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Effects of temperature and irradiance on a benthic microalgal community: A combined two-dimensional oxygen and fluorescence imaging approach

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Abstract

The effects of temperature and light on both oxygen (O2) production and gross photosynthesis were resolved in a benthic microalgal community by combining two-dimensional (2D) imaging of O2 and variable chlorophyll a (Chl a) fluorescence. Images revealed a photosynthetically active community with spatial heterogeneity at the millimeter scale. Irradiance strongly increased pore-water O2 concentration, sediment net O2 production, and gross photosynthesis. The latter was derived from measurements of the electron transfer rate (rETR) in Photosystem II. The onset of light saturation for gross photosynthesis was approximately twofold higher than for net O2 production, reflecting significant light-stimulated O2 consumption at higher light (> 75 μmol photons m−2 s−1). Temperature stimulated O2 consumption more than photosynthesis, turning the community more heterotrophic at elevated temperatures. Thus, the compensation irradiance (i.e., the irradiance at which community O2 production and consumption balance) increased fivefold (from 6 to 30 μmol photons m−2 s−1) with a temperature increase from 10 °C to 25 °C, corresponding to a temperature coefficient (Q10) of 2.9. Whereas net O2 production had a temperature optimum at ~ 20 °C, no optimum was observed for gross photosynthesis within the investigated range (10 °C to 25 °C). The resolved 2D net O2 production and rETR exhibited a significant exponential relationship, demonstrating predictable correlations between the net community production and gross photosynthesis for a complex microbial community, at different temperatures. The present imaging approach demonstrates a great potential to study consequences of environmental effects on photosynthetic activity and O2 turnover in complex phototrophic benthic communities.

Shallow-water benthic phototrophic communities have been shown to mediate a positive net community production over > 33% of the global shelf area and thereby play a crucial role in the global marine carbon cycling (Cahoon 1999; Gattuso et al. 2006). This underlines the importance of these communities as sources of organic carbon for sustaining the food web. The effects of fluctuating temperature and light on phototrophic sediments are complex, affecting biological, physical, and chemical processes, all of which influence the balance between the phototrophic and heterotrophic processes, with implications on both diel and seasonal scales (Barranguet et al. 1998; Glud 2008).

Benthic phototrophic communities often show a small-scale patchy variability on the distribution of microalgal biomass and activity. Microalgal communities consist of a dense physical matrix of autotrophic and heterotrophic organisms, which, at a micrometer scale distribution, exhibit close coupling between O2 production and consumption processes (Fenchel and Glud 2000). Community productivity is determined by the microalgal biomass and the photosynthetic activity, which, again, depend on the light availability. The light climate in sediments exhibits strong spatial variation due to absorption by microalgae themselves and scattering by inorganic particles, leading to a spectrally dependent light attenuation. In sandy sediments this typically results in a light penetration depth of only a few millimeters (Kühl et al. 1997). Studies on the distribution of microalgae and the tight coupling between light, photosynthesis, and community production require sophisticated techniques covering the variability at a high spatial resolution.

A good proxy for community production and sediment net metabolism is the oxygen (O2) exchange rate, because O2 is produced during photosynthesis and is consumed directly or indirectly by respiratory processes. In recent decades, microsensor studies have provided detailed insight about distribution and consumption of O2 in benthic communities (Revsbech and Jørgensen 1986; Glud 2008). However, the natural patchiness in activities may compromise alignment of traditional one-dimensional (1D) microsensor approaches, which can only provide a limited representation of the natural variability. Application of planar O2 optode images has added insight about the complexity, interaction, and balance between autotrophic and heterotrophic processes in benthic phototrophic communities (Glud et al. 1999; Fenchel and Glud 2000; Sevila et al. 2014). However, planar O2 imaging provides only indirect insight into the spatial distribution or the photophysiology of the phototrophic biomass. Such insight can be provided by chlorophyll a (Chl a) fluorescence imaging, because steady-state fluorescence represents a

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Methods

Experimental setup and microbial mat—Sediment was collected from Odense Fjord, Denmark (N 55.45, E 10.48) during low tide at ~ 0.5 m water depth, at an in situ temperature of 5°C, using sediment core liners to ensure an undisturbed vertical structure of the sediment. After gently removing larger shells and stones, the sediment was frozen at −18°C for 24 h to eliminate macrofauna. Keeping the overall integrity, the sediment was subsequently transferred to a custom-built mini flume (15 × 15 × 10 cm) that was connected to a recirculating thermostatted 8 liter water bath with water from the sampling site (salinity was 20), connected to a recirculating thermoregulated 8 liter water bath with water from the sampling site (salinity was 20), allowing the naturally existing microbial community to develop (Fig. 1). The mini flume was equipped with a flow collimator and provided a stable, unidirectional flow above the microbial mat (Glud et al. 1999). The height of water was 4 cm, and the mean current velocity at the surface was maintained at ~ 2 cm s⁻¹. The O₂ concentration in the water was kept at atmospheric saturation by continuous aeration.

At the front side of the flume, two fiber optic faceplates (FOFP) “windows” (75 × 50 × 3 mm, Schott Std. 47A Glass) were installed side-by-side (Fig. 1). The optic faceplates were constructed from bundled, 6 μm diameter optical fibers. The faceplate approach overcomes the problem of optical smearing experienced with plastic or glass walls and thereby improves the spatial resolution of recorded images (Fischer and Wenzhoefer 2010). One of the optic faceplates was coated with an O₂-sensitive luminophore functioning as a planar O₂ optode (details below), whereas the other was left uncoated for use in variable fluorescence imaging (details below).

The sediment was illuminated from above using a halogen lamp (KL2500, Schott) equipped with a heat filter and a collimating lens providing a homogeneous illumination via an optical fiber. A microbial mat was allowed to develop under ~ 150 μmol photons m⁻² s⁻¹ (16:8 h light : dark cycle), at 15°C for 4 weeks prior to the experiments. After completion of the experiments, subsamples of the upper 1 cm of the sediment were extracted in 96% ethanol for 24 h (5°C, darkness), and the Chl a concentrations were determined spectrophotometrically (Parsons and Strickland 1963) using an extinction coefficient of 83.4 L g⁻¹ cm⁻¹ (Wintermans and De Mots 1965).

During experiments, the photosynthetically available radiation (PAR) incident on the sediment surface (E_PAR) was set to 0, 20, 40, 80, 160, or 350 μmol photons m⁻² s⁻¹, whereas the spectral composition was kept constant. The incident irradiance was measured at the surface of the mat using a LiCor cosine-corrected quantum sensor (LiCor LI-190SA) and a datalogger (LiCor LI-1000). After a change in irradiance, a period of 60 min was allowed for the community to acclimate and the O₂ distribution to reach steady state. Temperature was kept constant during each irradiance “cycle” and after changes, allowing the sediment to acclimate overnight in the dark.

Preparation of the O₂-sensitive planar optode—The O₂-sensitive planar optode was constructed from an O₂-quenchable Iridium (III) complex (Ir(C₅H₅)₃acac) with blue light absorption (peaks at 445 and 475 nm) and yellow emission (peak at 564 nm and a shoulder at 610 nm, Staal et al. 2011). The sensor showed ultrabright luminescence and high sensitivity at supersaturating O₂ levels, making it an ideal O₂ indicator for phototrophic investigations. To manufacture a sensing cocktail, 1.5% wt:wt iridium(III) was dissolved in a 2% wt:wt polystyrene solution using toluene as solvent (Borisov and Klimant 2007). The solution was applied to the FOFP by spray coating, using a small airbrush. The sensing layer was ~ 5 μm after evaporation of the solvent, and the response time of the planar optode was < 5 s.

Planar optode imaging—Planar O₂ concentration imaging was based on the principle of modular luminescence imaging using a lifetime-based approach (Holst and Grunwald 2001; Frederiksen and Glud 2006). A fast-gateable, 12-bit, Peltier-cooled charge-coupled device (CCD) camera (PCO-Tech, Inc. SensiCam, www.pco.de) was mounted with a macro lens (Sigma Macro 50 mm F2.8 EX DG), equipped with a 530 nm long-pass filter (Schott OG-530). Images were recorded using an eight-image average and 2 × 2 binning to increase the signal-to-noise ratio. The final
The spatial resolution of the images was adjusted to 50 μm per pixel (i.e., 20 pixels mm⁻¹) to match the resolution that could be obtained with the adjacent imaging PAM system (www.walz.com). The excitation light was delivered by four 5W blue light-emitting diodes (LED) with a peak wavelength of 465 nm (LZ1-10DB05, www.ledengin.com), equipped with a 475 nm short-pass dichroic color filter (UQG optics, www.uqgoptics.com).

The camera and the LED power supply were synchronized with a custom-made trigger box and controlled by the software Look@Molli (Holst and Grunwald 2001). Recorded images were calibrated using CalMolli software, using the luminescent lifetime recorded in each pixel. The lifetime image was calibrated using the modified Stern-Volmer equation (Eq. 1; Klimant et al. 1995)

\[
\frac{\tau}{\tau_0} = f + (1-f) \left( \frac{1}{1+K_{SV}C} \right)
\]

Where \(\tau_0\) is the \(O_2\)-dependent, luminescent lifetime in the absence of \(O_2\) and \(\tau\) is the lifetime in the presence of any given \(O_2\) concentration \(C\), \(K_{SV}\) the bi-molecular quenching constant, and \(f\) the nonquenchable fraction of the luminescent signal. \(\tau\) was derived from two well-defined time frames on the luminescent decay curve, as configured by the Look@Molli software (Frederiksen and Glud 2006). Subsequent calibrations of the \(O_2\) concentration in each pixel were performed with two-point calibration, applying an experimentally determined \(f\) value of 0.09. All images were recorded during a brief darkening of the sample to avoid interference of ambient light. Calibrated images were further processed using the freeware ImageJ (http://rsb.info.nih.gov/ij/).

Variable fluorescence imaging—Two-dimensional images of variable Chl \(a\) fluorescence were obtained using a Walz Mini-Imaging-PAM fluorometer (Walz Mess- und Regel-
technik, GmbH), consisting of a high-speed CCD camera, an LED array, and a control unit that synchronized the LED light and the camera operation (Ralph et al. 2005). The Mini-PAM camera has a resolution of 640 × 480 pixels (F1.4, f = 16 mm lens) with a field of view of 32 × 24 mm, resulting in a spatial resolution of 50 μm per pixel (i.e., 20 pixels mm⁻¹), equivalent to the planar O₂ optode setup described above. Chlorophyll fluorescence was excited by 12 high-power blue LEDs (peak at 460 nm, equipped with short-pass filters) organized in four groups in a circle around the camera lens to provide a homogeneous illumination of the field of view. The blue LEDs both provided probing and saturating light pulses during measurements. When required, dark acclimation was ensured by covering the setup with a black cloth, and the room was darkened.

The imaging PAM fluorometer continuously measures the operational quantum yield of fluorescence (Fᵢ) by applying a weak probing light at 1 Hz, an intensity too low to induce photosynthetic activity. We applied the fluorescence nomenclature suggested by Van Kooten and Snel (1990). In the absence of actinic illumination, Fᵢ is equivalent to the minimum quantum yield of fluorescence (Fₒ), which is a good proxy for the 2D Chl a distribution.

By applying a strong saturating light pulse (0.8 s, at > 2000 μmol photons m⁻² s⁻¹), an image of the maximum fluorescence (Fₘ) was obtained, and the maximum quantum yield of PSII charge separation (Φ₉PSII) was calculated as Φ₉PSII,max = (Fₘ − Fₒ)/Fₘ Φ₉PSII,max is equal to the often used term Fᵥ/Fₘ (where Fᵥ = Fₘ − Fₒ). In the presence of actinic light, the steady-state fluorescence (Fₛ) and the maximum fluorescence after a saturation light pulse (Fₛₘ) were determined, from which the operational quantum yield in PSII (Φ₉PSII) was calculated; i.e., Φ₉PSII = (Fₛₘ − Fₛ)/Fₘ (Genty et al. 1989). The relative electron transfer rate in PSII was then calculated as rETR = Φ₉PSII × EPAR, where EPAR is the incident downwelling irradiance corrected for the vertical attenuation (see below).

Spectral irradiance measurements—Spectral scalar irradiance profiles (Eₐ,z) in the sediment were measured at the applied incubation irradiances using an optical microprobe (Kühl et al. 1994; Kühl et al. 1997) connected to a spectral irradiance meter (Ocean Optics, USB2000). The EPAR,z scalar irradiance was obtained from integrating the spectral irradiance from 400 nm to 700 nm, corrected for the spectral sensitivity of the detector system. The scalar irradiance was measured from 3 mm above the sediment surface down to the sediment depth, at which EPAR,z decreased below 0.01% of the surface irradiance, with a vertical resolution of 75 μm to 150 μm. The probe was positioned with a motor-driven micromanipulator (MUX2, PyroScience GmbH) controlled by a personal computer. Depth profiles were measured by inserting the microprobe from above at a 40 degree zenith angle to avoid self-shading by the sensor. The spectral distribution of the downwelling irradiance was measured after the positioning of the microprobe on top of a light trap of a black nonreflecting rubber plug.

The diffuse vertical spectral attenuation coefficient (Kₐ, Eq. 2) of the scalar irradiance was calculated as

\[ K(\lambda) = \frac{\ln(E_{\lambda1}/E_{\lambda2})}{z_2 - z_1} \]  

(2)

where E₁ and E₂ were measured at depths z₁ and z₂ in the sediment, and where z₂ represents a deeper penetration than z₁.

Flux calculations and curve fit regressions—The sediment net O₂ production (under illumination) and consumption rates (in darkness) were calculated from the shape of O₂ profiles extracted from the steady-state planar optode images, using the numerical software procedure “PROFILE” to arrive at depth-integrated O₂ flux rates (Berg et al. 1998). Only the subsurface parts of the O₂ profiles were included in these analyses to avoid potential wall-induced effects on the concentration gradient in the diffusive boundary layer (DBL). For the numerical procedure, the maximum vertical interpretation steps were set to 12 (the maximum achievable), and the boundary conditions were defined as setting both the bottom O₂ concentration and flux to zero. The sediment diffusivity (Dₛ) was calculated from the sediment porosity times the diffusivity in water, according to Broecker and Peng (1974), corrected for temperature and salinity (Li and Gregory 1974).

Both O₂ production and rETR vs. irradiance (P–E) relationships were fitted using the Webb equation because no sign of photoinhibition was observed (Eq. 3, Webb et al. 1974):

\[ P = P_{\text{max}} \left(1 - e^{-\left(\frac{z_1}{E_{\text{PAR},z}}\right)}\right) \]  

(3)

where the maximum O₂ production or photosynthetic activity (Pₘₐₓ) and the maximum light utilization coefficient (α) were fitted as a function of EPAR. The light-saturation coefficient (Eₛ) was calculated as Eₛ = Pₘₐₓ/α. Curve fitting was carried out using ordinary least-squares criterion in Origin 7.0 (OriginLab).

Results

Sediment and microalgal community characteristics—During the 4 weeks of pre-incubation, a 1–2 mm thick microalgal community dominated by pennate diatoms (Navicula, Nitzschia, Gyrosigma, and Pleurosigma) had colonized the sandy–silty sediment, which had a mean grain size of 195 μm. The top 1 cm of the sediment had a mean Chl a concentration of 104 ± 23 mg m⁻² (n = 10), and the surface appeared homogenous. However, the Fₒ values of the cross-section expressed an extensive horizontal patchiness of Chl a fluorescence (Fig. 2). Vertical profiles of Fₒ reflected variations in the biomass distribution, and representative profiles indicated an ~ 2.5-fold variation in the maximum biomass on a millimeter scale (Fig. 2C,D). Correspondingly, the O₂ distribution showed local maxima and minima (Fig. 2A), and extracted O₂ microprofiles verified a pronounced horizontal variability (Fig. 2B). However, as expected, the spatial patchiness in O₂ reflecting a diffusional smeared distribution was less
pronounced than for \( F_0 \), representing the direct distribution of microalgal biomass.

**Irradiance profiles and spectral irradiance attenuation**—The microalgae community experienced darkness and five incident surface irradiances, i.e., \( E_{\text{PAR}} = 20, 40, 80, 160, \) and \( 350 \) \( \mu \text{mol} \text{photons m}^{-2} \text{s}^{-1} \). Because light is absorbed strongly by microalgae and extensively scattered by sandy sediments, light is attenuated with sediment depth. This resulted in 10% and 1% irradiance depths of 800 ± 220 \( \mu \text{m} \) and 1300 ± 240 \( \mu \text{m} \) (\( n = \) seven microprofiles), respectively (Fig. 3A). Conventionally, these depths are considered to represent the midpoint and the lower limit of the euphotic zone. On average, the diffuse attenuation coefficient, \( K_{d,\text{PAR}} \), was 3.9 ± 0.8 \( \text{mm}^{-1} \).

The presence of microalgal light-harvesting carotenoids and chlorophylls shifted the spectral light composition toward green-yellowish colors with increasing sediment depth. This was observed as wavebands at 400 to 500 nm and 660 to 690 nm were quickly attenuated (Fig. 3B). This resulted in distinctive signatures for the spectral attenuation coefficient (\( K_{d,\lambda} \)) before and after the development of the microalgae community (Fig. 3C).

**Effects of irradiance on \( O_2 \) production and gross photosynthesis**—The sediment \( O_2 \) concentration increased with irradiance as a response to photosynthetic activity (Fig. 4A). The \( O_2 \) concentration in the immediate subsurface increased from atmospheric saturation (252 \( \mu \text{mol} \text{L}^{-1} \)) in the dark to supersaturation (> 1000 \( \mu \text{mol} \text{L}^{-1} \)) at \( E_{\text{PAR}} > 80 \) \( \mu \text{mol} \text{photons m}^{-2} \text{s}^{-1} \). All images in Fig. 4 were recorded at steady-state conditions. To deduce the response of \( O_2 \) penetration depth and net \( O_2 \) production to irradiance in sediment with intermediate to high photosynthetic activity, five \( O_2 \) profiles were selected in sequence between positions 2 and 3 in Fig. 2A, each representing a 500 \( \mu \text{m} \) horizontal mean.

At 20°C, the \( O_2 \) penetration depth almost tripled with increasing irradiance, from 0.13 ± 0.05 cm in the dark to 0.35 ± 0.17 cm at the maximum irradiance (Figs. 4A, 5A). Correspondingly, the net \( O_2 \) production increased from −0.020 ± 0.002 nmol cm\(^{-2}\) s\(^{-1}\) to 0.117 ± 0.012 nmol cm\(^{-2}\) s\(^{-1}\); a negative production indicating a net \( O_2 \) consumption. The net \( O_2 \) production behaved according to a classical P–E relationship, and no photoinhibition was observed (Fig. 5B). The light-acclimation coefficient (\( E_k \)) for the net \( O_2 \) production was ~ 75 \( \mu \text{mol} \text{m}^{-2} \text{s}^{-1} \) at 20°C (Table 1).

In parallel to the increasing \( O_2 \) concentration, \( \Phi_{\text{PSII}} \) decreased with increasing irradiance, from ~ 0.6 in the dark to < 0.1 at the highest irradiance (Fig. 4B). \( \Phi_{\text{PSII}} \) occasionally dropped below detection limit at the highest irradiance, which can be observed as “black zones” just beneath the sediment–water interface in Fig. 4B. The patchiness of \( F_0 \) remained unchanged with irradiance, and no vertical migration of the microalgal biomass was
observed. To compare the microalgal gross photosynthetic response with the net O₂ production, rETR was calculated from five vertical profiles of WPSII representing intermediate to high photosynthetic activity, extracted from the images as was done for O₂, i.e., between positions 2 and 3 in Fig. 2C. The extracted profiles of $\Phi_{PSII}$ showed a mean value of 0.6 at low irradiances, which first decreased at irradiances $> 80 \mu$mol m$^{-2}$ s$^{-1}$.

Fig. 3. (A) Scalar irradiance (mean $E_{PAR,z}$ ± standard deviation [SD], n = 4) as a function of sediment depth and (B) the spectral light composition at different depths above and below the benthic diatom community, respectively, illustrating the light attenuation and the spectral behavior, with sediment depth. (C) The computed spectral light attenuation ($K_{d,PAR}$) in sediment with and without the presence of microalgae, respectively. Note that in (A) the absolute SD is higher in the top 0.5 mm of the sediment (ranging from 10% to 15%) than below 1 mm (< 5%), reflecting the patchiness in algal biomass; however, this is not obvious from the plot due to the log scale. The apparent increase in $K_{d,PAR}$ at depth > 0.3 mm is caused by the inherent spectral dependence of light attenuation.

Fig. 4. (A) Images of the steady-state O₂ distribution and (B) $\Phi_{PSII}$ at six incident irradiance intensities, from 0 to 350 $\mu$mol photons m$^{-2}$ s$^{-1}$, at 20°C. The sediment surface is indicated by a black line in (A) and a white line in (B). The O₂ concentration, as well as the O₂ penetration depth in the sediment, increased with increasing irradiance, whereas $\Phi_{PSII}$ decreased gradually with increasing irradiance.
Fig. 5. Vertical profiles of (A) O$_2$ and (C) $\Phi_{\text{PSII}}$ at different incident irradiances at 20°C. Integrated sediment (B) net O$_2$ production and (D) rETR, representing gross photosynthesis, are shown as a function of incident irradiance ($\mu$mol photons m$^{-2}$ s$^{-1}$). All data are mean ± SD. Profiles of the O$_2$ concentration are averaged over five sequential profiles between positions 2 and 3 in Fig. 2A, each representing a 10-pixel horizontal mean (500 μm). O$_2$ flux rates were computed from the mean profiles using the numerical model PROFILE and are volume specific (Berg et al. 1998). Profiles of $\Phi_{\text{PSII}}$ were averaged over five sequential profiles between positions 2 and 3 in Fig. 2C (five pixel horizontal average), and rETR were depth-integrated by correcting for the light attenuation and the vertical distribution of $\Phi_{\text{PSII}}$, as seen in example (C). The observed decrease in $\Phi_{\text{PSII}}$ with depth at the high irradiance (C) possibly can be ascribed to a decrease in the fraction of functional to total pool of light-harvesting pigments. Further investigations of this were beyond the scope of this work.

Table 1. Photosynthesis–irradiance (PE) coefficients for the sediment net O$_2$ production and the relative electron transport rate (rETR), fitted with the equation by Webb et al. (1974). Data are derived from images of O$_2$ and variable Chl $a$ fluorescence, respectively (data in Fig. 5). SE is standard error. For O$_2$ production, P$_{\text{max}}$ is in units of nmol O$_2$ cm$^{-2}$ s$^{-1}$, $\alpha$ in nmol O$_2$ cm$^{-2}$ s$^{-1}$ [$\mu$mol photons m$^{-2}$ s$^{-1}$]$^{-1}$, and E$_k$ in μmol photons m$^{-2}$ s$^{-1}$; whereas for rETR, P$_{\text{max}}$ and $\alpha$ are in relative units and E$_k$ in μmol photons m$^{-2}$ s$^{-1}$.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Net O$_2$ production</th>
<th>rETR</th>
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<tr>
<td></td>
<td>P$_{\text{max}}$</td>
<td>SE</td>
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<tr>
<td>10</td>
<td>0.053</td>
<td>0.002</td>
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<tr>
<td>15</td>
<td>0.099</td>
<td>0.004</td>
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<tr>
<td>20</td>
<td>0.121</td>
<td>0.005</td>
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<tr>
<td>25</td>
<td>0.064</td>
<td>0.003</td>
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* Probably overestimated due to the lack of data at the highest irradiance.
The deduced depth-integrated rETR correspondingly increased with increasing irradiance, showing a linear response at low irradiances (Fig. 5D). The fitted Ek values ranged from 150 \( \mu \text{mol photons m}^{-2} \text{s}^{-1} \) to 200 \( \mu \text{mol photons m}^{-2} \text{s}^{-1} \) (Table 1). Gross photosynthesis was never completely light saturated within the investigated irradiance range; thus, values of \( P_{\text{max}} \) and Ek have to be evaluated with care even though the computed SD is small (Table 1).

Effects of temperature on O\(_2\) production and gross photosynthesis—Increasing temperature decreases O\(_2\) solubility, increases the molecular diffusion rate, and affects the microbial process rates. The net result at \( E_{\text{PAR}} = 80 \mu \text{mol m}^{-2} \text{s}^{-1} \) was that the O\(_2\) concentration and the O\(_2\) penetration depth in the sediment both decreased with increasing temperature (Fig. 6A). The same trend applied to the entire irradiance range that was investigated. This was also reflected in the calculated net O\(_2\) production rates. In darkness and at low irradiance, net O\(_2\) production decreased gradually with increasing temperature (Fig. 7A). However, at high irradiance, the net O\(_2\) production increased toward an optimum at 20°C, after which it decreased. Temperature had a negligible effect on the net O\(_2\) production at 80 \( \mu \text{mol photons m}^{-2} \text{s}^{-1} \) up to 20°C, after which it decreased (Fig. 7A).
In contrast to the decreasing net O2 production, there was no significant change in gross photosynthesis, as expressed by the rETR, with increasing temperature at low irradiance ($\leq 80 \, \mu\text{mol m}^{-2}\text{s}^{-1}$). However, gross photosynthesis increased with temperature at irradiances above $80 \, \mu\text{mol m}^{-2}\text{s}^{-1}$ (Figs. 6B, 7B). No temperature optimum was observed for gross photosynthesis within the investigated range. These findings demonstrated a temperature independence of rETR under light-limited conditions (Fig. 7B), whereas gross photosynthesis was stimulated by temperature during light-saturated conditions (Table 1).

Discussion

The combined application of a planar O2 optode and variable fluorescence imaging provided novel insight into the turnover of O2 and the corresponding gross photosynthesis in phototrophic sediments. The approach facilitated direct investigations of the balance between the autotrophic and heterotrophic processes, as well as the biomass and patchiness of phototrophs on micrometer scales, in two dimensions.

Microalgal biomass and spatial distribution—The Chl a concentration in the experimental setup ($\sim 104 \, \text{mg Chl a m}^{-2}$) was on the high end of typical coastal benthic microalgal communities (Cahoon 1999) but was typical for semiprotected shallow-water systems, such as the Odense fjord system from which we sampled ($133 \pm 60 \, \text{mg Chl a m}^{-2}, n = 86$, across seasons, K. Hancke unpubl.)

Traditionally, microalga-dominated sediments are considered to be horizontally stratified structures characterized by a vertical zonation of successive primary producers, with a suite of underlying O2-consuming organisms and pathways (Canfield et al. 2005). In natural ecosystems, this zonation is often disturbed by faunal activity and bioirrigation, leaving shallow-water sediments horizontally and vertically heterogeneous. In the present study, we deliberately attempted to eliminate or reduce the spatial variation of microalgae by prefreezing to eliminate faunal activity. The microalgal abundance still, however, exhibited a pronounced horizontal heterogeneity, as observed in the fluorescence images (Fig. 2C,D). The spatial variation in microalgal biomass was supported by the O2 images, although these images only reflected a “smeared” distribution of production due to the diffusivity of O2 in sediment pore water (Fig. 2A,C). These observations underpin the extensive natural heterogeneity of benthic microalgal communities even without the presence of macrofauna (Fenchel and Glud 2000). We speculate that the small-scale variations reflect microniches governed by scattering and absorption of light in complex media that induce highly dynamic growth patterns, which again affect solute and nutrient transport. The observation also underlines the advantage of 2D-imaging approaches that allow small-scale variations during biogeochemical and photophysiological investigations to be overcome. Our speculations are supported by observations of large natural variability in sediment light propagation (Kühl and Jørgensen 1994) and the close coupling between phototrophic and heterotrophic process in phototrophic mats (Glud et al. 1999).

To our surprise, we did not observe any vertical migration of the microalgal biomass at the investigated timescales (Figs. 4, 6), even though bentic microphytes are known to vertically migrate (Serodio et al. 1997; Jesus et al. 2006). Motility of microphytes has been documented to exhibit complex responses to spectral light regimes and environmental factors (McLachlan et al. 2009); however, this study offers no apparent explanation for this phenomenon.

Autoheterotrophic coupling and balance—The present study confirms that increasing temperature gradually stimulates the heterotrophic activity more than gross photosynthesis in benthic microalgal communities (Hancke and Glud 2004). Moreover, here we observed that this community response is strongly light dependent. At low irradiance ($< 80 \, \mu\text{mol m}^{-2}\text{s}^{-1}$), this certainly occurred...
Compensation irradiance—Compensation irradiance is a key parameter when assessing the importance of benthic communities for system productivity and the contribution of mixed phototrophic communities to the ecosystem carbon turnover. Compensation irradiance is here defined as the irradiance at which photosynthetic gross O2 production balances community O2 consumption. The temperature response, Q10, was calculated according to Davis and McIntire [1983]. This compensation irradiance scales with the lower range of estimated global compensation irradiances in benthic communities, which range from 5.6 \mu mol photons m^{-2} s^{-1} to 102 \mu mol photons m^{-2} s^{-1} (recalculated from Gattuso et al. 2006). The temperature response, Q10,seems reasonable to extrapolate to natural communities because the experimental conditions mimicked typical in situ conditions. Extrapolating the data to 0 °C reveals that the compensation irradiance would be only ~ 3.3 \mu mol photons m^{-2} s^{-1}. This corresponds to findings for Arctic benthic microalgal communities at similar low temperatures of ~ 4.7 \mu mol photons m^{-2} s^{-1} (Glud et al. 2009), and also for some temperate regions at low temperature, although data are sparse (Gattuso et al. 2006). The strong temperature sensitivity of the compensation irradiance infers that net autotrophy is inhibited during low irradiance at temperatures that are typical for most temperate benthic microalgal ecosystems during summer conditions.

Comparison between net O2 production and rETR—Comparing the sediment net O2 production with the rETR, for each investigated temperature, showed an exponential relationship \( R^2 = 0.87 \). The consistent relationship showed a proportionally stronger increase of rETR relative to the net O2 production with increasing irradiance. The nonlinear relationship was induced by an increasing concurrent O2 consumption with increasing light availability. This relation was further enhanced at elevated temperatures (Fig. 9). The observations are consistent with the progressive increase in the net O2 consumption when both irradiance and temperature increased (see above and
Glud et al. 1999; Hancke and Glud 2004). Photosynthetic O$_2$ production and rETR (or $\Phi_{PSII}$) have been compared in a number of studies of benthic microalgae (Barrangouet and Kromkamp 2000), pelagic phytoplankton (Hancke et al. 2008a,b, ice algae (Glud et al. 2002), and macroalgae (Longstaff et al. 2002). However, most studies have been carried out on cultured algae or suspended material, applying different procedures and experimental conditions.

The majority of studies have found a linear relationship between O$_2$ evolution and $\Phi_{PSII}$ under moderate irradiance, sometimes with deviation from linearity at very low or high irradiance conditions. Apparent explanations for deviations include changes in O$_2$ consumption in the light, cyclic electron transport around PSII, or Mehler-type reactions (Flameling and Kromkamp 1998; Morris and Kromkamp 2003). The relationship between PSII electron transport and photosynthetic activity, and O$_2$ turnover in sediments kept in the laboratory. The techniques have previously been applied separately in the field (Glud et al. 2001; Hawes et al. 2012), and further optimization will enable the combined application for in situ studies using, e.g., inverted periscopes (Rhoads and Germano 1982; Fan et al. 2011).

**References**


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