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Advective relief of CO_2 limitation in microphytobenthos in highly productive sandy sediments

Perran L. M. Cook[1] *and Hans Røy*
Max Planck Institute for Marine Microbiology, Celsiusstr. 1, Bremen 28359, Germany

Abstract

Following field observations of increased photosynthesis at increased rates of sediment flushing in sandy sediments, we conducted a series of laboratory experiments to elucidate the mechanism behind these observations. Column experiments in which water was pumped though sand at rates ranging from 0 to 613 L m^{-2} d^{-1} showed that carbon (C) fixation, as measured using carbon-14 (^{14}C) incorporation, increased from 6.4 to 8.6 mmol m^{-2} h^{-1} with increasing rates of flushing. Bottle incubations showed that the addition of inorganic nutrients [ammonium ion (NH_4^+), inorganic phosphate (HPO_4^-), silicic acid $Si(OH)_4$] did not stimulate C fixation over short-term incubations. Microprofiles of pH showed that the pH within the photic zone increased to 8.9, reducing free carbon dioxide (CO_2) concentrations to ~0.5 μmol L^{-1}. Further bottle incubations, where pH and total inorganic carbon (TCO_2) were manipulated, showed that high pH (9.6) did not affect photosynthesis if free CO_2 was present at concentrations of 10 μmol L^{-1}, suggesting a direct effect of low free CO_2 concentrations. ^{14}C fixation profiles at a resolution of 100 μm recorded by β-radiation imaging showed that while the depth specific maximum rates of C fixation were the same under both diffusive and advective (flushed) conditions, the integrated rates of photosynthesis were highest under flushed conditions because of a thickening of the photosynthetic zone. We conclude that advective pore-water transport can enhance benthic photosynthesis in shallow permeable sand sediments by counteracting CO_2 limitation.

Introduction

In nearshore shallow water environments, microphytobenthos may exhibit high areal rates of photosynthesis (often >8 mmol m^{-2} h^{-1}) and contribute significantly to total system primary production compared with phytoplankton (Heip et al. 1995; Macintyre et al. 1996; Underwood and Kromkamp 1999). Central to the process of photosynthesis is the acquisition of CO_2. Even though there are relatively high concentrations of TCO_2 in seawater (~2 mmol L^{-1}), only a small fraction of this is present as free CO_2 (~1%), which is the form of inorganic carbon directly assimilated by the enzyme ribulose-1,5-biphosphate carboxylase (RUBISO) into the Calvin Benson cycle (Cooper et al. 1969). In general, the concentrations of free CO_2 (10–20 μmol L^{-1}) are too low to allow diffusion of CO_2 into the cell at rates sufficient to match measured rates of photosynthesis. Hence many classes of microalgae may actively transport CO_2 and bicarbonate ion (HCO_3^-) across the cell membrane. Such processes are referred to as "carbon concentration mechanisms" (Raven 1997). In the ocean, CO_2 availability is generally not considered to be limiting to photosynthesis by pelagic algae (Raven 1997), although studies do exist that suggest that this might possibly occur at times (e.g., Riebesell et al. 1993).

While there has been recent discussion in the literature on inorganic carbon limitation in pelagic algae, there has been much less focus on inorganic carbon limitation in microphytobenthos (MPB). In comparison to pelagic algae, MPB have extremely high volume-specific rates of primary production, and their position at the sediment–water interface means that transport of solutes to and from the cells will be limited by diffusion across the benthic boundary layer (Jørgensen 2001). A combination of both these factors results in extreme conditions within the photosynthetic layer of MPB, including oxygen (O_2) partial pressures of up to 1 bar (e.g., Revsbech and Jørgensen 1986) and pH values in excess of 9 (Revsbech and Jørgensen 1986; de Jong et al. 1988) caused by CO_2 assimilation. Under such conditions, the concentrations of free CO_2 will be <0.5 μmol L^{-1}, at which point CO_2 assimilation may be significantly reduced even in microalgae with a high affinity for inorganic C (Raven and Johnston 1991). Admiraal et al. (1982) provided direct experimental evidence of inorganic carbon limitation in benthic diatom films cultured in the laboratory, and Larkum et al. (2003) concluded that dissolved inorganic carbon (DIC) was likely to be a major limiting factor for photosynthesis in epilithic algal communities on coral reefs. Thus there is strong evidence to suggest that inorganic

[1] To whom correspondence should be addressed. Present address: CSIRO Land and Water, 120 Meiers Rd, Indooroopilly, Queensland 4068, Australia (Perran.Cook@csiro.au).

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carbon limitation may occur in natural communities of MPB at times of high productivity.

In permeable sediments, water may be advected through the sediment as a consequence of small-scale pressure gradients created by water movement over sediment topography (Webb and Theodor 1968; Savant et al. 1987; Thibodeaux and Boyle 1987). This advective movement of solutes can result in transport rates orders of magnitude higher than diffusion (Precht and Huettel 2003) and hence dramatically enhance the transport of solutes such as O_2 into the sediment (Precht et al. 2004). As a consequence of this, it is plausible that in permeable sediments, the advection of TCO_2 into the zone of photosynthesis will allow higher rates of photosynthesis to occur in microphytobenthos inhabiting these environments. Experimental evidence for this comes from recent observations in chamber experiments, which showed that effluxes of O_2 and influxes of TCO_2 increased with stirring speed in illuminated sediments (F. Wenzhöfer unpubl. data).

In the next section, we present a set of experiments to reproduce field observations that increasing rates of pore-water advection lead to increased photosynthesis under laboratory conditions, and we elucidate the mechanism behind this phenomenon.

Methods

Sampling site—The sand used in all the following experiments was collected from a sandy sublittoral site located off the "Hausstrand" beach on the Island of Sylt, Northern Germany, in July and November 2004. The organic matter content of the sediment is low (0.1–0.2% w/w) with a carbon–nitrogen (C : N) ratio of 5–8. Chlorophyll *a* (Chl *a*) concentrations are extremely high with an annual range of 13–21 μg g sed^{-1} (dry weight), and it is estimated that microphytobenthos (MPB) account for ~50% of the organic carbon in the upper 5 cm of sediment (Hedtkamp 2005). Measurements of Chl *a* in the sediments after the experiments performed below were in the range of 17–27 μg g sed^{-1} and as such, the algal biomass used in these experiments was similar to that present in situ. Diatoms dominated the MPB community at the sample site throughout the year with the following species being commonly observed: *Odontella aurita, Cerataulus turgidus, Subsilicea fragilarioides, Dimeregramma minor, Plagiogramma staurophorum, Opephora pacyfica, Fragilaria schulzii, Amphora coffeaeformis, Amphora pediculus, Auliscus sculptus, Navicula gregaria, Planothidium delicatulum, Achnanthes lemmermannii, Navicula germanopolonica,* and *Nitzschia frustulum* (Agnieszka Tatarek and Jozef Wiktor pers. comm.). The in situ permeability of the sediment was between 3×10^{-11} and 8×10^{-11} m^2 in the top 5 cm of sediment (Hedtkamp 2005). The sand was either used immediately (nutrient addition experiments), or transported back to the laboratory, where it was stored at 4°C in the dark for up to 3 months before use. Several days before experiments were commenced, the sand was placed in a tray, covered with seawater, and illuminated with a light intensity of ~300 μmol quanta m^{-2} s^{-1} (PAR) at 20°C. Previous experiments have shown that photosynthesis in these sediments is saturated at 200–300 μmol quanta m^{-2} s^{-1} (F. Wenzhöfer unpubl. data). Unless otherwise specified, the seawater used in these experiments was collected from the North Sea and stored in tanks in the dark for >9 months. Nutrient concentrations at the start of preincubation were NH_4^+ 1 μmol L^{-1}, nitrate ion (NO_3^-) 13 μmol L^{-1}, HPO_4^- 2 μmol L^{-1}, $Si(OH)_4$ 8 μmol L^{-1}.

^{14}C tracer column experiments—Column experiments were conducted to investigate the effect of pore-water advection (hereafter referred to as sediment flushing) on photosynthesis rates of MPB inhabiting permeable sandy sediments. The experiments were set up in six 100 mm long × 36 mm internal diameter core liners; a layer of sand 2 cm high was supported on filter sponge resting on a perforated rubber stopper at the base of the core liner. Seawater was added to the core to a height of 4 cm and was recirculated through the sand at a rate of ~30 mL h^{-1} (equivalent of 710 L m^{-2} d^{-2}) via a peristaltic pump that drew water out of the column base and returned it to the overlying water column. The columns were illuminated at a light intensity of ~300 μmol quanta m^{-2} s^{-1} at room temperature and preincubated for 3 days on a 9 h : 15 h light–dark cycle. During the preincubation, the cores were each given two additions of 5 μmol NH_4^+ and $Si(OH)_4$ and 0.5 μmol HPO_4^-.

Effect of flushing rate—After the preincubation period, the seawater was drained out of the columns and replaced with seawater to which ^{14}C had been added (600 kbq L^{-1}). The tracer was thoroughly flushed through the columns and the flow rate of seawater through each column adjusted to 0, 47, 94, 188, 330, and 613 L m^{-2} d^{-1}. After being pumped through the column, the tracer was directed to waste; the water column height in the cores was maintained by pumping fresh replacement tracer into the columns at the same rate. In the three cores with the lowest flow-though rate (0, 47, 94 L m^{-2} d^{-1}), the water column was flushed at a rate of 8 mL h^{-1} to ensure that no significant depletion of TCO_2 occurred in the water column. The column treatment with no sediment flushing was stirred gently with a small magnetic stirrer bar to keep the water column mixed. The columns were then incubated in the light for ~3 h before being processed as follows.

The sand was removed from the column, homogenized by vortexing, and 3 subsamples of ~1 g weighed into 20-mL scintillation vials. All ^{14}C remaining in the sample was removed by acidification to pH < 2 with hydrochloric acid (HCl), and subsequent purging with nitrogen (N_2). Blanks (water samples to which only ^{14}C were added) were run to ensure the efficiency of this procedure. Fifteen milliliters of scintillation cocktail (Ultima Gold) was added to the sample, and the radioactivity of the samples was immediately counted in a Packard, Tri-Carb 2900TR or 2500TR liquid scintillation counter. Counts were corrected for self-quenching, which was determined by adding known amounts of radiation (^{14}C acetate) to sand from the same site. Photosynthesis rates were calculated using the ratio of radioactivity to TCO_2 concentrations measured in the tracer solutions.

Effect of bicarbonate addition—After the preincubation period, the flow rate in all the columns was adjusted to 165 L m^{-2} h^{-1}. Three of the columns were percolated with a seawater tracer (766 kbq L^{-1}, TCO_2 = 2.2 mmol L^{-1}); the remaining three columns were percolated with a seawater tracer (1,533 kbq L^{-1}) to which bicarbonate had been added to give a TCO_2 concentration of 8 mmol L^{-1}. As described previously, the tracer was thoroughly flushed through the columns before the incubations commenced. The columns were then incubated in the light for ~3.5 h, after which the cores were processed as described above for scintillation counting.

Two-dimensional β*-imaging of fixed* ^{14}C—After the preincubation period, the flow in three of the columns was adjusted to 330 L m^{-2} d^{-1}, and the remaining three columns had their flow stopped and the water column mixed by gently blowing air over the surface of the water. All the columns were then thoroughly percolated with tracer (860 kbq L^{-1}) as described previously and incubated in the light for 4.5 h. At the conclusion of the incubation, two cores from each treatment were processed for scintillation counting as described previously. The remaining core from each treatment was percolated with seawater, which had its pH lowered to 4.5, and a final concentration of 2% formalin was added to remove all inorganic ^{14}C and fix the MPB cells. The cores were then percolated with a methacrylate resin and allowed to set overnight. The solidified sand columns were sliced, polished, and the β emissions counted in two dimensions using a Biospace Measures Micro Imager. Vertical profiles of β counts through the photosynthetic zone, 400 μm wide, were analyzed from the images of two slices from each treatment (n = 11 per slice) at a vertical resolution of 100 μm.

Microdistribution of light, pH, and CO_2—Columns for microsensor measurements were set up and preincubated as described above for the ^{14}C column experiments. Light distribution was measured with an Ocean Optics USB2000 fiber-optic spectrometer through 100-μm tapered fibers with a 75-μm spherical diffuser on the tip (Kühl and Jørgensen 1992). Spectra were measured each 100 μm from 2 mm above the sediment–water interface to 5 mm below. Scalar PAR irradiance was calculated by integrating the spectra from 400 to 700 nm and calibrating against a PAR scalar-irradiance meter (LI-COR LI 250A / US-SQS/L).

Profiles of pH were measured at 100-μm resolution using LIX pH microsensors with a tip diameter of ~10 μm (de Beer et al. 1997). At the start of the light cycle where pH was measured, the preincubation flushing was replaced by gentle stirring by blowing air over the surface of the water. After 6 h in light and the absence of flushing, three profiles were measured through different spots on the sediment surface. Subsequently, flushing at 330 L m^{-2} d^{-1} was established and the cores allowed to equilibrate for 1 h, before three profiles were measured. Based on measured nutrient consumption rates in the bottle incubations, we expected all of the added nutrients to have been consumed when the microsensor measurements commenced.

The speciation of the carbonate system was calculated from pH alone, assuming alkalinity was conserved (Zeebe and Wolf-Gladrow 2001). In contrast to most other situations in sediments, this assumption is expected to hold: The cores were oxic throughout the region of interest, which ruled out anaerobic respiration, and the cores were flushed with oxic seawater for 3 days in advance of the incubations, which assured that no labile reduced metal or sulfur compounds were present. Because the uncatalyzed reaction between CO_2 and $H_2CO_3^-$ is slow, strict equilibrium cannot be assumed. Thus, the calculated CO_2 concentrations should be considered as maximum values.

Bottle incubations—Nutrient addition experiments: Freshly collected sand was sieved through 500-μm mesh and rinsed with seawater on 21 July 2004. Fifteen grams of sand was weighed into eight flat 50-mL glass culture vials. These bottles were divided into two treatments: (1) plus nutrients; which had filtered seawater amended with 100 μmol L^{-1} NH_4^+, 100 μmol L^{-1} $Si(OH)_4$, and 10 μmol L^{-1} HPO_4^-, and (2) minus nutrients, which simply had filtered seawater added, ambient nutrient concentrations at this time of year were NO_3^-, nitrous ion (NO_2^-), NH_4^+, Si <0.5 μmol L^{-1}; phosphorus (P) <0.1 μmol L^{-1}. The bottles were placed on a shaker table and shaken at a rate such that the sand was slightly fluidized. The bottles were illuminated at ~200 μmol quanta m^{-2} s^{-1} and preincubated for 2 days with ~8 h light being provided each day; the seawater was replaced at the start of each day for both treatments. After the preincubation period, the seawater for both treatments was again replaced; half of the bottles for each treatment were sealed, and TCO_2 consumption and nutrient assimilation were measured in a time series by sampling a bottle in each treatment approximately every 2 h as described below. The other half of the bottles had ^{14}C tracer added (114 Kbq) and were incubated in parallel with the other bottles, and ^{14}C uptake was measured by sampling a bottle every 2 h. For the ^{14}C incubations, the supernatant and sediment were frozen for later scintillation counting as described above. For the net TCO_2 assimilation incubations, TCO_2 samples were taken and fixed immediately with mercuric chloride ($HgCl_2$) (0.01% final concentration) for later analysis using a UIC inc. CM5130 acid module in line with a CM5012 coulometer. Nutrient samples were filtered through 0.2-μm filters and frozen for later analysis using a Skalar Continuous-Flow-Analyzer according to Grasshoff (1983).

Effect of pH and TCO_2 concentration on photosynthesis rates: Four 35-g L^{-1} sodium chloride (NaCl) solutions with different bicarbonate concentrations were prepared and pH adjusted using sodium hydroxide (NaOH), as follows:

1. pH = 9.3, TCO_2 = 1.0 mmol L^{-1}, CO_2 = 0.3 μmol L^{-1}
2. pH = 8.2, TCO_2 = 0.07 mmol L^{-1}, CO_2 = 0.5 μmol L^{-1}
3. pH = 8.2, TCO_2 = 1.5 mmol L^{-1}, CO_2 = 10 μmol L^{-1}
4. pH = 9.6, TCO_2 = 97 mmol L^{-1}, CO_2 = 11 μmol L^{-1}

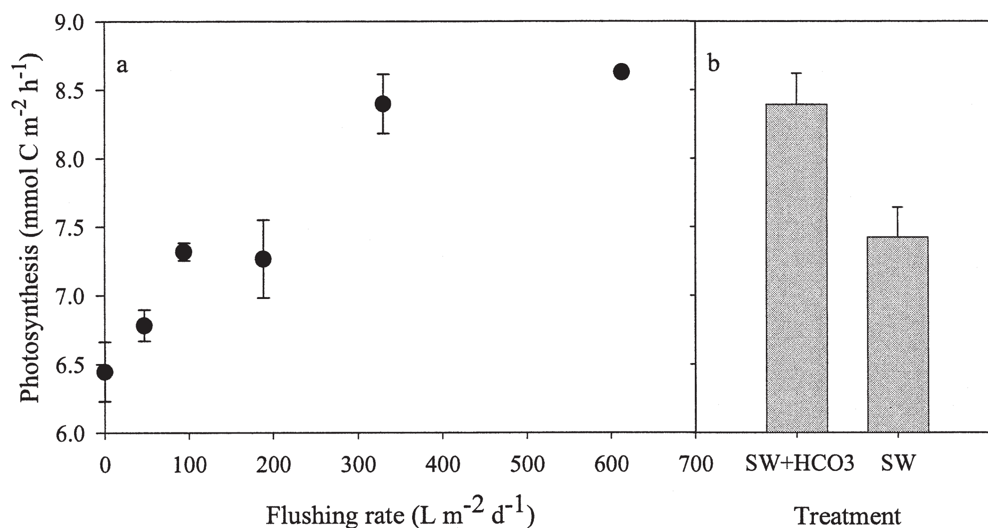

Fig. 1. (a) The photosynthesis rate of microphytobenthos versus column flushing rate and (b) the effect of bicarbonate addition (SW + HCO_3^- = seawater + 6 mmol L^{-1} HCO_3^-, SW = seawater, 2.2 mmol L^{-1} TCO_2) to the seawater tracer mixture in columns flushed at 165 L m^{-2} d^{-1}. The two treatments were significantly different (*t*-test, $p < 0.05$). For (a) the error bar represents the standard error of replicate samples taken from 1 column ($n = 3$), and for (b) the error bars represent the standard error of replicate column measurements ($n = 3$).

Fifteen grams of sand was weighed into 16 50-mL flat culture bottles and rinsed with 35-g L^{-1} NaCl solution. The bottles were divided into four treatments of four replicates and filled bubble free with the solutions detailed in the previous list. The bottles were placed on a shaker table as described previously and illuminated at ~300 μmol quanta m^{-2} s^{-1}. The concentration of O_2 in the bottles was then measured using a PreSens oxygen optode connected to a PreSens microx TX3 oxygen meter.

Results

Column experiments—Increased flushing rates (Fig. 1a) had a clear effect on photosynthesis rates, with rates increasing from 6.5 mmol m^{-2} h^{-1} (no sediment flushing), up to a rate of 8.5 mmol m^{-2} h^{-1} at a flushing rate of 600 L m^{-2} d^{-1}. The effect of flushing tapered off above rates of 300 L m^{-2} d^{-1}. The addition of HCO_3^- to the seawater tracer mix (Fig. 1b) resulted in a significant increase ($p < 0.05$, *t*-test) in the rate of photosynthesis from 7.4 up to 8.4 mmol m^{-2} h^{-1}. The rate of photosynthesis in the seawater treatment of the bicarbonate addition experiment (7.4 mmol m^{-2} h^{-1}, Fig. 1b) was similar to that observed at the similar flushing rate (188 L m^{-2} d^{-1}) in the flushing gradient experiment (7.3 mmol m^{-2} h^{-1}, Fig. 1a). The addition of HCO_3^- to the seawater increased photosynthesis up to a similar rate observed at the maximum flushing rate in the flushing gradient experiment (8.3 and 8.6 mmol m^{-2} h^{-1}, respectively). Thus, the addition of TCO_2 produced the same response in photosynthesis as did the increased rate of flushing.

Nutrient addition bottle experiments—The measured rates of C fixation based on TCO_2 assimilation were 0.87 ± 0.06 and 1.1 ± 0.1 μmol mL sed^{-1} h^{-1} in the plus nutrient and minus nutrient treatments, respectively, while the rates based on ^{14}C assimilation were 0.84 ± 0.04 and 0.83 ± 0.08 μmol mL sed^{-1} h^{-1} for the plus nutrient and minus nutrient treatments, respectively (Fig. 2). Concurrent measurements of nutrient concentrations in the bottles showed that the added nutrients (NH_4^+, Si, and P) were linearly assimilated over the light period (data not shown) and that no nutrients were present in the no nutrient treatment.

Effect of pH and TCO_2 on photosynthesis rates—The O_2 concentrations increased linearly in all treatments over the incubation period used (~ 3 h, raw data not shown). The O_2 production rates were clearly the lowest in the treatments with low initial free CO_2 (<0.5 μmol L^{-1}, first two bars of Fig. 3) compared with the treatments with high initial free CO_2 (~10 μmol L^{-1}, second two bars of Fig. 3). High pH apparently had no inhibitory effect on photosynthesis provided that the initial free CO_2 concentration was high (final bar of Fig. 3).

Microprofiles—In the absence of flushing, the pH increased rapidly with depth reaching a maximum pH of 8.9 at 1.5 mm below the sediment–water interface (Fig. 4), reflecting the assimilation of CO_2 by microphytobenthos (MPB). The result was a zone of minimum CO_2 concentration of ~0.6 μmol L^{-1} between 1 mm and 2 mm sediment depth (Fig. 5). Under flushed conditions, the pH remained relatively constant within the sediment. The bulge toward higher pH seen from −2 mm was mainly driven by a single deviating profile (Fig. 4). The light distribution is seen in Fig. 5.

Two-dimensional β imaging—Bulk photosynthesis rates as measured in the replicate flushed and diffusive cores

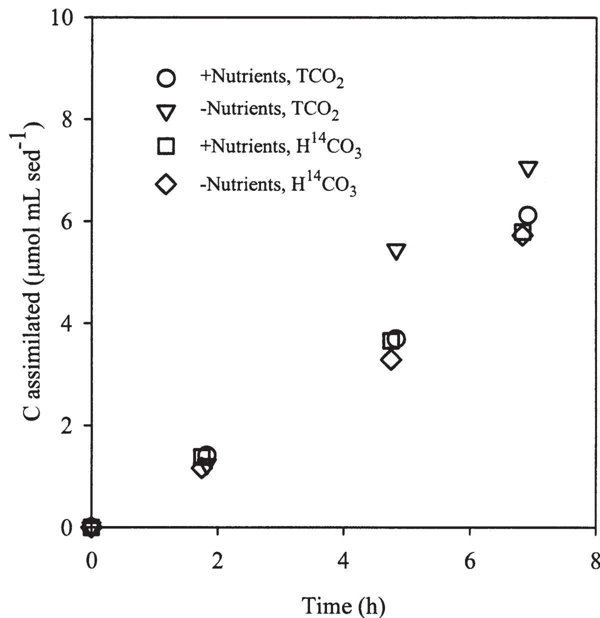

Fig. 2. Net assimilation of TCO_2 and ^{14}C incorporation versus time in bottle incubations of sand with (+Nutrients), and without (−Nutrients) nutrient additions.

averaged 8.0 and 6.8 mmol m^{-2} h^{-1}, respectively, in agreement with the previous experiments (Fig. 1). The depth integrated relative rates of C fixation based on profiles taken from the β images (see following) showed a similar difference in their magnitude, with the rates of C fixation being 22% higher in the flushed treatment,

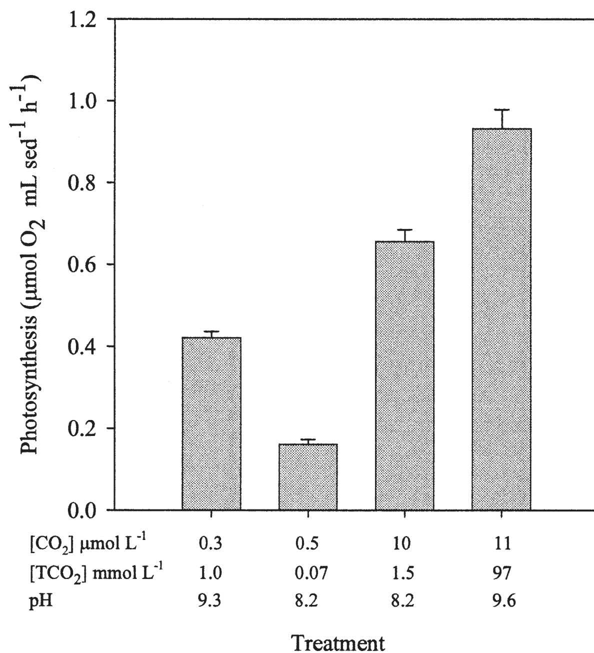

Fig. 3. Photosynthesis rates in bottle incubations treated with different initial pH and calculated free CO_2 concentrations. The error bars represent the standard error of the photosynthetic rate measured in replicate bottles (n = 4).

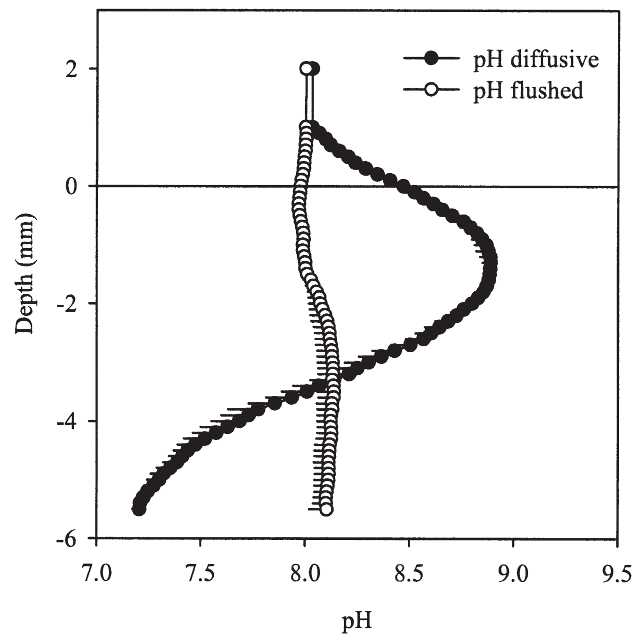

Fig. 4. Profiles of pH measured in columns that were flushed at 330 L m^{-2} d^{-1} (flushed) and stirred only (diffusive). Error bars represent the standard error of replicate profiles (n = 3) measured within one core.

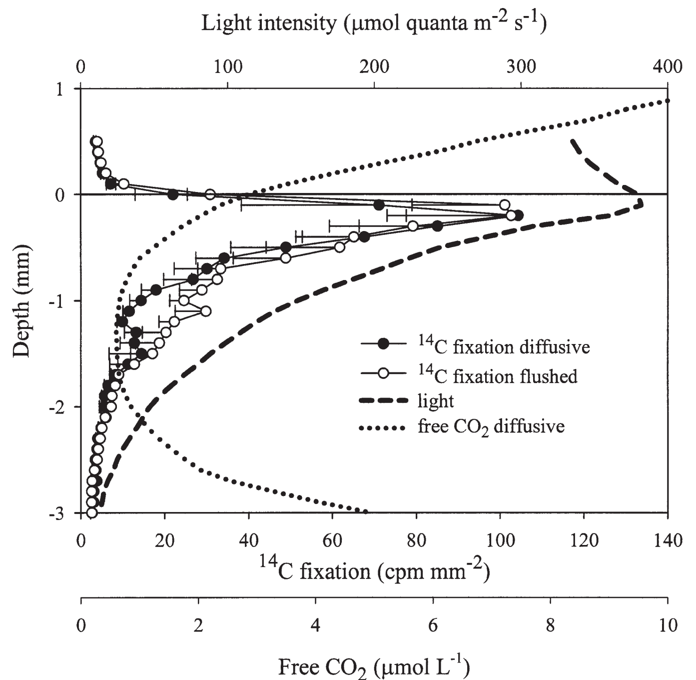

Fig. 5. Profiles of the relative rates of ^{14}C fixation measured in columns that were flushed at 330 L m^{-2} d^{-1} and stirred only (diffusive) relative to the light profile (measured in a separate core) and calculated free CO_2 in the diffusive treatment based on pH profiles shown in Fig. 4. Because the free CO_2 concentrations were calculated based on pH, assuming equilibrium of the system, the CO_2 concentrations shown here should be considered maximum estimates. Error bars represent the 90% confidence interval of 22 profiles taken randomly from 2 slices per treatment.

compared with an 18% increase for the flushed treatment where photosynthesis was measured in the bulk cores.

The two-dimensional β images gave a clear picture of both the distribution and rate of photosynthesis within the sediment, with the sediment surface being clearly defined (raw two-dimensional images not shown). Averaged profiles from two slices in each treatment normalized to the sediment surface show the distribution of ^{14}C fixation with depth (Fig. 5). It can be clearly seen that maximum relative rates of ^{14}C fixation were the same for both the flushed and diffusive treatments, and coincided with the maximum light intensity measured at the sediment surface. In the flushed treatment, rates of ^{14}C fixation then decreased rapidly to a depth of 0.8 mm at a light intensity of $\sim$100 μmol quanta m^{-2} s^{-1}, where the decrease in ^{14}C fixation rates slowed, before decreasing rapidly again below a depth of $\sim$1.5 mm, where the light intensity was less than 50 μmol quanta m^{-2} s^{-1}. In the diffusive treatment, rates of ^{14}C fixation decreased similarly to those in the flushed treatment to a depth of 0.8 mm, where the rates of ^{14}C fixation fell markedly below those in the flushed treatment, coinciding with the point at which the free CO_2 concentration calculated from pH profiles fell below $\sim$1 μmol L^{-1}. A minimum rate of C fixation in the diffusive treatment was reached at a depth of 1.2 mm in the sediment, coinciding with the center of the free CO_2 minimum. A secondary peak is seen at a depth of 1.5 mm where the light intensity was still $\sim$50 μmol quanta m^{-2} s^{-1}. Below this depth, the rate of C fixation rate tailed off to zero in the same manner as the flushed treatment.

Discussion

Methodological artifacts—Before discussing our results, we first address some potential artifacts inherent in our approach. Two potential problems with using the ^{14}C technique to measure photosynthesis in sediments are (1) ensuring the tracer is evenly distributed initially and (2) dilution of the ^{14}C tracer pool through the remineralization of unlabeled C in the sediment. In our system, we can confidently discount the first problem because the sand allows thorough flushing of the tracer through the sediment before the experiments are commenced.

The second problem is significant in our experiments because tracer dilution will only pose a problem in the diffusive treatment and hence the observed differences in rates between treatments could be an artifact arising solely from tracer dilution. The extent to which tracer dilution affected our diffusive treatment is dependent upon the rate of sediment respiration and the speed at which the freshly fixed ^{14}C enters the pool of C being respired. Dark respiration rates in bottle incubations were consistently $\sim$0.08 μmol mL^{-1} h^{-1} in both sieved and unsieved sediments (data not shown). We did not measure respiration in the light; however, increases due to illumination of respiration rates of up to twofold in benthic phototrophic communities have been reported, most likely as a consequence of photorespiration (Glud et al. 1992; Epping and Jørgensen 1996). While we cannot rule out effects of other increases in light respiration, photorespiration would not lead to a significant dilution of the $^{14}C:^{12}C$ ratio in the organic pool: Ribulose-1,5-biphosphate (the substrate for photorespiration) is an active part of the Calvin–Benson cycle (Falkowski and Raven 1997), and hence will rapidly approach the same $^{14}C:^{12}C$ ratio as the inorganic pool. This is supported by the close agreement between the rates of net TCO_2 assimilation and ^{14}C fixation (Fig. 2), which suggests that either respiration is only a very small fraction of C fixation or that any C respired is of a similar $^{14}C:^{12}C$ ratio to that fixed. That is to say, any increase in light respiration is fed by freshly fixed C of the same $^{14}C:^{12}C$ ratio as the inorganic C pool and is not derived from previously fixed C with an unenriched ^{14}C pool. In either case, an increased rate of the tracer pool dilution over that estimated for the dark respiration rates is highly unlikely.

Looking at the pH profiles (Fig. 4), one can observe a DIC drawdown to 6 mm. Based on the volumetric rates of DIC production, the integrated rate of production in the top 6 mm of sediment is 0.48 mmol m^{-2} h^{-1}, compared with a measured total DIC uptake by MPB of 6.5 mmol m^{-2} h^{-1} under diffusive conditions (Fig. 1). Therefore, we estimate that MPB derive 93% of their DIC uptake from the water column where tracer dilution will not occur at all; as such, the effect of any tracer dilution within the sediment will have a negligible effect on the measured rates of photosyntheis.

Effects of flushing on photosynthesis—The flow-through column experiments clearly showed that increased rates of flushing in the sandy sediments studied leads to higher rates of photosynthesis (Fig. 1a). The effect observed in the laboratory closely mirrored the effect seen in the field in benthic chambers, with rates in the field climbing from $\sim$5.5 up to $\sim$8 mmol m^{-2} h^{-1} across a flushing gradient (F. Wenzhöfer unpubl. data).

Given that sediment flushing can enhance primary production, the question then arises as to what the underlying cause of this phenomenon is. Here we directly considered three factors documented in the literature as having a negative effect on pelagic and benthic algal productivity, and which could conceivably be rapidly relieved by sediment flushing. These factors were (1) nutrient limitation (e.g., Beardall et al. 2001; Clavier et al. 2005), (2) inorganic C limitation (Admiraal et al. 1982), and (3) physiological effects of high pH (as distinct from inorganic C limitation).

Photosynthesis in pelagic algae may respond to nutrient additions within hours (Beardall et al. 2001), and within hours to days for benthic algal communities (Nilsson et al. 1991; Clavier et al. 2005). It has been shown that the porewater flow fields that develop in permeable sediments may provide a rapid supply of nutrients from deeper within the sediment (Huettel et al. 1998). As a consequence, the increased rates of photosynthesis observed may be a consequence of increased nutrient supply from within the sediment (in the field) or from the seawater tracer (column experiments). The nutrient addition bottle experiments performed here (Fig. 2), however, clearly demonstrated that nutrient additions did not stimulate photosynthesis in the short term.

The inhibiting effects of pH on photosynthesis have also been documented in the literature (Hansen 2002) and may be due to physiological effects on the cell other than inorganic carbon limitation. It is therefore conceivable that the increased rates of photosynthesis occur solely as a consequence of the reduced pH within the photosynthetic zone of the sediment under flushed conditions (Fig. 4). The bottle incubations performed with modified pH and free CO_2 concentrations (Fig. 3), however, show that photosynthesis by the benthic algae in these sands can proceed at unaffected rates even at pH values in excess of 9, provided that there is an availability of free CO_2.

Inorganic carbon limitation in diatom films cultured in the laboratory as well as coral reef epilithic algal communities has previously been suggested to occur (Admiraal et al. 1982; Larkum et al. 2003). The experiments conducted here strongly suggest that the MPB present in these sandy sediments may become CO_2 limited in the absence of sediment flushing. Several lines of evidence support this hypothesis. First, the addition of HCO_3^- to the column experiments resulted in the same stimulation in photosynthesis as did increasing the flushing rates of the sediment (Fig. 1b). The effect could not be demonstrated with nutrients. Second, the bottle experiments with pH and TCO_2 amendments (Fig. 3) clearly showed that the inhibitory effect could be induced by low free CO_2 concentration, but not by high pH alone. And third, the microsensor studies showed that the pH in the photosynthetic zone increased to 8.9, which would result in a free CO_2 concentration of ~0.5 μmol L^{-1}, which is less than or similar to the $K_{1/2}$ values documented for CO_2 in the diatom species *Phaeodactylum tricornatum* (Raven and Johnston 1991). Our bottle experiments also showed that such low free CO_2 concentrations limit photosynthesis irrespective of total inorganic C and pH (Fig. 3).

The community scale mechanism behind the enhanced rates of C fixation under flushed conditions becomes apparent when looking at the ^{14}C profiles (Fig. 5). In the absence of flushing, photosynthesis was inhibited between a depth of ~0.6–1.5 mm, coinciding with the minimum free CO_2 concentrations. By relieving this constraint, sediment flushing allowed photosynthesis to take place deeper within the sediment, where light intensities were still sufficient (~100–200 μmol quanta m^{-2} s^{-1}). On the cellular scale, the effect can be attributed to kinetic constraints, photorespiration at unfavorable $O_2 : CO_2$ ratios, and possibly to energetic constraints on a HCO_3^--based carbon concentration mechanism when the CO_2 concentration outside the cell is low. The exact cellular mechanism cannot be elucidated from our measurements and is therefore left for further studies.

The extent to which sediment flushing enhances photosynthesis in natural settings will be determined primarily by the photosynthetic potential of the sediments. At another, more oligotrophic, shallow sandy site in the Baltic Sea, where rates of photosynthesis were ~1–3 mmol C m^{-2} h^{-1}, a flushing gradient apparently had no effect on photosynthesis (F. Wenzhöfer unpubl. data). Similarly, Berninger and Huettel (1997) found no evidence of increased O_2 evolution by MPB with increasing flow in flume experiments where photosynthesis rates were 2.5 mmol m^{-2} h^{-1}. On highly productive hard substrates Larkum et al. (2003) observed an increase in photosynthesis rates from ~9 up to 16 mmol m^{-2} h^{-1} when flow over the substrates surface was increased from stagnant conditions up to 0.08 m s^{-1}, an observation which was also ascribed to relief of inorganic carbon limitation. The high rate of photosynthesis observed under stagnant conditions in the study of Larkum et al. (2003) was attributed to the direct assimilation of HCO_3^- by the microalgae present. The relatively low rate of photosynthesis observed under stagnant conditions in our study (5 mmol m^{-2} h^{-1}) is consistent with the community being dominated by diatoms (Bacillariophyceae), which use CO_2 as their immediate carbon source in contrast to the taxa Cyanobacteria and Rhodophyta present in the study of Larkum et al. (2003), which are capable of directly assimilating HCO_3^- (Raven 1997). We therefore suggest that the threshold and extent to which sediment flushing or flow processes enhance photosynthesis will depend both on the rates of photosynthesis and the algal taxa (carbon acquisition mechanism) present. Based on the results of this study, we suggest that MPB, which use CO_2 as their C source, may become C limited when rates of photosynthesis under diffusive conditions exceed ~5 mmol m^{-2} h^{-1}.

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