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Effect of nutrient availability on carbon and nitrogen incorporation and flows through benthic algae and bacteria in near-shore sandy sediment

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ABSTRACT: Carbon and nitrogen uptake in a microbial community comprising bacteria and microalgae in a sandy marine sediment under nutrient-limited and -replete conditions was studied using a mesocosm approach. After 2 wk of incubation, a pulse of $H^{13}CO_3^-$ and $^{15}NH_4^+$ was added to the mesocosms, and subsequent uptake of ^{13}C and ^{15}N by bacteria and microphytobenthos (MPB) was traced by analysis of ^{13}C and ^{15}N incorporation into hydrolysable amino acids, including the bacterial biomarker D-alanine. The results confirm that MPB communities are capable of sustained high rates of photosynthesis despite nutrient limitation. Under these conditions cellular growth stops (as defined by the synthesis of chlorophyll *a* and amino acids) and the carbon fixed under such conditions consists predominantly of carbohydrates produced through 'overflow metabolism'. In the treatment with nutrient addition, algal growth was stimulated and label incorporation was more balanced, with carbohydrates accounting for a much smaller fraction of newly fixed organic carbon. There was close agreement between net C fixation based on O_2 fluxes and the increase of particulate organic carbon in the sediment under both nutrient-limited and -replete conditions. This finding suggests that very little fixed C was lost to the water column as dissolved organic carbon (DOC), consistent with direct measurements of DOC release using ^{14}C. There was a significant and rapid transfer of ^{13}C from the MPB to bacterial biomass in both treatments within 24 h of label addition, revealing that fixed carbon excreted by MPB was rapidly utilised by bacteria. In both treatments, bacteria incorporated a significant fraction of ^{15}N from $^{15}NH_4^+$, with the greatest incorporation being observed under nutrient-limited conditions.

KEY WORDS: Amino acid · Isotope label · Nitrogen · Carbon · Algae · Bacteria · Sediment · Excretion

INTRODUCTION

Primary production by phytoplankton is generally considered to underpin the productivity of oceanic and near-shore food webs, with nitrogen-rich biomass providing a rich resource for higher trophic levels. In near-shore euphotic sediments, microphytobenthos (MPB) may have similar, if not higher, rates of total primary production compared to phytoplankton (Underwood & Kromkamp 1999), and may also be a significant source of carbon for higher trophic levels (Kang et al. 2003). In contrast to pelagic algae, however, MPB appear to direct a large fraction of primary production into synthesis of carbohydrates, accounting for as much as 70% of this production (Goto et al. 1999, de Brouwer & Stal 2001). A number of functions of this carbohydrate production have been suggested. It is thought that extracellular polymeric substances (EPS) are produced for a number of reasons, including locomotion, adhesion to sediment particles and sediment cohesion (Underwood

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& Paterson 2003). Carbohydrate production may also play a role in the maintenance of cell nutrient balances by excreting and/or storing carbon assimilated in excess of the ratio to nutrients required for cell growth. This is consistent with the observation that both EPS and intracellular carbohydrate (ICH) production increase under nutrient-limited conditions (in particular N) in culture studies of benthic and pelagic algal species (Staats et al. 1999, 2000b, Engel et al. 2002).

The fact that a large fraction of carbon fixed by MPB is directed into EPS synthesis (a labile, carbon-rich, nitrogen-poor substrate; Biddanda & Benner 1997, Wetz & Wheeler 2003) has a number of profound ecological consequences relevant to our understanding of the coupling of the C and N cycles in near-shore benthic ecosystems. Firstly, one would expect the assimilation of dissolved inorganic C and N to be well above the expected 'Redfield' C:N ratios of cellular algal material (~7). Indeed, a recent field study of a shallow water mesotrophic ecosystem dominated by MPB suggested a significant deviation from Redfield C:N ratios in benthic primary production (Cook et al. 2004). It should be noted, however, that such estimates of N assimilation by MPB in a field setting are highly uncertain, because MPB derive a large fraction of N from within the sediment — a flux which is difficult to accurately constrain. Constantly changing light regimes in natural settings further complicate efforts to scale up the relative rates of CO_2 and N assimilation, because they are often temporally decoupled. As such, controlled experimental approaches are necessary to further elucidate the relative assimilation rates of C and N within benthic microbial communities.

Substrate C:N ratio will also affect the amount of N released upon mineralisation, and above a critical threshold of C:N ≈ 20 there will no longer be a net release of N (Blackburn 1986). For example, it has been shown that marine bacteria growing on labile, high C:N ratio substrates will assimilate inorganic nitrogen to synthesise their low C:N ratio biomass (e.g. Goldman & Dennett 2000), and, thus, it seems likely that EPS with high C:N ratios derived from MPB will have the same effect on sediment bacterial communities. Once again, this is consistent with field data from mesotrophic estuaries, which show an extremely low release of N from the sediment relative to C, compared to that expected from 'Redfield' stoichiometry (Cook et al. 2004, Ferguson et al. 2004). Thus, the input of EPS into the sediment is likely to give rise to a situation of intense microbial competition for available nitrogen. It has been suggested that low rates of nitrification in sediments colonised by MPB may be due to competition for NH_4^+ between nitrifiers and heterotrophic bacteria, possibly stimulated by the extracellular carbohydrate production (Risgaard-Petersen 2003). One might expect an ecologically paradoxical situation under nutrient-limited conditions, in which MPB create an intense competition for nitrogen between themselves and bacteria, because of increased rates of EPS production. In the pelagic environment, it has been found that bacteria assimilate inorganic nutrients and even outcompete algae under low nutrient conditions (Bratbak & Thingstad 1985, Goldman & Dennett 2001), confirming that nutrient status may play an important role in determining the coupling between C and nutrient cycling and in the pathways of these elements through the microbial food web. To date, however, no studies have investigated whether this may be the case within benthic algal and bacterial communities.

The lack of studies investigating the relative flows of C and nutrients through benthic microbial communities is partly due to difficulties in distinguishing between bacterial and algal pools of carbon and nitrogen and the rates of transfer between them. Fatty acid biomarkers have proved to be a useful tool for tracing C through the algal and bacterial compartments of food webs (Boschker & Middelburg 2002); however, they give no information about N flows. The recent development of a new method for stable isotope analysis of hydrolysable amino acids, including the bacterial biomarker D-alanine (D-Ala) (Veuger et al. 2005), now makes it possible to trace both C and N through the algal and bacterial compartments of benthic food webs. A further advantage of this method is that EPS extraction procedures are not needed, which may extract intracellular carbon as well as excreted carbon (Chiovitti et al. 2004).

The objective of the present study was to assess the effect of nutrient limitation and excess nutrients on the composition of organic matter generated by the benthic primary production, and the fate of C and N within the algal and bacterial pools in sandy sediment. Based on previously published findings addressing pelagic microbial communities, we tested the hypothesis that carbon fixed by microphytobenthos is rapidly transferred to the benthic bacterial community, with N limitation leading to production of extracellular polymeric substances depleted in N and a shift in the relative rate of N uptake from algae towards bacteria. To this end, we conducted a dual stable-isotope pulse-chase experiment in a mesocosm and followed the pathways of ^{13}C and ^{15}N within the bacterial and algal pools in the incubated near-shore sandy sediment.

MATERIALS AND METHODS

Experimental setup. At the study site on the Island of Sylt, Germany, surface sand (upper 5 cm) was collected during July 2004 from mobile sublittoral sands,

characterised by high rates of benthic primary production (~5 to 7 mmol m^{-2} h^{-1} O_2). For a detailed description of the study site see Cook & Røy (2006) and Cook et al. (2007). For convenience, the sand was stored for 2 mo at 4°C in the dark prior to use. This was justified on the basis that (1) *in situ*, MPB survive for many months buried in the sediment over winter, and (2) we found that areal rates of photosynthesis in (unsieved) sediment preserved in this way rapidly (1 d) returned to rates measured in the field (Cook & Røy 2006). The sediment was sieved (500 µm mesh, to remove macrofauna and large particles), rinsed (to remove detritus and non-attached cells) and transferred to 2 aquaria (390 × 230 mm) to form a ~5 mm layer of sand overlaid by 11 cm (10 l) of seawater collected from the North Sea. Microscopic analysis of the sand showed that the MPB community was dominated by episammic diatoms *in situ*; a list of the species present is given in Cook & Røy (2006). The aquaria were placed in a climate-controlled room (15°C) and illuminated at a uniform irradiance of ~300 µE m^{-2} s^{-1} (saturating in these sediments; F. Wenzhöfer unpubl. data) on a 12:12 h light:dark cycle. These temperature and light conditions were chosen as they were considered to be most representative of the regime occurring *in situ* during times of low nutrient concentrations in the water column and, hence, of nutrient limitation (late spring to early autumn). Water was recirculated through the aquaria at a rate of ~1 l min^{-1}, thus ensuring that the water column was well mixed. To 1 aquarium NH_4^+, $Si(OH)_4$ and HPO_4^- were added daily in amounts equivalent to 0.8 mmol of N and Si, and 0.05 mmol of P (enough to support a daily production of ~60 mmol C m^{-2} d^{-1} assuming Redfield C:N:Si:P stoichiometry of 106:16:16:1); this treatment is referred to as the (+) treatment. The other aquarium did not receive additional nutrients; this was referred to as the (–) treatment. No algal growth was observed in the water of the (+) treatment; 14 d into the incubation and at the start of the dark phase, pulses of $^{15}NH_4^+$ (100 µM final conc.) and $H^{13}CO_3$ (1000 µM final conc.) were added to the aquaria. (This timing was based on the observation that photosynthesis in the 2 treatments had diverged and stabilised at this point.) After a complete light:dark cycle (24 h) following the additions, the water in each aquarium was replaced with unlabeled fresh seawater containing 0.08 mmol Si, 0.14 mmol NO_3^- and 0.02 mmol P.

Primary production and respiration were measured by placing a lid over the water surface of the respective aquarium and measuring the O_2 concentration change over time. The O_2 fluxes were corrected for the O_2 leak rate for each aquarium, which was determined in aquaria filled only with water after O_2 had been depleted in the water by purging with N_2 gas. After primary production and respiration had been measured, a sediment sample equivalent to ~5% of the sediment surface area of the aquarium was taken in 1 spot from the top ~3 mm of sediment (using a spatula). The sample was homogenised and subsampled for chlorophyll *a* (chl *a*), bacterial production, extracellular carbon production rates, sediment carbohydrates, sediment hydrolysable amino acid (HAA) concentrations and label incorporation, total label incorporation and content analyses of the percent C and N in the sediment. Bacterial production and extracellular carbon production rates were measured immediately. Sediment samples for all other parameters were frozen at –20°C and then freeze dried before analysis within 6 mo.

Only 1 tank for each treatment was used, and, hence, there was no true replication in this experimental design. We justify this on the basis that nutrient addition to a phototrophic community will clearly elicit a growth response that is well known and was indeed observed. Accepting that the 2 tanks are different, our aim was to make measurements of a range of parameters which are less well understood in relation to algal growth and nutritional status, including carbohydrates, carbon excretion, bacterial production and the relative assimilation of C and N by algae and bacteria. These parameters were replicated over time in the aquaria, so we were able to test for significant differences between the tanks using a Wilcoxon matched pairs test. In doing this we assume that the difference in algal growth and nutrition between the 2 aquaria was the only variable giving rise to these differences. Other potentially important variables such as the light field, water circulation and temperature were carefully controlled to ensure that they were the same in both aquaria.

DOC production. In order to measure the proportion of fixed carbon lost from the sediment as dissolved organic carbon (DOC), duplicate ~0.5 g subsamples of sediment were placed in 15 ml clear polypropylene centrifuge vials with 3 ml of filtered seawater and $H^{14}CO_3^-$ tracer (~2 kBq). The vials were incubated under the same light field as the aquaria for 2 to 3 h, after which the samples were stored overnight in the dark at 4°C. The following day, the vials were centrifuged at 1000 × *g* for 1 min and a 2 ml sample of supernatant was acidified with 100 µl of 6 M HCl and purged with N_2 to remove all CO_2 before scintillation counting. For the determination of ^{14}C fixed into the sediment, the sediment was placed in 20 ml scintillation vials. All $^{14}CO_2$ remaining in the sample was removed as described above. Blanks (water samples to which only ^{14}C were added) were run to ensure the efficiency of this procedure. Scintillation cocktail (Ultima Gold) was added to the super-

natant and sediment samples, and the radioactivity was measured in a Packard Tri-Carb 2900TR or 2500TR liquid scintillation counter. Counts for the sediment samples 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 (CO_2 + H_2CO_3 + HCO_3^- + CO_3^{2-}) concentrations measured in the tracer solutions. The fraction of DOC released from the sediment was then calculated in relation to total ^{14}C fixed into the sediment.

Bacterial production. Bacterial production was estimated by measuring ^{14}C leucine incorporation using L-[U-^{14}C]leucine (specific activity 303 mCi mmol^{-1}, Amersham). Triplicate samples of approximately 1 ml sediment were taken from each aquarium 3 to 4 h into the light phase of the daily cycle and mixed with 1 ml of sterile, filtered seawater. Then, 100 µl of aqueous leucine solution (containing 0.5 nmol ^{14}C-leucine and 399.5 nmol leucine) was added to each of the slurries, and they were incubated for 1 to 2 h at 15°C in the dark. The incubations were terminated upon the addition of 100 µl of 37% formalin, and the samples were stored at 4°C until analysis. An additional blank sample from each aquarium was supplied with 100 µl of 37% formalin prior to the addition of leucine.

Following the methodology of Findley et al. (1984) and Michel & Bloem (1993), samples were washed 3 times with 2 ml of sterile, filtered, 5% formalin seawater by mixing, centrifuging and decanting the supernatant. The sediment was resuspended in 2.5 ml of 0.5 M NaOH, and the samples were heated for 2 h in a water bath at 60°C. After centrifugation, the supernatant was decanted into ice-cooled tubes and the sediment was washed with 2.5 ml of 0.5 M NaOH. The supernatants were pooled, supplied with 480 µl of 3 M HCl, 150 µl BSA (50 mg ml^{-1}) and 800 µl of 50% TCA (trichloroacetic acid) and heated for 30 min in a water bath at 80°C. After cooling on ice, the samples were filtered through cellulose-nitrate filters (0.4 µm mesh size) and the filters were washed 5 times with 5% ice-cold TCA. The filters were transferred to scintillation vials, and 12 ml of Ultima Gold scintillation cocktail was added. After 12 h, the radioactivity was counted on a 2500TR liquid scintillation counter. Benthic bacterial production rates were estimated according to Kirchman (1993). This approach assumes that all leucine incorporation is by bacteria, which may not necessarily be the case (Veuger & Middelburg 2007). Uptake of leucine by algae would mean that the bacterial production rates, and hence bacterial production: primary production ratios, measured here were higher than the true values.

Hydrolysable amino acids. Samples were processed and analysed according to the protocol presented in Veuger et al. (2005). Briefly, samples (~1 g) of freeze-dried sediment were washed with HCl (2 M) and Milli-Q water (removing dissolvable HAAs), followed by hydrolysis of the sediment pellet in HCl (6 M) at 110°C for 20 h. After purification by cation exchange chromatography, amino acids were derivatized with isopropanol and pentafluoropropionic anhydride and samples were further purified by solvent extraction. Stable isotope ratios for carbon (R = ^{13}C:^{12}C) and nitrogen (R = ^{15}N:^{14}N) in the derivatized D- and L-amino acids were measured by gas chromatography combustion isotope ratio mass spectrometry (GC-c-IRMS) and were used to calculate δ-values: $\delta X(‰) = [(R_{sample}/R_{standard}) - 1] \times 1000$, where X = ^{13}C or ^{15}N. Standards were Vienna Pee Dee Belemnite and atmospheric nitrogen for ^{13}C and ^{15}N analyses, respectively. δX was used to calculate the atomic percent of X: at $\%X = [100 \times R_{standard} \times (\delta X_{sample}/1000) + 1]/[1 + R_{standard} \times (\delta X_{sample}/1000) + 1)]$, which was used to calculate excess X (absolute amount of incorporated ^{13}C or ^{15}N): excess X = [(at δX_{sample} − at.$\%X_{control}$)/100] × AA concentration$_{sample}$ (with amino acid [AA] concentrations expressed in mol C or N). Excess ^{13}C was calculated directly from δ^{13}C values for derivatized amino acids (using AA-C concentrations for derivatized amino acids), which bypasses correction of δ^{13}C values for added C during derivatization.

Bulk sediment and isotopic analysis. The carbon and nitrogen isotopic composition of freeze-dried sediment samples were analysed (single sample) using a Fisons CN elemental analyser coupled on-line via a Conflo 2 interface with a Finnigan Delta S mass spectrometer. Samples for C analysis were first acidified to remove carbonates. Bulk particulate organic carbon and nitrogen were analysed after acidification using a Fisons NA1500 elemental analyser.

Carbohydrates and chlorophyll *a*. Total sediment carbohydrates and EDTA-extractable (hereafter referred to as colloidal) carbohydrates were measured in triplicate on freeze-dried samples as described by Underwood et al. (1995). For total carbohydrate analysis, 2 ml of Milli-Q water was added to ~50 mg of sediment, followed by 1 ml of 5% phenol and then 5 ml concentrated sulphuric acid. The absorbance of the supernatant was measured at 485 nm and quantified against a glucose standard. Colloidal carbohydrates were extracted from 100 mg sediment with 3 ml of 100 mM EDTA for 15 min. After extraction the samples were centrifuged at 3000 × *g* for 15 min, then 2 ml of the supernatant was analysed for carbohydrates as described above. Chl *a* and pheo-pigments were measured (single sample) by colorimetry as described by Lorenzen (1967) using 0.5 ml of wet sediment.

Respiration, primary production and nutrient uptake. O_2 concentrations during the flux measurements were measured using Winkler titration (as described by Grasshoff 1983); the leak rate of O_2 into the aquaria was measured using a PreSens oxygen optode connected to a PreSens microx TX3 oxygen meter. Nutrient samples were filtered through 0.2 µm filters and frozen for later analysis using a Skalar Continuous-Flow-Analyser and the chemistry described by Grasshoff (1983). C assimilation rates were calculated assuming a 1:1 O_2:CO_2 stoichiometry, consistent with 1:1 stoichiometry measured at the site from which the sediment was derived (P. L. M. Cook unpubl. data). Net daily C fixation was calculated by subtracting dark respiration (12 h) from the net C fixation rate in the light (12 h). Daily gross C fixation rates were calculated by adding the dark respiration rate to the measured net light C fixation rate. Cumulative C fixation was calculated by adding net daily rates of C fixation. For days on which C fixation and respiration were not measured, rates were estimated as the average of the rates of the preceeding day and following day. Rates of N assimilation were calculated from daily measurements of NH_4^+ concentrations before and after NH_4^+ additions. We estimate the loss of N through nitrification and subsequent denitrification to be negligible, based on the fact that benthic algae inhibit nitrification (Risgaard-Petersen 2003); this is supported by our observation that no NO_3^- was detected in any of the samples analysed for nutrients. Furthermore, *in situ* rates of nitrification and denitrification at this study site are low (F. Wenzhoefer unpubl. data).

RESULTS

Chlorophyll *a*

The chl *a* content of the sediment in the (–) treatment remained within a relatively narrow range of 47 to 60 mg m^{-2} over the course of the experiment (Fig. 1a). For the (+) treatment, the chl *a* content of the sediment increased steadily from the start of the experiment to Day 16, when chl *a* content reached 270 mg m^{-2}. Pheophytin, a chlorophyll degradation product, was usually undetectable or it was only a small fraction of chl *a* (data not shown).

Total organic carbon

The organic carbon content of the sediment increased from an initial concentration of 600 mmol m^{-2} to 1000 and 1240 mmol m^{-2} at the conclusion of the experiment in the (–) and (+) treatments, respectively (Fig. 1b). In the (+) treatment, very high sediment carbon concentrations in excess of 1600 mmol m^{-2} were measured on Days 15 and 16, coinciding with the high chl *a* values observed at this time in a particularly dense patch of diatoms sampled on these dates. The average increases in sediment carbon content over the 21 d incubation calculated using linear regression were 470 ± 70 and 900 ± 200 mmol m^{-2} for the (–) and (+) treatments, respectively. These increases balanced closely with the calculated cumulative C fixation based on the O_2 flux measurements, assuming a production quotient of 1:1, which were 410 ± 30 and 1000 ± 70 mmol m^{-2} for the (–) and (+) treatments, respectively. The C:chl *a* ratio of the sediment also showed clear differences between the 2 treatments, with the ratio increasing up to ~300, just before the pulse in the (–) treatment, compared to a decrease to ~60 in the (+) treatment (Fig. 1c). The N:chl *a* ratio was always highest in the (–) treatment, generally varying between 20 and 30, while in the (+) treatment the N:chl *a* ratio generally remained between 10 and 20 (Fig. 1d). The C:N ratio of the (–) treatment increased markedly from a minimum value of 4 to 5 at the start of the experiment to up to 10 on Day 14, just before the pulse of $^{15}NH_4^+$ was added, after which the sediment C:N ratio dropped down to ~6. The sediment C:N ratio for the (+) treatment remained within the range of 4.5 to 6.5 (Fig. 1e).

Carbohydrates

Total carbohydrates in the sand increased steadily over the first 2 wk, reaching ~400 and 380 mmol C m^{-2} in the (–) and (+) treatments, respectively, just before the pulse of $^{15}NH_4^+$, after which the concentrations decreased, before increasing again on Day 21 (Fig. 2a); there was no significant difference between the 2 treatments ($p > 0.05$ Wilcoxon matched pairs test). Colloidal carbohydrates increased rapidly in the (–) treatment, reaching 120 to 140 mmol C m^{-2} between Days 7 and 14, after which the concentration dropped abruptly to ~70 mmol C m^{-2}, coinciding with the addition of the NH_4^+ pulse (Fig. 2b). In the (+) treatment, the colloidal carbohydrate concentrations increased more gradually and erratically to a maximum concentration of 120 mmol C m^{-2} on Day 11, before dropping rapidly to 90 mmol C m^{-2}, coincident with the addition of the ^{15}N pulse. There was no significant difference in the colloidal carbohydrate concentrations between the 2 treatments ($p > 0.05$, Wilcoxon matched pairs test). When the carbohydrate fractions are normalised to chl *a*, the differences between the 2 treatments are clear, with the carbohydrate to chl *a* ratio rapidly climbing in the (–) treatment, before dropping back

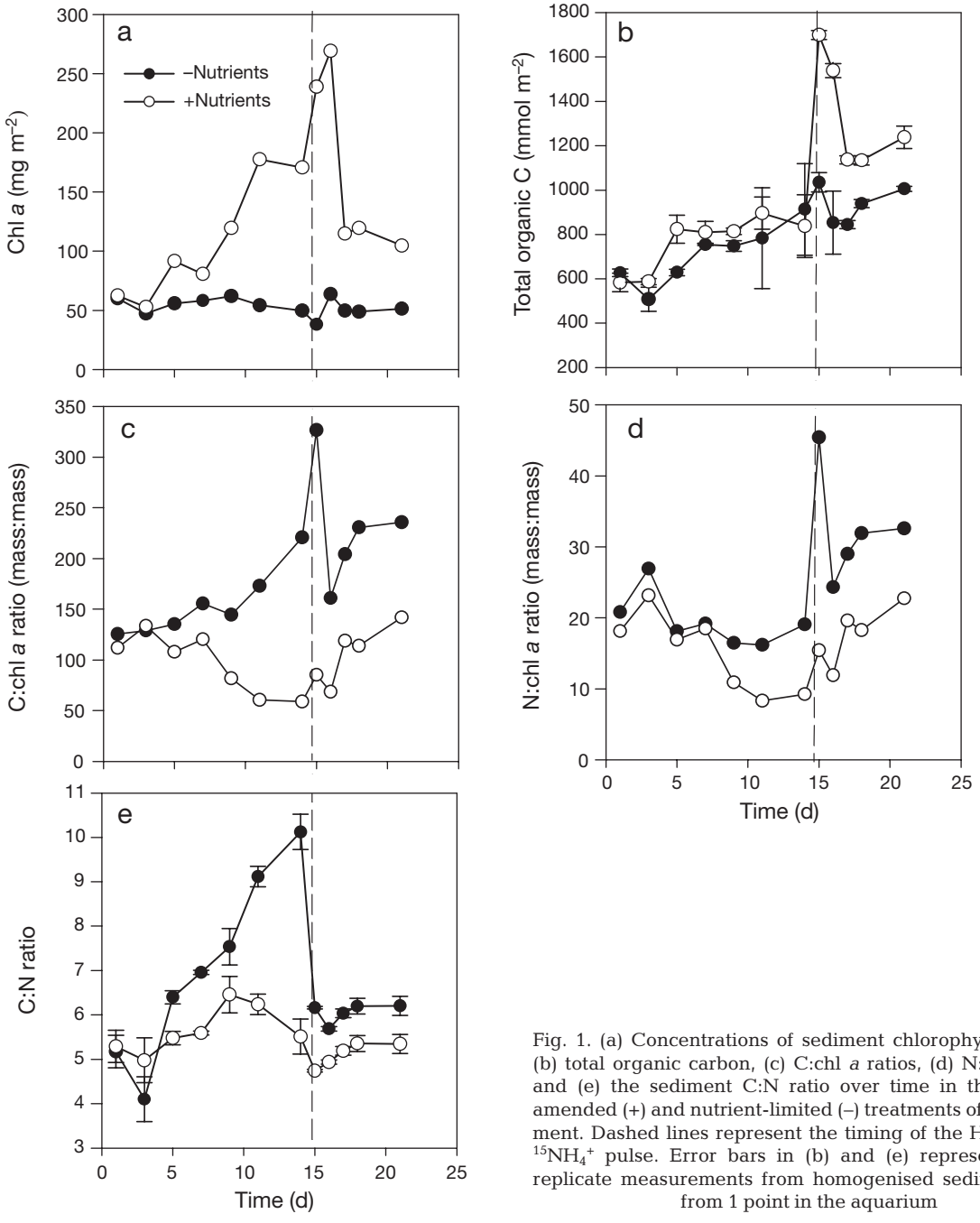

Fig. 1. (a) Concentrations of sediment chlorophyll *a* (chl *a*), (b) total organic carbon, (c) C:chl *a* ratios, (d) N:chl *a* ratios and (e) the sediment C:N ratio over time in the nutrient-amended (+) and nutrient-limited (–) treatments of the experiment. Dashed lines represent the timing of the $H^{13}CO_3^-$ and $^{15}NH_4^+$ pulse. Error bars in (b) and (e) represent ±SD of replicate measurements from homogenised sediment taken from 1 point in the aquarium

again after the $^{15}NH_4^+$ and nutrient pulse (Fig. 2c,d). The proportion of newly fixed carbon present as carbohydrates (the concentration of total C or carbohydrates at each time point minus the initial total C or carbohydrate concentration) was often 100% between the start of the experiment and Day 10 in the (–) treatment, before dropping to between 30 and 70% towards the end of the experiment (Fig. 2e). In the (+) treatment, carbohydrates generally made up between 20 and 50% of the newly fixed carbon.

Metabolism and nutrient assimilation

In the (–) treatment, net daily C fixation decreased from an initial rate of 28 to 9 mmol m^{-2} d^{-1}, just before the pulse of $^{15}NH_4^+$, after which C fixation increased to 31 mmol m^{-2} d^{-1} within 2 d (Fig. 3a). In the (+) treatment, net C fixation increased steadily over the first 9 d of the experiment, from an initial rate of 25 up to 40 mmol m^{-2} d^{-1}, after which the rates stabilised until the day after the pulse addition (Day 15), when the

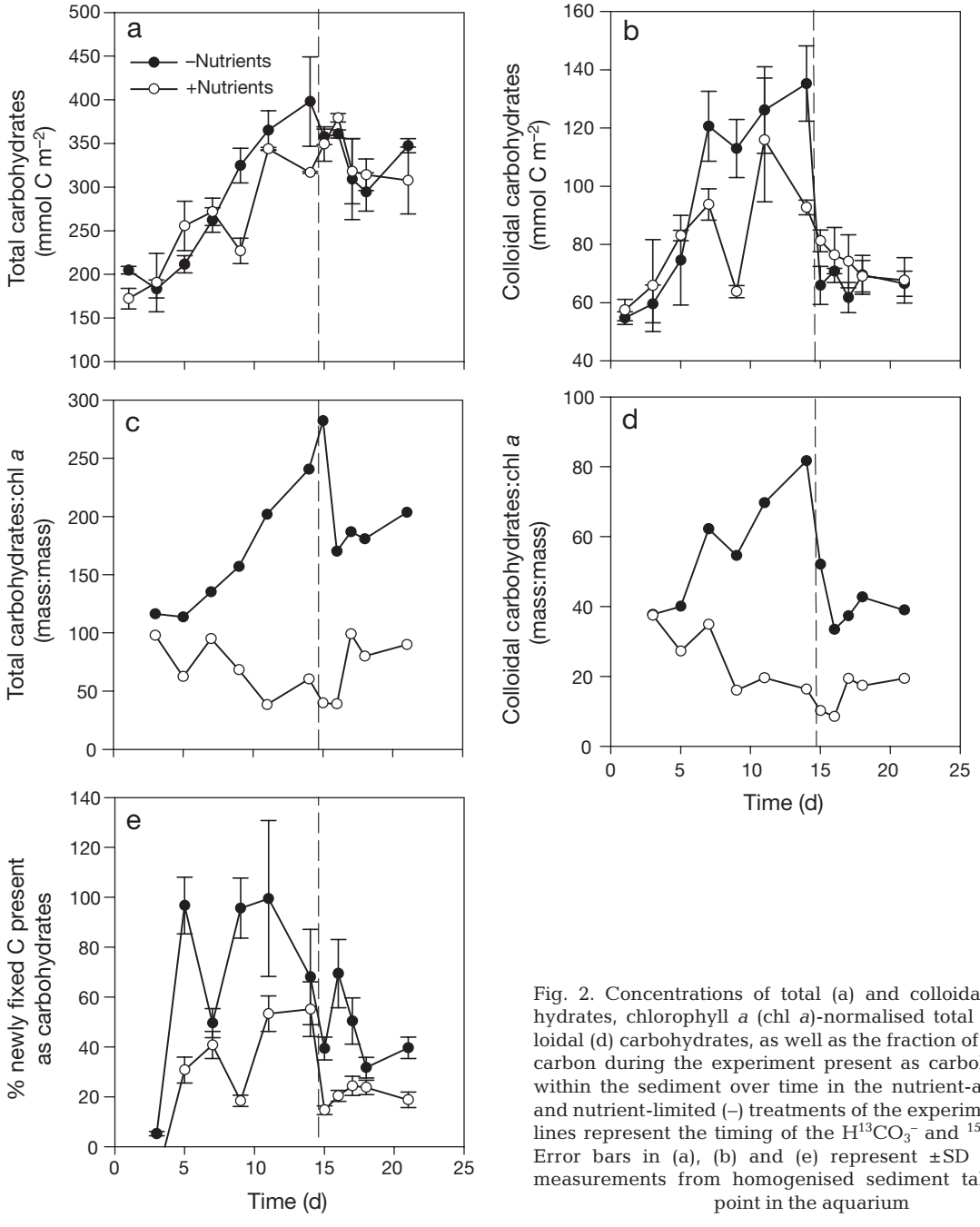

Fig. 2. Concentrations of total (a) and colloidal (b) carbohydrates, chlorophyll *a* (chl *a*)-normalised total (c) and colloidal (d) carbohydrates, as well as the fraction of newly fixed carbon during the experiment present as carbohydrates (e) within the sediment over time in the nutrient-amended (+) and nutrient-limited (–) treatments of the experiment. Dashed lines represent the timing of the $H^{13}CO_3^-$ and $^{15}NH_4^+$ pulse. Error bars in (a), (b) and (e) represent ±SD of replicate measurements from homogenised sediment taken from 1 point in the aquarium

rates of O_2 production jumped up to ~80 mmol m^{-2} d^{-1} and remained high until the conclusion of the experiment. Respiration in the (–) treatment followed no clear trend and generally remained between 10 and 23 mmol m^{-2} d^{-1} over the course of the experiment (Fig. 3b). For the (+) treatment, respiration increased dramatically over the first 5 d of the experiment, from an initial rate of 23 to up to 60 mmol m^{-2} d^{-1}, after which it stabilised, before increasing to 80 mmol m^{-2} d^{-1} on Day 21.

Nutrient measurements showed that the daily addition of N and P was assimilated in the (+) treatment, with concentrations of NH_4^+ dropping from 80 to between 0.5 and 6 µM and of PO_4^- from ~5 to ~0.05 to 1 µM in 24 h. Concentrations of $Si(OH)_4^-$ decreased to a much lesser extent, and never fell below 10 µM. However, there was no long-term build up, suggesting that the added Si was ultimately assimilated. In the (–) treatment, NH_4^+ and PO_4^- were generally <1 and 0.05 µM, respectively, and concentrations of Si remained <5 µM. Concentrations of NO_3^- remained <0.5 µM in both treatments.

Daily gross C to N assimilation stoichiometry in the (+) treatment increased to 10 within 5 d of the com-

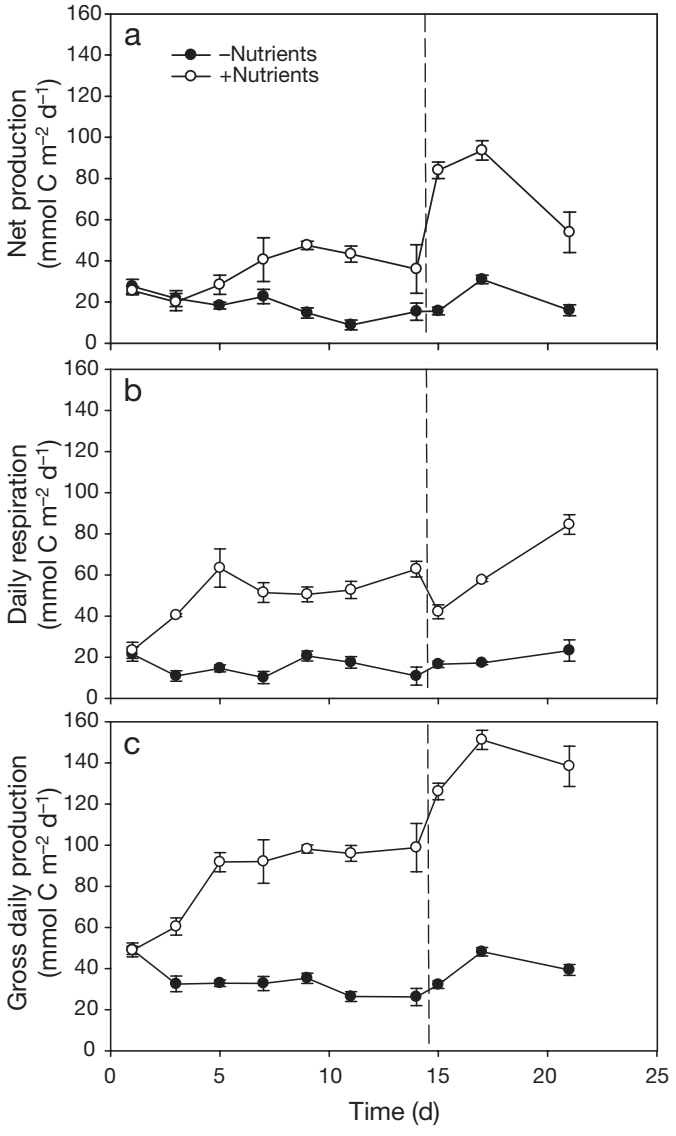

Fig. 3. Daily (a) net primary production, (b) respiration and (c) gross primary production in the nutrient-amended (+) and nutrient-limited (–) treatments of the experiment. Dashed lines represent the timing of the $H^{13}CO_3^-$ and $^{15}NH_4^+$ pulse. Error bars represent ±SE of the linear regression analysis of O_2 concentration changes versus time

mencement of the experiment, and subsequently increased to ~20 by Day 10, reaching its maximum value of ~40 on Day 18 (Fig. 4). Net C to N assimilation stoichiometry increased similarly, although the magnitude of the increase was much smaller, reaching a value of 13 on Day 14, when the isotope pulse was added, and climbing to as high as 27 by Day 18. The net cumulative C:N ratio of organic matter assimilated steadily increased from a value of ~3 at the start of the experiment to a value of ~5.5 at the point of the isotope pulse addition and to a maximum value of ~8 by the conclusion of the experiment.

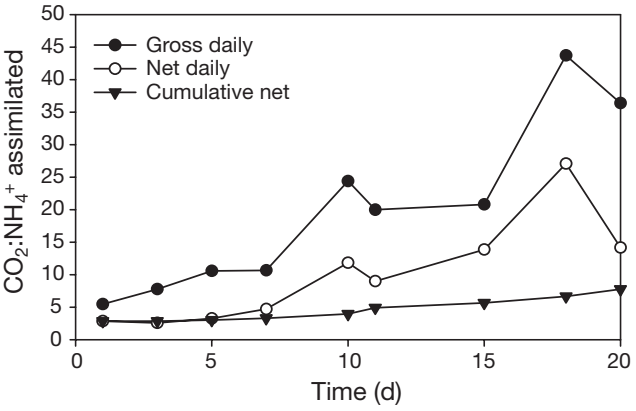

Fig. 4. Ratio of gross (Gross daily) and net (Net daily) CO_2 assimilation to NH_4^+ assimilation on a daily basis, as well as the cumulative ratio of net CO_2 to NH_4^+ assimilated over the course of the (+) nutrient experiment (Cumulative net)

Dissolved organic carbon release from the sediment

The loss of DOC from the sediment was assayed on 6 occasions for each treatment between Days 1 and 11. The loss of DOC from the sediment was very low, being 3 ± 2 and 1.4 ± 0.7% of total C fixation for the (–) and (+) treatments, respectively.

Bacterial production

Bacterial production measured using the leucine incorporation method showed a very similar pattern for both treatments, with the rates of production being generally slightly higher in the (+) treatment, although this difference was not significant ($p > 0.05$, Wilcoxon matched pairs test). Rates of production showed an initial drop after Day 1, followed by a period of stable production (5 to 9 mmol m^{-2} d^{-1}) between Days 3 and 15, before bacterial production again rose in both treatments towards the end of the experiment (Fig. 5a). Bacterial production was generally 10 to 30% of primary production, and this proportion was always higher in the (–) treatment, by a factor of about 2 for the majority of the experiment (Fig. 5b).

Label incorporation into hydrolysable amino acids

The total hydrolysable amino acid (THAA) content of the sediment ranged between 270 and 400 mmol C m^{-2} in the (+) treatment compared to between 140 and 204 mmol C m^{-2} in the (–) treatment (Fig. 6a). THAAs made up a smaller fraction of the carbon pool in the (–) treatment (16 to 22%) compared to the (+) treatment (22 to 33%; Fig. 6b). THAAs always comprised a

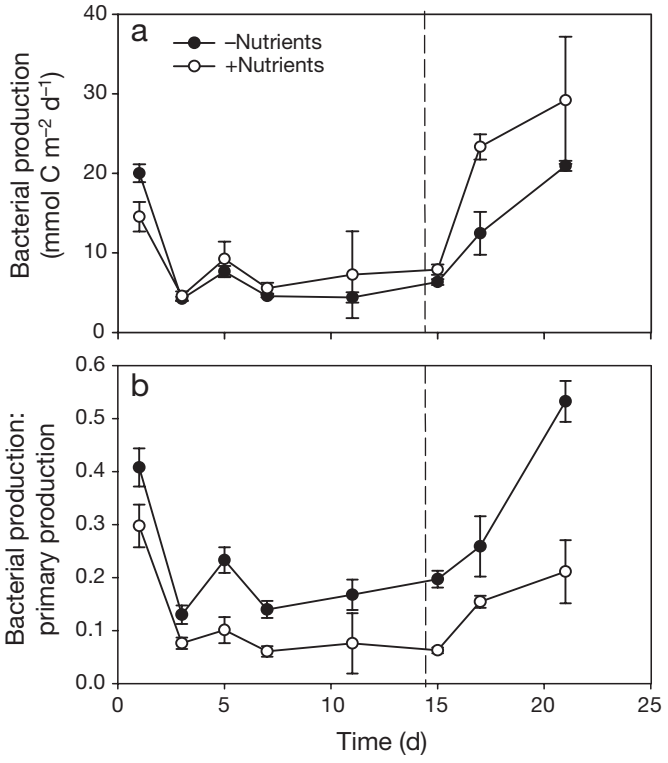

Fig. 5. (a) Bacterial productivity and (b) ratios of bacterial to primary production over time in the nutrient-amended (+) and nutrient-limited (–) treatments of the experiment. Dashed lines represent the timing of the $H^{13}CO_3^-$ and $^{15}NH_4^+$ pulse. Error bars represent ±SD of 3 replicate analyses of sediment taken from 1 point in each aquarium

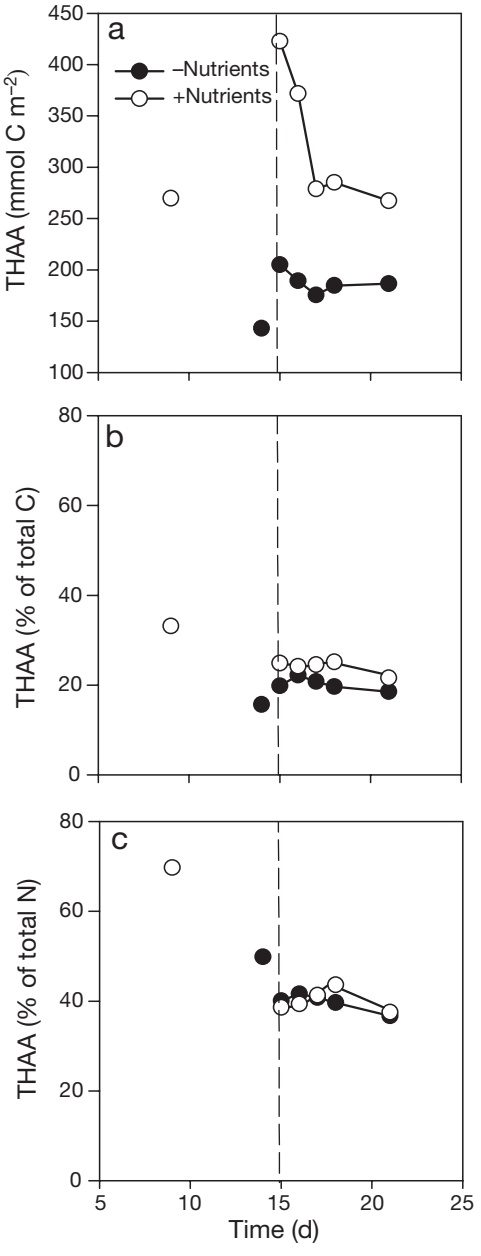

Fig. 6. (a) Concentrations of total hydrolysable amino acids (THAA), their contribution to (b) total C and (c) total N within the sediment over time in the nutrient-amended (+) and nutrient-limited (–) treatments of the experiment. Dashed lines represent the timing of the $H^{13}CO_3^-$ and $^{15}NH_4^+$ pulse

greater fraction of total sediment N than C, with the highest fraction observed being 70% of total N in the (+) treatment on Day 9. After the isotope pulse, THAAs comprised just over 40% of total N for both treatments.

After ^{13}C label addition and illumination, the incorporation of ^{13}C into THAAs was higher in the (+) treatment (~6 to 12 mmol m^{-2}) compared to the (–) treatment (~2 mmol m^{-2}; Fig. 7a), consistent with the differences in net C fixation measured between the treatments during this phase of the experiment (Fig. 3a). By contrast, the initial incorporation of ^{15}N into THAAs in the dark was highest in the (–) treatment, where ~2 mmol m^{-2} of N was incorporated into THAAs compared to ~1 mmol m^{-2} for the (+) treatment (Fig. 7b). In general, however, no clear distinction could be made between the 2 treatments for $^{15}NH_4^+$ incorporation. The proportion of total ^{13}C in the sediment incorporated into THAAs after the light phase of the experiment was between 19 and 25% for the (–) treatment and between 25 and 43% for the (+) treatment (Fig. 7c). In general, a much greater fraction of ^{15}N was incorporated into THAAs, with 50 to 60% of the total label in the sediment being incorporated into THAAs within 2 d of the label addition (Fig. 7d). Excess ^{13}C and ^{15}N in D-Ala mirrored that for THAA (Fig. 7e,f). After the first illumination cycle, excess ^{13}C D/L-Ala ratios were 0.029 and 0.032 in the (+) and (–) treatments, respectively, followed by a gradual increase in both treatments, with the highest values being consistently observed in the (–) treatment (Fig. 7g). After 12 h in the dark, the excess ^{15}N D/L-Ala

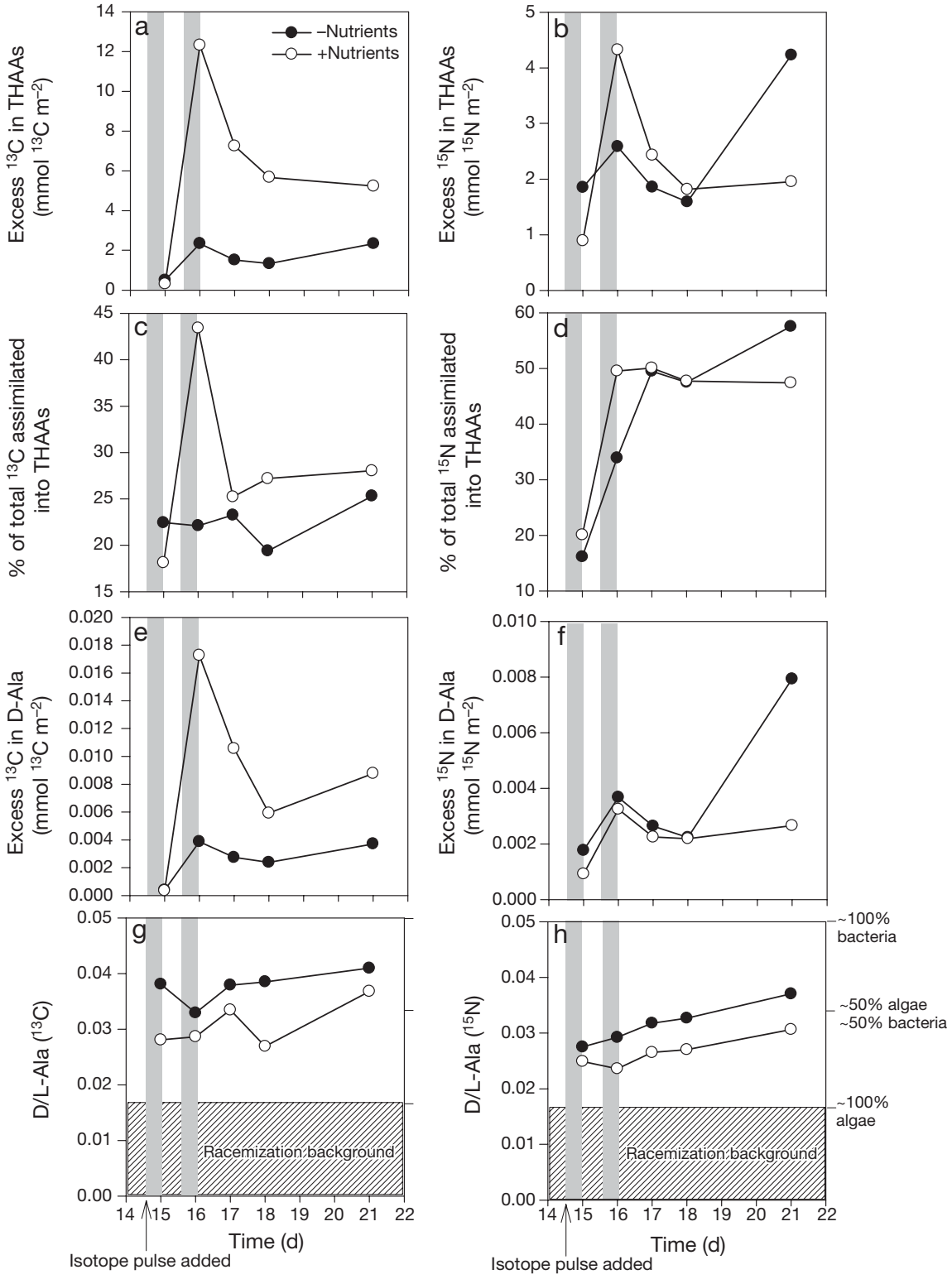

Fig. 7. Excess (a) ^{13}C and (b) ^{15}N in total hydrolysable amino acids (THAAs); the fraction of (c) total ^{13}C and (d) total ^{15}N in the sediment (or bulk ^{13}C and ^{15}N) incorporated into THAAs; excess (e) ^{13}C and (f) ^{15}N in D-alanine (D-Ala); and the excess label D/L-Ala ratio of (g) ^{13}C and (h) ^{15}N after the addition of a pulse of $H^{13}CO_3^-$ and $^{15}NH_4^+$ in the nutrient-amended (+) and nutrient-limited (–) treatments of the experiment. The approximate proportions of ^{13}C and ^{15}N incorporated into bacterial and algal biomass are shown on the *y*-axis in (g) and (h); details of this estimate are described in 'Discussion; ^{15}N and ^{13}C in D-alanine'. For clarity, the first 2 dark phases of the illumination cycle are denoted with grey bars. Hatched areas indicate racemization background

ratios were ~0.025 and 0.027 in the (+) and (–) treatments, respectively, followed by a gradual increase, with the highest ratios being consistently observed in the (–) treatment (Fig. 7h).

DISCUSSION

Conditions in mesocosms as used here may deviate significantly from *in situ* conditions in terms of grazing, advective porewater movement and re-suspension. Grazing was not quantified in these experiments; however, no macrofauna were present and the lack of pheophytin suggests that grazing rates were minimal. We cannot, however, rule out the presence of microscopic grazers such as ciliates, flagellates and meiofauna. Grazing may lead to cell lysis, releasing DOC, and represents an additional mechanism through which carbon fixed by photoautotrophs can be transferred to heterotrophic bacteria; we therefore refer to DOM (dissolved organic matter) release as opposed to excretion throughout this section. Grazing and resuspension may act in concert to remove MPB biomass, maintaining the algal cells in a constant phase of growth rather than forming a dense biofilm where growth becomes limited (Blanchard et al. 2001). Advective porewater transport may have a significant influence on the microbial community, for example, through enhanced supply of TCO_2 to primary producers, which may be limited by CO_2 in these sediments (Cook & Røy 2006). Advection and sediment resuspension may play an important role in the release of DOC from the sediment through mechanical action not captured in these experiments (see below). Despite these differences we note that the essential observations and conclusions of this study are the same as those from a similar study conducted at the same site run under close to *in situ* conditions (V. Evrard et al. unpubl. data). We are therefore confident that our results are ecologically meaningful.

The use of mesocosms, however, offers the distinct advantage of having well-controlled conditions, allowing budget calculations without interference from stochastic uncertainties, which occur *in situ*. Furthermore, mesocosms are well suited to the regular and simultaneous sampling of many parameters, which together give a deeper insight into the functioning of microbial communities. We first discuss the functioning of the algal compartment in terms of its loss of DOC to the water column and its response to nutrient limitation. We then address the use of D-Ala as a bacterial biomarker and its application to following the relative incorporation of ^{13}C and ^{15}N into bacteria and algae under N-limited and -replete conditions.

Loss of DOC to the water column

Microphytobenthos are known to excrete a large fraction of fixed carbon as low and high molecular weight compounds (Underwood & Paterson 2003). In short-term incubations of cultured benthic diatoms it has previously been shown that up to 60% of carbon assimilated may be excreted into the water column within 1 h (Smith & Underwood 2000). Our assays were methodologically very similar in terms of the incubation time frame and the extraction procedure. The major difference between these experiments was that the diatoms in our study were predominantly epipsammic as opposed to the epipelic species studied by Smith & Underwood (2000). The fact that very little (~1 to 5%) of the fixed ^{14}C in undisturbed sediment samples could be recovered in the dissolved phase, and the good agreement between the net particulate organic carbon fixation with that calculated from O_2 production in our experiment, indicates that virtually all of the excreted carbon is bound to the sediment and not released to the water column. Thus, it seems that the proportion of excreted carbon that becomes dissolved in the water column depends on the type of carbon excreted (i.e. algal type/species). We note also that the algal growth phase has a marked effect on the amount of carbon excreted, with the fraction of carbon excreted increasing markedly in the transition from the logarithmic to the stationary growth phase (Underwood & Paterson 2003). The DOC excretion incubations were terminated at 11 d at around the time the stationary phase of the (+) treatment was reached (see below); it is therefore possible that DOC excretion in the (+) treatment increased after 11 d. We consider this unlikely, however, because little excretion was observed in the (–) treatment, which was in the stationary phase when the DOC excretion incubations were undertaken. We also note that the conditions used here were very different to *in situ* in terms of hydrodynamic energy. *In situ* resuspension and advective processes may play an important role in disaggregating and dissolving EPS, which possibly lead to DOC release from the sediment.

Effect of nutrient limitation on algal growth dynamics

The effect of nutrient status on pelagic algal growth dynamics has received much attention, and sophisticated models of unbalanced algal growth have been developed to describe experimental observations (Van den Meersche et al. 2004). Under nutrient-replete conditions, virtually all of the carbon incorporated will be directed into cellular growth, and very little DOC will

be lost from the cell (the bloom phase). Upon exhaustion of the external nitrogen pool, carbon assimilation will continue, resulting in a gradual increase in the C:N ratio of the algal cellular material produced, concomitant with an increase in the fraction of C assimilation exuded as DOC (the intermediate phase). Eventually, the cellular C:N ratio will increase to a point (~20) where C acquisition becomes limited by the physiological condition of the cell, and most of the C fixed is exuded as DOC (the stationary phase), at this point the algal biomass decreases as grazing and mortality exceed growth (Van den Meersche et al. 2004).

For microphytobenthos grown in mesocosms, however, such growth dynamics are tempered by the changing physical environment of the growing biofilm, where CO_2 concentrations, light and space may eventually become limiting factors. As such, biofilm growth typically follows a sigmoidal curve, where a stationary phase is reached after 10 to 12 d and the community is not necessarily limited by nutrients (Blanchard et al. 2001, Morris 2005). During the first 16 d of the (+) treatment, chl *a* increased steadily, but after this, there was a marked drop, suggesting either senescence of the algae or a high degree of spatial variability. We ascribe this trend primarily to spatial variability, based on visual observations and the fact that no pheophytin was detected in any of the samples. Furthermore, rates of photosynthesis were highest on Days 15 to 21, supporting the contention that the algal community had not senesced. An alternative line of evidence to suggest that the community was in a stationary phase of growth after ~10 d comes from the C:chl *a*, N:chl *a* and total carbohydrate:chl *a* ratios, which all showed a minimum at 10 d followed by a gradual increase. These observations are all consistent with a decrease in the allocation of resources to cellular growth relative to C excretion after 10 d, suggesting that the cells had entered a more stationary phase of growth. It is unclear in our experiments whether the apparent slowing in biofilm growth was due to nutrient, light, or CO_2 limitation. However, it seems unlikely that the MPB community in our experiment were N limited, because the net cumulative ratio of C and N incorporated was 5.5, which is relatively low (Fig. 4); furthermore, the continued addition of nutrients after Day 15 should have allowed further cellular growth. Thus, at the point of the label addition, the MPB community was most likely in a stationary phase of growth, but not necessarily limited by nutrient availability. In the (–) treatment, no growth took place, as indicated by the relatively constant chl *a* concentrations, and, as such, this MPB community remained in a stationary phase due to severe nutrient limitation.

The different growth phases of the algae over the first 15 d of the experiment are also clearly seen in the ratios of C and carbohydrates to chl *a*, which climbed steadily in the (–) treatment, but dropped in the (+) treatment (Figs. 1 & 2). Virtually all of the C fixed in the (–) treatment was directed into carbohydrate synthesis compared to <50% in the (+) treatment (Fig. 2e). Thus, at the point of isotope addition, the 2 treatments produced 2 biofilms: one in a stationary phase severely limited by nutrients [(–) treatment], where there was a large relative build-up of high C:N ratio material such as carbohydrates, and the other also in a stationary phase of growth, but in a relatively nutrient-replete state [(+) treatment], with low cellular C:N ratios and carbohydrate concentrations (relative to biomass). Hence, the findings here should be considered relevant to relatively mature and undisturbed biofilms.

Interestingly, there was a rapid drop in the colloidal carbohydrate concentration upon the addition of the ^{15}N label (Fig. 2b,d), suggesting a rapid consumption of colloidal carbohydrates by either bacteria or algae. No jump in bacterial productivity was observed at the time of the drop in colloidal carbohydrates, suggesting that the consumption of this fraction was not by bacteria. It has been suggested that benthic diatoms may hydrolyse, then consume excreted carbohydrates; however, the ecological controls and relevance remain poorly understood (Staats et al. 2000a). In this instance, it is possible that the addition of NH_4^+ to the aquarium stimulated the algae to hydrolyse and/or consume the excreted carbon.

^{15}N and ^{13}C in D-alanine

As D-Ala is an amino acid unique to bacteria, label incorporation into D-Ala represents label incorporation by bacteria. The most useful information can be derived from the ratio between excess label in D-Ala versus that in L-Ala (a common HAA in all organisms), which provides a direct indication of the bacterial contribution to total microbial label incorporation. Quantitative interpretation of these data requires knowledge about the D/L-Ala ratio of the active bacterial community in the sediment. The D/L-Ala ratio of bacterial communities roughly ranges between 0.05 and 0.1, where relatively high values are associated with high abundance of Gram-positive bacteria and/or cyanobacteria (Veuger et al. 2007). Since the contribution of Gram-positive bacteria to the total bacterial community mainly seems to be relevant in deeper (anaerobic) sediment, while the present study concerned a thin layer of sandy (aerobic) sediment, the contribution of Gram-positive bacteria to the D/L-Ala ratio of the total bacterial community was probably negligible. Moreover, microscopic examination of the composition of the MPB community showed that cyanobacteria com-

prised only a minor fraction (<10%) of the benthic microbial community. Therefore, the D/L-Ala ratio of the bacterial community in the sediment (dominated by Gram-negative bacteria) was most likely around 0.05. This is supported by measured excess ^{13}C D/L-Ala ratios from additional sediment incubations with ^{13}C-labeled extract from the original labelled sediment, which yielded excess ^{13}C D/L-Ala ratios of ~0.05. Furthermore, the rates of bacterial production using ^{14}C leucine incorporation were consistent with the estimated uptake rates of ^{13}C by bacteria. Assuming a D/L-Ala racemization background of 0.017 and that a D/L-Ala ratio of 0.05 represents 100% bacterial C, then, based on the D/L-Ala incorporation ratios of 3.3 and 2.9 in the (–) and (+) treatments, respectively, after 24 h, we estimate that 48 and 35% of the ^{13}C uptake into amino acids was by bacteria in the (–) and (+) treatments, respectively (for a more detailed description of these calculations please see Veuger et al. (2007). Given a total ^{13}C assimilation into amino acids of 2.3 and 12.3 mmol ^{13}C m^{-2} d^{-1} for the (–) and (+) treatments, respectively, after 24 h, a ^{12}C:^{13}C ratio for dissolved inorganic carbon in the water column of 3.2 and assuming that amino acids comprise 50% of the bacterial biomass, this equates to a total bacterial C assimilation of ~7 and ~28 mmol C m^{-2} d^{-1} in the (–) and (+) treatments, respectively, 24 h after the start of the first illumination cycle following isotope addition. This compares to concurrently measured bacterial production rates of 6 to 12 and 8 to 23 mmol C m^{-2} d^{-1} on Days 15 and 17 for the (–) and (+) treatments, respectively, using the more conventionally employed leucine incorporation method. Thus, while these calculations are based on quite speculative assumptions, they give a reasonable agreement with more conventionally used methods.

Algal–bacterial interactions

Having established clear differences between the algal growth dynamics between the 2 treatments leading up to the isotope pulse, the question then arises as to how these differences affect the interaction between the bacteria and algae. According to the concepts put forward by Caron (1994), Legendre & Rassoulzadegan (1995), and Anderson & Ducklow (2001), one would expect that with increasing nutrient limitation there would be an increased coupling between algae and bacteria through the increased exudation of DOC by algae leading to a scenario resembling the 'microbial loop' (Legendre & Rassoulzadegan 1995). Consistent with this, the ratio of bacterial production to primary production (BP:PP) was generally twice as high in the (–) treatment (Fig. 5b), a difference which was significant at $p < 0.05$ (Wilcoxon matched pairs test). Evidence for the tighter coupling between algal and bacterial productivity in the (–) treatment also comes from the ^{13}C D/L-Ala ratios, which suggest that a significantly higher fraction (Wilcoxon matched pairs test $p < 0.05$) of the fixed C was initially transferred to the bacterial pool as indicated by the higher ^{13}C D/L-Ala ratios in this treatment (Fig. 7g). Furthermore, the excess ^{13}C D/L-Ala ratios also suggested that ~50 and 40% of the C incorporated by MPB was transferred to the bacteria in the (–) and (+) treatments, respectively, after the first illumination cycle (Day 16), and that there was a subsequent, albeit slower, transfer of C to the bacteria (Fig. 7g). This strongly suggests that the carbon release in this instance was ~50% of C assimilation under both nutrient-replete and -limited conditions. Such a high rate of EPS production in the (+) treatment is surprising given that the cumulative ratio of CO_2 and NH_4 incorporation was 5.5 at this point (Fig. 4). According to the model of Van den Meersche et al. (2004), excretion of fixed carbon would only become significant when the C:N ratio of the algal cells approaches 20. This discrepancy is, however, consistent with observations that MPB excrete a greater fraction of C-assimilated than of pelagic algae (Goto et al. 1999). Moreover, these results are also consistent with field and mesocosm studies of EPS dynamics, which show dramatic diurnal variations in EPS concentrations, suggesting a rapid production of EPS by MPB and consumption by bacteria (van Duyl et al. 1999). As already mentioned, it is also possible that the transfer of C from the algae to the bacteria is enhanced by cellular lysis through grazing, although we suggest that this is a minor contributor in this instance.

As for C, the N dynamics showed broad internal consistency with current concepts in microbial food web dynamics (sensu Caron 1994, Legendre & Rassoulzadegan 1995). Under N-replete conditions, the C:N ratio of excreted DOM is low and the bacteria utilising this DOM release NH_4, which then can be utilised again by the algae, resulting in a commensal relationship. Under N-limited conditions, the C:N ratio of exuded DOM increases, and bacteria using this high C:N ratio DOM will increasingly rely on inorganic nitrogen to meet their N requirements, putting them directly in competition with algae for available N (Bratbak & Thingstad 1985, Van den Meersche et al. 2004). The excess ^{15}N D/L-Ala ratios in our experiments suggested that bacteria initially incorporated ~20 and 30% of the ^{15}N added to the (+) and (–) treatments, respectively, after 12 h in the dark (Fig. 7h). This initial rapid enrichment was then followed by a slower transfer of ^{15}N from the algal to the bacterial pool in the days following the isotope pulse. About 50% of all ^{15}N in the sediment was in THAAs (Fig. 7d), indicating that all

the added $^{15}NH_4$ had been incorporated in biomass. It is unlikely that MPB could have first incorporated the added $^{15}NH_4^+$ and then released ^{15}N-DON (dissolved inorganic nitrogen) within 12 h in the dark. Thus, it seems that there was an initial direct assimilation of $^{15}NH_4^+$ by bacteria, followed by a slower continued assimilation of ^{15}N, most likely in the form of DON derived from algae.

In the case of the (+) treatment, we would expect the DOM released to have a low C:N ratio, resulting in bacteria being able to rely solely on this for their N source, according to the model and observations of Van den Meersche et al. (2004). The C:N ratio of DOM exuded by algae has rarely been directly measured. The limited data available suggest that under nutrient-replete conditions, the C:N ratio of DOM is quite variable (~5 to 14) (Biddanda & Benner 1997, Wetz & Wheeler 2003, Van den Meersche et al. 2004), but that it rapidly increases beyond 16 under nutrient-limited conditions (Wetz & Wheeler 2003, Van den Meersche et al. 2004). Other measurements suggest that protein and amino acids comprise only 5 to 10% of the DOM exuded by algae (Staats et al. 1999, Granum et al. 2002). Thus, the limited data available support our observation that the DOM derived from MPB can be relatively N poor, and that bacteria utilising this carbon source would most likely derive a large proportion of their N requirements through the direct assimilation of inorganic forms of N.

Additional support for the concept of MPB maintaining a low cellular C:N ratio by excreting high C:N ratio material, which is subsequently incorporated by bacteria, comes from an examination of gross C fixation to N assimilation ratios in the (+) treatment (Fig. 4). It can be seen that a large excess of C is fixed relative to N, but that, because a significant fraction of the gross C fixation is respired, the net ratio of C:N fixed is significantly lower. Assuming that diatoms respire on average 21% of the daily gross production (Langdon 1993), then, based on the gross primary production estimates, we can only account for 30 to 50% of the measured respiration rate, suggesting that bacteria account for 50 to 70% of the C respired. These estimates of bacterial respiration are also consistent with the measurements of bacterial production, suggesting an average bacterial growth efficiency of ~0.5 over the course of the experiment in the (+) treatment, which is quite high, but consistent with a value of ~0.6 expected for growth on excreted organic carbon (del Giorgio & Cole 1998). The implications of this are that, whilst MPB do produce low C:N ratio cellular material, the substrate used by bacteria may actually be of a much higher C:N ratio as a consequence of the exudation of high C:N ratio EPS. Therefore, the C:N ratio of the pools of organic matter present in the sediment gives limited information on the C:N ratio of the substrate being used by bacteria, because it is rapidly turned over. We suggest that, paradoxically, the C:N ratio of the substrate being used by bacteria may actually be higher than the bulk C:N ratio of the sediment, which is the opposite of what is conventionally assumed.

Comparing our results to other field studies is problematic because of the highly artificial system used here. Perhaps, most significantly, rates of grazing were low, owing to an absence of macrofauna as also indicated by the absence of pheophytin. Within the microbial loop, grazing has an important role in N recycling, particularly in the release of DON (Glibert et al. 1991). Indeed, an absence of grazing may explain the extremely low loss of DOC to the water column in our experiment, which is at odds with some field observations of high DON fluxes in systems with high MPB biomass (Cook et al. 2004, Ferguson et al. 2004). Furthermore, given that grazing and resuspension may maintain algae in a higher state of production (Blanchard et al. 2001), and, hence, possibly lower excretion rates, the extremely high transfer of C from the algae to the bacteria observed here may not be applicable to the field. Our results are, however, in broad agreement with previous studies, which indicate that bacteria in the surface sediment of intertidal flats rely heavily on MPB as a C substrate (Middelburg et al. 2000), and that the recycling of N relative to C is very conservative in highly productive sediments colonised by MPB (Cook et al. 2004, Ferguson et al. 2004). Furthermore, the findings here are consistent with the suggestion that the presence of MPB reduces nitrification and denitrification rates as a consequence of competition between nitrifiers and heterotrophic bacteria for available NH_4^+ (Risgaard-Petersen 2003).

Our experiments suggest that MPB release a large fraction of C assimilated under both nutrient-limited and -replete conditions, but that an insignificant fraction of the carbon released is lost to the water column when fluxes are dominated by molecular diffusion. Consistent with current concepts of microbial food web dynamics, N limitation led to a tighter coupling between algal and bacterial production and an increased competition for inorganic N. Our data suggest, however, that even under relatively nutrient-replete conditions, MPB exude a large fraction of the C assimilated.

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