enhanced the extent of the effect of N limitation on the cell physiology in comparison with N-limited FL conditions. At the level of the cellular composition, this becomes evident from the doubling of the C:N ratio and the drastic increase in the ratio of carbohydrates to proteins from 0.8 to 2.5 under nutrient-replete and N-limited conditions, respectively. As the biosynthesis of carbohydrates requires fewer electrons per carbon than the synthesis of proteins, the degree of reduction in the biomass decreased under N limitation. To re-balance the photosynthetic energy supply and the drastically decreased metabolic energy demand under N limitation, the higher total light dose in SL and the resulting photosynthetic excitation pressure required a much more pronounced physiological acclimation as observed under the N-limited FL conditions. Therefore, N-limited cells in SL were strongly forced to lower the electron supply by the photosynthetic process. This was achieved at the levels of light absorption, of the dissipation of excessively absorbed light, and of the dissipation of excessively released photosynthetic electrons. Thus, \( Q_{\text{phar}} \) was reduced by the large decrease in the amount of cellular Chla under N limitation. In addition, lowering of the photosynthetic efficiency of oxygen evolution led to the decrease of the photosynthesis electron supply by 75% in N-limited conditions. The decrease in \( \Phi_O \) in response to N limitation is in accordance with the results of Geider et al. (1998). In that study, the lower efficiency of photosynthesis was attributed to the increase in the ratio of photoprotective carotenoids per Chl and to a related decrease in the effective cross-section for photosynthesis. The indirect correlation of the amount of photoprotective pigments and the quantum yield of photosynthesis was demonstrated by Arbones et al. (2000). In the present study with \( P. \) tricornutum, no increase in the concentration of the pool of photoprotective pigments in response to N limitation was observed (data not shown). Nevertheless, the lower \( \Phi_O \) in N-limited cells of \( P. \) tricornutum can be attributed to the increased non-photochemical quenching due to a higher degree of de-epoxidation of diadinoxanthin to the photoprotective pigment diatoxanthin (data not shown) and, additionally, to the enhanced activity of alternative electron-consuming reactions.

With respect to the substantial decrease in the degree of reduction of the biomass, the drastic decrease in the photosynthetic electron supply by the mechanisms described above yielded a \( \Phi_C \) which was even slightly higher under N limitation compared with nutrient-replete conditions.

Metabolic costs of carbon assimilation

The metabolic costs of carbon assimilation can be defined as the ratio of the photosynthetically produced energy
(photosynthesis rate) per carbon-related biomass production. If the metabolic costs are given as \( P_{\text{el}}/B_C \), this ratio includes energy losses by respiration, alternative electron consumption, and the electron requirement for biomass production (dependent on the macromolecular composition of the biomass). Interestingly, the highest and the lowest metabolic costs of carbon production have been observed under SL in combination with nutrient-replete and N-limited conditions, respectively. Fluctuating light conditions yielded metabolic costs in between the values obtained under SL; however, the nutrient status did not influence the \( P_{\text{el}}/B_C \) ratio. The large deviation in metabolic costs (16–29 mol PSII electrons per mol C) in the present study with \( P. tricornutum \) confirms data of Suggett et al. (2006) where the energetic requirement of C fixation in Atlantic tropical and subtropical gyres ranged between 5 and 25 mol PSII electrons per mol C fixed.

The comparison of the ratios \( P_{\text{el}}/B_C \) and \( P_{\text{me}}/B_C \) in \( P. tricornutum \) reveals that the metabolic costs of carbon production are differentially regulated depending on the nutrient status of the cells. Under nutrient-replete carbon conditions, the high degree of reduction in the biomass increased the metabolic costs. This effect also depended on the light conditions. On the other hand, under N-limited conditions, where the degree of reduction in the biomass was low, the photosynthetic energy was lost by respiration and by alternative electron consumption. Here, the light conditions obviously did not influence the metabolic costs of carbon production.

**Conclusions**

The investigation of the effects of N limitation on the energy balance of \( P. tricornutum \) under dynamic light conditions showed that under high nutrient conditions, the light climate is the major factor controlling \( \Phi_C \) and also the metabolic costs of carbon production. Under N-limited conditions, the light climate has less or even no influence on \( \Phi_C \) and the metabolic costs, respectively. Therefore, it can be expected that stratified conditions in natural bodies of water will show the highest variability in the quantum yield of primary production depending on the trophic state. Despite the fact that \( P. tricornutum \) is a rather atypical diatom, we believe that the results obtained in this study are important with respect to the investigation of high nutrient areas, where the strongest deviations from the measured to the modelled primary production have been observed (Carr et al., 2006). The data are important for the interpretation of fertilizing experiments (Gervais et al., 2002; Street and Paytan, 2005), and also in light of the expected environmental changes (stratification, wind stress, and nutrient availability) due to the global climate warming (Sarmiento et al., 2004; Kamykowski and Zentara, 2005). Furthermore, the approach revealed the contribution of basic cellular processes that influence \( \Phi_C \) under simulated natural light conditions. Thus, under nutrient-limited conditions, the respiratory losses were most important in a fluctuating light climate whereas non-fluctuating sine light conditions had a stronger impact on the changes in the amount of alternative electrons, the non-photochemical quenching (both of which determine the photosynthetic efficiency), and the degree of reduction in the biomass. In this context, the FTIR method for the rapid determination of the biomass composition of phytoplankton cells can be a valuable complement to the fluorescence method.

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