**Photosynthetic activity and community shifts of
microphytobenthos covered by green macroalgae**

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SUMMARY

Macroalgae blooms, a frequent consequence of eutrophication in coastal areas, affect the photosynthetic activity of sediments dominated by microphytobenthos (MPB). Light spectra, steady-state (after 1h) microprofiles of O_2 , gross photosynthesis (P_g), community respiration in light (R_L) and net community photosynthesis (P_n) were measured in diatom- and cyanobacteria- dominated communities below increasing layers of *Ulva*. Photosynthetic photon flux (PPF) decreased exponentially with increasing layers of algae and the light spectrum was increasingly enriched in the green and deprived in blue and red regions. Sediment P_g , P_n and R_L decreased as the number of *Ulva* layers increased, however 1.6 times higher macroalgal density was necessary to fully inhibit cyanobacteria P_g compared to diatoms, indicating that cyanobacteria were better adapted to this light environment. Long-term (3 weeks) incubations of diatom-dominated sediments below increasing layers of *Ulva* resulted in a shift in the taxonomic composition of the MPB towards cyanobacteria. Hence, changes in the light climate below macroalgal accumulations can negatively affect the photosynthetic activity of sediments. However spectral niche differentiation of MPB taxonomic groups and concurrent in changes in the MPB community may provide sediments with increased resilience to the detrimental effects of eutrophication.

INTRODUCTION

The increase in magnitude and frequency of ephemeral macroalgal blooms is a widespread symptom of chronic eutrophication in coastal areas (Morand and Briand, 1996; Schramm, 1999). Nevertheless in shallow systems, even at relatively high nutrient loads enough light reaches the benthos to support primary producers, which continue to play a role in benthic-pelagic nutrient cycling (McGlathery et al., 2007). Still, as opportunistic macroalgal blooms accumulate, they increasingly become strong structuring components, often negatively affecting several other ecosystem compartments and functions (Sundbäck et al., 1990; Raffaelli et al., 1998).

Microphytobenthos (MPB), which include benthic diatoms, green algae, dinoflagellates, euglenoids, cyanobacteria and photosynthetic bacteria (Admiraal, 1984), are the most ubiquitous benthic primary producers, extending from the high intertidal to depths of up to 200 m (Cahoon, 1999; McGee et al., 2008). MPB can represent a substantial proportion of coastal primary production (MacIntyre et al., 1996), are important for coastal foodwebs (Evrard et al., 2010) and strongly control benthic-pelagic nutrient transport (Sundbäck and Miles, 2000; Dalsgaard, 2003) meaning they play an important role in the biogeochemistry of shallow coastal areas throughout the globe (Duarte and Cebrian, 1996). Hence, understanding how macroalgal blooms affect benthic microbial communities is important for defining the response of shallow coastal ecosystems to eutrophication (Sundback and McGlathery, 2005).

Generally, increasing macroalgal densities result in lower sediment light levels due to shading and a decrease or complete inhibition of MPB photosynthetic activity (Sundbäck et al., 1990; Corzo et al., 2009; Garcia-Robledo and Corzo, 2011). Sediments become increasingly anoxic, resulting in altered benthic-pelagic nutrient fluxes (Krause-Jensen et al., 1999) and negative effects on the faunal community (Raffaelli et al., 1998). On the other

hand, enhancements in MPB production related to photoacclimation and an increase in heterotrophic metabolism have also been reported (Trimmer et al., 2000; Garcia-Robledo and Corzo, 2011), suggesting that the mechanisms driving these changes are complex and not fully resolved. Previous studies have not distinguished between the effects of shading and other biogeochemical interactions between macroalgae and the sediment community, such as direct competition for, and exchange of, organic and inorganic nutrients (McGlathery et al., 2001; Garcia-Robledo and Corzo, 2011). Furthermore, MPB and macroalgae can also interact indirectly, via the accumulation and remineralisation of macroalgal organic matter in the sediment and the resulting release of nutrients (García-Robledo et al., 2008; Corzo et al., 2009).

In the present study, we aimed to exclude these interactions and focus only on the effect of changes in the magnitude and spectra of down-welling irradiance caused by different densities of green macroalgae. The changes in light climate are likely to favour MPB functional groups with a suitable light-niche, such as cyanobacteria (Jorgensen et al., 1987; Stomp et al., 2007). This will potentially produce a shift in community composition that will affect the photosynthetic activity of the sediment (Barranguet, 1997). Acting as a type of community photoacclimation, this mechanism may help to keep sediments net autotrophic during shading by macroalgae and, thus provide increased resilience against the detrimental effects of eutrophication. We conducted 2 experimental manipulations of sediment cores (short term; 1 h and long term; 3 weeks) so as to examine two specific hypotheses; 1) the light spectrum below increasing densities of green macroalgae favours the photosynthetic performance of cyanobacteria compared to diatom dominated MPB communities, 2) this change in light climate causes a shift from a diatom to a cyanobacteria-dominated community that results in relatively higher rates of benthic photosynthesis at comparable macroalgal densities.

RESULTS

Light attenuation by *Ulva*

Photosynthetic photon flux (PPF) decreased exponentially with increasing layers of *Ulva* (Fig. 2A). Fitting the light attenuation curves to the Beer-Lambert equation, resulted in an attenuation coefficient of $0.47 \pm 0.02 \text{ layer}^{-1}$ or $0.019 \pm 0.001 \text{ m}^2 \text{ g DW}^{-1}$. Spectral bands corresponding to Chlorophyll *a*, *b* and carotenoids were preferentially absorbed, with the blue (400 – 490 nm) and red regions of the spectra (620 – 690 nm) almost completely extinguished below 3-4 layers of *Ulva* (Fig. 2B). Light attenuation coefficients between 400 – 490 and 620 – 690 nm were > 3 times higher than at 750 nm (Fig. 2C). Between 490 and 620 nm attenuation was reduced, with a local minimum of $0.47 \pm 0.02 \text{ layer}^{-1}$ observed at 560 nm (Fig. 2C).

Short term response of microphytobenthos to shading by *Ulva*

The diatom- and the cyanobacteria-dominated communities were both autotrophic in the absence of *Ulva* (Fig. 3). However, maximum photosynthetic rates were 3 times higher in the cyanobacteria compared to the diatom-dominated community ($t = 2.71$, $df = 7$, $p < 0.05$), resulting in higher oxygen concentrations below the sediment surface (Fig. 3). The photic depth (z_{ph}) of the cyanobacteria-dominated community (850 μm) was also significantly deeper than the diatom-dominated community (500 μm) ($t = 3.52$, $df = 7$, $p < 0.05$) (Fig. 3, Table 1). Although sediment [Chl *a*] were not significantly different, areal gross production rate (P_g^A) was about 5 times higher in the cyanobacteria compared to the diatom-dominated community ($t = 3.49$, $df = 7$, $p < 0.05$, t-test) (Table 1). However, because of the high rates of

community respiration in the light (R_L) of the cyanobacteria-dominated community (Table 1), the difference in net community production rates (P_n) was smaller (about 3 times, $t = 3.10$, $df = 7$, $p < 0.05$, t -test).

Under short-term shading by increasing densities of *Ulva*, the typical subsurface O_2 maximum progressively disappeared and oxygen depletion became stronger with depth (Fig. 3A and B). For both communities, the successive addition of *Ulva* layers significantly reduced P_g (cyanobacteria-dominated: $F_{7, 207} = 7.63$, diatom-dominated: $F_{4, 324} = 41.45$, both $p < 0.01$, two-way ANOVA, factor *Ulva* layers) and z_{ph} (cyanobacteria-dominated: $F_{7, 15} = 4.32$, diatom-dominated: $F_{4, 34} = 10.77$, both $p < 0.05$, one-way ANOVA) (Fig. 3C and D). However, the number of *Ulva* layers needed to completely inhibit P_g in the two communities was different; 5 layers (120 ± 5 g DW m^{-2} , Fig. 3C and 4A) and 8 layers (195 ± 9 g DW m^{-2} , Fig. 3D and 4B), for the diatom-dominated and cyanobacteria-dominated communities, respectively.

Decreases in R_L of both communities were correlated with increasing densities of *Ulva* and thus decreases in P_g^A (Fig. 4E and F). Whilst, initial values of R_L were clearly higher for the cyanobacteria-dominated community (Table 1), rates of sediment community respiration in the dark (R_D) were not significantly different ($t = 0.51$, $p > 0.05$, t -test) (Table 1).

Net community production rates (P_n) significantly decreased with increasing number of *Ulva* layers (Fig. 4C and D, cyanobacteria-dominated: $F_{8, 17} = 6.80$, diatom-dominated: $F_{5, 25} = 11.14$, both $p < 0.01$, one-way ANOVA). For the diatom-dominated community, P_n became negative (-0.44 ± 0.08 mmol O_2 m^{-2} h^{-1}) with 3 *Ulva* layers (72 ± 3 g DW m^{-2}), whereas, for the cyanobacteria-dominated community 6 layers of *Ulva* (145 ± 6 g DW m^{-2}) were required to cause negative P_n (-0.50 ± 0.12 mmol O_2 m^{-2} h^{-1}).

Long-term response of microphytobenthos to shading by *Ulva*

The initial MPB community used in this experiment was an autotrophic, intertidal diatom-dominated community (both in terms of biomass and biovolume), similar to that described in the previous section. After 3 weeks, the sediment remained net autotrophic in both the control and 2 *Ulva* layers treatments, with clearly visible subsurface O₂ and P_g peaks at 200-300 µm depth (Fig. 5). However, O₂ profiles in cores with 4 and 6 *Ulva* layers showed no apparent oxygen production peak, although P_g was still measurable (Fig. 5B). Significant differences in P_g^A, R_L and P_n (Fig. 6A) were observed between the lower (0 and 2 layers) and higher (4 and 6 layers) shading treatments ($p < 0.05$, Tukey's HSD).

Shading by *Ulva* canopies significantly changed sediment pigment concentrations (Fig. 6 B). Both Chl *a* and Chl *c* were higher in cores shaded by 2 and 4 *Ulva* layers compared to the control treatment ($p < 0.05$, Tukey HSD test), whereas, no difference was found between the control and cores shaded with 6 *Ulva* layers (Fig. 6B).

The abundance of diatoms did not change between treatments ($F_{3,7} = 0.11$, $p > 0.05$, one-way ANOVA) (Fig. 6C). The most common genera were: *Gyrosigma*, *Navicula*, *Pleurosigma* and *Pseudonitzschia*. On the contrary, the abundance of cyanobacteria, mainly *Leptolyngbya*, was significantly higher in the 4 and 6 layer compared to the 0 and 2 layer treatments ($p < 0.05$, Tukey HSD test). The ratio of diatoms to cyanobacteria abundance (D:C ratio, Fig. 6C), was close to 1 (1.1 ± 0.1) for cores incubated with 0 layers of *Ulva*, whereas for treatments with 4 and 6 *Ulva* layers the ratio decreased to 0.3 ± 0.1 .

DISCUSSION

Our experimental design allowed us to clearly demonstrate how the taxonomic composition and photosynthetic activity of intertidal microphytobenthos communities changed in response to reductions in the quantity and quality of light below macroalgal blooms. Any mass transfer or competition for nutrients between the macroalgae and the benthic community, by direct contact or through the water phase, was deliberately avoided.

PPF at the sediment surface decreased exponentially with increasing numbers of *Ulva* layers (Fig. 2A) and extinction coefficients were similar to previous values found for planar *Ulva* species (Vergara et al., 1997). Nevertheless, attenuation coefficients of macroalgae are strongly controlled by photo-physiological processes such as chloroplast movement, changing pigment contents and thalli structure (Britz and Seliger, 1973; Britz and Briggs, 1987; Vergara et al., 1997). Hence, these attenuation coefficients probably vary daily, seasonally and between locations. Light reaching the sediment surface was progressively impoverished in the blue (430 nm) and red regions (650 and 675 nm) of the spectrum (Fig. 2C), corresponding to the in vivo absorption peaks of chlorophyll *a*, *b* and carotenoids typical of *Ulva*, and relatively enriched in green and near infrared (NIR) as shading by macroalgal biomass was augmented. Based on the measured attenuation coefficients and assuming a maximum intertidal sediment surface PPF ($2000 \mu\text{mol photons}^{-1} \text{ s}^{-1}$), an *Ulva* biomass of 450 g m^{-2} would be necessary to completely attenuate all light reaching the sediment surface, which is in the mid-range of the densities reported for natural algal blooms (Hernández et al., 1997; Pihl et al., 1999; Corzo et al., 2009).

The reductions in the magnitude and quality of light below increasing numbers of *Ulva* layers caused a decrease in the rates of gross photosynthesis of both MPB communities (García-Robledo et al., 2008; Corzo et al., 2009). However, 8 *Ulva* layers (about 200 g DW m^{-2}) were needed to completely inhibit photosynthetic activity in the cyanobacteria-dominated

sediment compared to only 5 layers (corresponding to about 120 g DW m⁻²), in the diatom-dominated sediment (Fig. 3, 4). These densities were roughly equivalent to a sediment surface PPF of 10 and 50 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$, respectively. Whilst these light levels are not particularly low considering the wide range of photo-acclimation observed in benthic diatoms (Sundback and Granéli, 1988; Mercado et al., 2004), the available light was mostly green; a region of the spectrum where diatoms have relatively low absorption cross-sections (Mercado et al., 2004; Morris et al., 2008).

Benthic cyanobacteria, due to their photosynthetic pigments (phycocyanin and phycoerythrin), are better adapted to the light environment below green macroalgal mats (Jorgensen et al., 1987). Comparison of photosynthetic action spectra between a diatom and *Microcoleus* cyanobacterial community showed that maximum photosynthesis rates were measured at 430 and 670 nm and at 580 and 650 nm, respectively (Jorgensen et al., 1987). Hence, cyanobacteria have a higher photosynthetic efficiency compared to diatoms below *Ulva* canopies, which results in higher rates of P_g^A at equivalent macroalgal densities and, presumably, a competitive advantage.

On the other hand, rates of community respiration in the light were much higher for the cyanobacteria-dominated sediment. R_L represents the fraction of P_g^A that is consumed in the light (Epping and Jørgensen, 1996), plus the basal respiration rate in dark (R_D). Hence, R_L was linearly dependent on P_g^A in both communities (diatom-dominated: $R_L = 0.51 + 0.36 P_g^A$, $r^2 = 0.81$, $p < 0.001$; cyanobacteria-dominated: $R_L = 0.54 + 0.69 P_g^A$, $r^2 = 0.89$, $p < 0.001$). The slope of these regressions represents the proportion of P_g^A that was consumed within each community: 36 % and 69 % for the diatom- and cyanobacteria-dominated communities respectively. Because oxygenic photosynthesis and aerobic respiration are not carried out in separate organelles, cyanobacteria tend to have higher rates of R_L compared to other algae

(Schmetterer, 2004). Still, considering the very tight coupling between heterotrophic bacteria and primary producers in sediments, differences in the stimulation of bacterial respiration (and thus R_L) via rapid transfer of C-rich exudates may also help explain this effect (Epping and Kühl, 2000).

The ratio of P_g^A to R_L ($P_g^A:R_L$) for the cyanobacterial-dominated sediment did not decrease as steeply as the diatom community with increasing *Ulva* shading, remaining autotrophic (> 1) up to 5 *Ulva* layers (Fig. 7). The critical PPF values for the switch from net autotrophic to heterotrophic in the diatom- and cyanobacteria dominated sediments were roughly 140 and 35 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$, respectively. Therefore, assuming an incident PPF of 2000 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$, cyanobacteria-dominated sediments would be able to remain net autotrophic under a larger macroalgal biomass (216 g DW m^{-2}) than diatom-dominated sediments (135 g DW m^{-2}). Although as mentioned above, differences in algal attenuation coefficients need to be considered, this biomass is in the low range of values (200 - 420 g DW m^{-2}) reported typically in many coastal areas affected by seasonal or permanent green macroalgal blooms (Hernández et al., 1997; Pihl et al., 1999; Corzo et al., 2009). Thus, even at low densities shading by macroalgal canopies can negatively affect the primary production of MPB and in consequence the redox status of surface sediments.

The temporal scales over which macroalgal blooms may affect the microphytobenthic community may vary considerably. At time scales of hours to days, MPB species may photo-acclimatize to the light conditions prevailing below the macroalgal canopy increasing the cell content of photosynthetic pigments and their effective absorption cross section (Blanchard and Cariou-Le Gall, 1994; Barranguet et al., 1998). At longer time scales, changes in the taxonomic composition of the MPB community are expected (Barranguet, 1997). Indeed, our results suggest that incubation of sediments below different densities of macroalgae for a

period of 3 weeks was long enough to induce both of these acclimation mechanisms. Cyanobacteria increased in abundance (30 to 160 %) when shaded by *Ulva*. This contributed to an increase in sediment Chl *a* in the intermediate shading treatments, but not at the highest density, suggesting adjustments in cell pigment contents. For diatoms this was confirmed by a doubling of Chl *c* in the 2 and 4 layers shading treatments with no changes in cell abundance. These photo-acclimation responses increased the light-capture capacity of the MPB community, resulting in no change in sediment P_g^A , P_n or R_L when shaded by 2 *Ulva* layers (sediment surface PPF reduced to about 100 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) for 3 weeks.

At higher *Ulva* densities (4 and 6 layers, corresponding to PPF of about 60 and 20 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) increases in diatom photosynthetic pigment contents were unable to compensate for the reduction in light availability; essentially because diatoms have no specialised photosynthetic pigments in the green region of the spectrum and pigment packaging eventually limits cell absorption capacity (Mercado et al., 2004). Hence, long-term shading resulted in decreases in sediment P_g^A , causing P_n to become negative. Nevertheless, $P_g^A:R_L$ ratios were significantly higher than in the diatom-dominated community and similar to the cyanobacteria-dominated community shaded by 6 *Ulva* layers in the short term experiment (Fig. 7). This compensatory effect could be mainly attributed to the change in taxonomic composition of the MPB community, particularly in the highest density shading treatment. Hence, the photosynthetic activity of cyanobacteria appeared to lessen the detrimental effects of shading by *Ulva* canopies on sediments.

In natural systems this general response will also be affected by feedbacks between macroalgae and MPB such as competition for inorganic nutrients, transfer of dissolved organic matter and stimulation of sediment heterotrophic metabolism by labile particulate organic matter. Indeed MPB are readily able to metabolise labile DOM and thus, may well

benefit from organic nutrients exuded by macroalgae. Furthermore, interactions with sediment fauna are also likely to be important; diatoms seem to be better adapted to intense grazing than cyanobacteria, then again, sub-oxic sediments negatively affect grazers.

In summary our results demonstrate that changes in the quantity and quality of light caused by shading of sediments by green macroalgae may induce a change in the taxonomic composition of natural MPB communities, favouring MPB functional groups with more suitable spectral niches, such as cyanobacteria (Stomp et al. 2007). This community photo-acclimation may provide sediments with increased resilience to the detrimental effects of eutrophication, supporting the hypothesis that the diversity of benthic primary producers is critical for determining the response of shallow bays and intertidal areas to environmental perturbations.

EXPERIMENTAL PROCEDURES

Sample collection and preparation

Sediment cores (i.d. 5.4 cm, length 20 cm) and planar *Ulva* sp. were collected in July 2009 from inner Cadiz Bay (SW Spain). Sediment was a silty-mud with no macroscopic macroalgal or seagrass material. Cores and macroalgae were maintained separately in flow through aquariums with in situ sea water. Cores were incubated at a photosynthetic photon flux (PPF) of 300 and 500 $\mu\text{mol photons m}^{-2} \text{ h}^{-1}$ (long term and short term experiment, respectively) with a 14 light : 10 dark photoperiod at about 20 °C until treated. Planar *Ulva* sp. was maintained with the same photoperiod and temperature at a PPF of 200 $\mu\text{mol photons m}^{-2} \text{ h}^{-1}$.

Short term experiment

Six cores were incubated as previously described until analysis. In situ seawater was amended with inorganic nutrients ($20 \mu\text{mol NO}_3^- \text{ L}^{-1}$ and $5 \mu\text{mol PO}_4^{3-} \text{ L}^{-1}$). Three cores were processed within 3 days after collection whereas the other three were incubated for 2 weeks to allow the development of a cyanobacteria-dominated community. Addition of nitrate and phosphate, but not silicate, and the absence of macrofaunal grazers favour the development of cyanobacteria-dominated communities (Fenchel et al., 1998; Garcia de Lomas et al., 2005). After 2 weeks, dense cyanobacterial patches composed mainly by *Microcoleus sp.* developed in two of the cores.

Cores were processed at a rate of one per day. The upper 2 cm of sediment was carefully transferred to a Plexiglas tube (i.d. 5.4 cm and 5 cm length) and the bottom of the tube was sealed using parafilm and aluminium foil. The tube was filled with sea water to a height of 2 cm above the sediment surface (Fig. 1) and continuously renewed using an open water flow system (approx. 10 min hydraulic retention time). A Petri dish with thick white paper on the bottom was placed on top of the core and filled with sea water (1 cm height) to help diffuse the downwelling irradiance. The system was illuminated from above with a halogen lamp (Novaflex, World Precision Instruments) at a PPF of $470 \pm 20 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$, measured at the sediment surface (spherical sensor, LiCor). The spectrum of light reaching the sediment surface were measured using a USB2000 spectrometer (Ocean Optics) connected to an optic fibre (300 μm diameter and a light acceptance angle of 180° , i.e. cosine, BFL22-200, Thorlabs) positioned close to the sediment surface through the bottom of the core, (Fig. 1). After setting up the core, the system was left for one hour to reach steady state before measurements of O_2 and gross photosynthesis (P_g) profiles were initiated. Once these were completed under the initial conditions (0 layers), the oxygen microsensor was

positioned below the oxic-anoxic interface. A single layer of a large, planar *Ulva* sp. frond was placed so as to fit perfectly within the Petri dish diffuser above the core. After one hour, the downwelling irradiance spectrum was recorded, followed by O₂ and P_g profiles. This procedure was repeated, adding successive disks of *Ulva*, until no P_g could be measured. The core was then immediately subsampled in the same position where the profiles were measured using a truncated syringe (i.d. 1.6 cm, depth 2cm) in order to measure Chlorophyll *a* and *c*.

Long-term experiment

Twelve sediment cores wrapped in black plastic on the sides were placed inside a temperature controlled aquarium at 20 °C and maintained under a 14 h light: 10 h dark photoperiod for one day before the start of the experiment. Light was provided by a halogen lamp (Philips 7748S, 250W), at a PPF of $320 \pm 15 \mu\text{mol photon m}^{-2} \text{s}^{-1}$ measured at the sediment surface (LiCor spherical light sensor). The lower PPF used in this experiment was chosen to avoid strong desiccation and bleaching of the macroalgal cover. A continuous open flow system (approx. 6 hours hydraulic retention time) was established in each core using in situ seawater which had low nutrient conditions (ca. 2, 0.5 and 6 $\mu\text{mol L}^{-1}$ for NH₄⁺, PO₄³⁻ and SiO₃²⁻, respectively) typical of Cadiz Bay in summer.

As for the short term experiment a glass Petri dish with 1 cm of seawater and white paper was placed on top of each core. Three cores were used as Controls (0 layers), whereas in the remaining 9 cores, 2, 4 and 6 *Ulva* layers were added to the Petri dishes to have 3 cores per macroalgal density. *Ulva* layers were replaced with fresh material every week to minimize the effects of *Ulva* deterioration and the consequent modification of light conditions below the canopy (Vergara et al., 1997). Based on the short term experiment, the densities of *Ulva* were chosen to roughly inhibit MPB photosynthetic rate by 0, 50, 100 and 150 %.

After 3 weeks, 2 vertical profiles of O_2 and P_g were measured in each core without disturbing the light climate of the treatment. The Petri dish was displaced laterally less than 1 cm just enough to introduce the oxygen microsensor with an angle of 30° . After completion of the profiles the upper 5 mm of the sediment was removed, homogenized and sub-sampled to measure Chlorophyll and microalgal cell abundance.

MPB Chlorophyll and cell densities

Chlorophylls *a* and *c* were extracted as in (Thompson et al., 1999) and estimated using the equations of Ritchie (2008). Sediment sub-samples were fixed with glutaraldehyde (2.5 % in 0.22 μ m filtered sea water) and later analyzed by optical microscopy. Individual diatoms and cyanobacterial colonies were counted by epifluorescence microscopy of a diluted subsample. Empty diatom frustules were not counted in order to avoid overestimation of the diatom community.

Oxygen micro-profiles and calculation of photosynthetic rates

In order to minimize the disturbance by making repeated measurements in the same position, during measurement of oxygen profiles the specially built sensors had a tip diameter of 10 μ m, a long and very slender shaft (4 mm) and a fast response time (< 0.3 seconds) (Unisense, Denmark). In addition, the microsensors were filled up with an electrolyte immobilized in agarose in order to avoid bubbles in the sensor tip which would modify the sensor response when used upside down. Oxygen microsensors were connected to a picoammeter (PA2000, Unisense) and the signal was recorded using an A/D converter.

Profiles were automated using a motorized micromanipulator connected to a computer controlled using the software Sensor TracePro (Unisense).

Gross photosynthesis (P_g) profiles, net community production (P_n) and Community respiration in light (R_L) were calculated according to (Revsbech and Jorgensen, 1983; Glud et al., 1992). Community respiration in darkness (R_D) was calculated from the O_2 gradient at the diffusive boundary layer in darkness, i.e., under a sufficient number of macroalgal layers where P_g became zero. The photic depth (z_{ph}) was estimated as the maximum depth with measurable P_g .

Statistical analysis

Significant effects of treatments were examined using 1-way and 2-way analysis of variance (ANOVA) followed by a Tukey's HSD (honest significant difference) test for multiple pairwise comparison. Non-linear curve fitting was used to estimate light attenuation coefficients of algal material. Analyses were considered significant at $p < 0.05$. Statistics were performed using the software Statgraphics Centurion XVI.

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TABLES

Table 1. Comparison of diatom-dominated and cyanobacteria-dominated communities illuminated at 500 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ without *Ulva* cover. Chlorophyll *a* concentration (Chl *a*) expressed as mg m^{-2} ; areal gross photosynthesis rate (P_g^A), areal net photosynthesis rate (P_n), areal dark respiration rate (R_D) and areal light respiration rate (R_L) expressed as $\text{mmol O}_2 \text{ m}^{-2} \text{ h}^{-1}$ and photic depth (z_{ph}) expressed in millimeters. Data are mean $\pm$ standard deviation of 4 and 2 cores for the diatom-dominated and the cyanobacteria-dominated community, respectively. Significant differences are marked by asterisks (*t*-test, $p < 0.05$).

	Diatom-dominated	Cyanobacteria-dominated
Chl <i>a</i>	25 ± 5	33 ± 4
P_g^A	3.26 ± 0.62	$15.65 \pm 6.41^*$
P_n	1.69 ± 0.34	$4.86 \pm 1.60^*$
R_D	0.52 ± 0.04	0.54 ± 0.11
R_L	1.90 ± 0.42	10.79 ± 8.01
P_g^A / R_L	1.83 ± 0.23	2.25 ± 1.08
P_n / R_D	3.01 ± 0.90	$8.76 \pm 1.23^*$
z_{ph}	0.49 ± 0.05	$0.85 \pm 0.05^*$

FIGURE LEGENDS

Figure 1. Set-up for short term experiment. A core with 2 cm of sediment (A) was covered with 2 cm of seawater (B) in an open water flow system. A Petri dish (C) under an increasing number of *Ulva* layers was positioned on top of the core. The oxygen microsensor (D) was moved from the bottom to the top and steady-state O₂ and Pg profiles were measured. An optic fibre (E) connected to a spectrometer was positioned close to the sediment surface.

Figure 2. Photosynthetic Photon Flux (A) measured at the sediment surface under an increasing number of *Ulva* layers in the short-term (black dots) and long term experiment (white dots). Light spectra reaching sediment surface under increasing *Ulva* layers (B) measured in the short-term experiment. Numbers in the panel B represent *Ulva* layers. Data are means $\pm$ SE ($n = 2 - 3$). Variation of light extinction coefficients (k) for the *Ulva* sp. canopy along light spectra (C), expressed as number of layers⁻¹ $\pm$ SE ($n = 5$).

Figure 3. Oxygen concentration and gross production rates (P_g) profiles under increasing number of *Ulva* layers in diatom-dominated (left column) and cyanobacteria-dominated sediment (right column). Profiles are means $\pm$ SE ($n = 2 - 3$).

Figure 4. Areal gross production (P_g^A), net production in light (B) and light respiration (R_L) under an increasing number of *Ulva* layers (expressed as biomass of *Ulva* in g DW m⁻²) for a diatom-dominated community (left column) and a cyanobacteria-dominated community (right column). Data are means $\pm$ SE ($n = 2 - 3$).

Figure 5. Mean O₂ (A) and gross production (Pg) microprofiles (B) in the sediment after 3 weeks under an increasing number of *Ulva* layers (numbers in legend). Profiles are means $\pm$ SE ($n = 3$).

Figure 6. Areal gross production (P_g^A), net production (P_n) and light respiration (R_L) (A); Chlorophylls *a* and *c* contents in the sediment (B); and microphytobenthic cell abundance (C) in the sediment under an increasing number of *Ulva* layers (also expressed as biomass density in brackets) after 3 weeks. Data are means $\pm$ SE ($n = 3$).

Figure 7. Relationship between P_g^A/R_L ratio for diatom- (diatoms) and cyanobacteria-dominated (cyanobacteria) communities and the number of *Ulva* layers in the short term experiment and after three weeks in the long term experiment (long term). Data are means $\pm$ SE ($n = 2 - 3$).

537 **FIGURES**

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546 **Fig. 1**

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