International Journal of Astrobiology 1 (4): 295–304 (2003) Printed in the United Kingdom
DOI: 10.1017/S1473550403001320

Photosynthesis below the surface in a cryptic microbial mat

Lynn J. Rothschild[1] and Lorraine J. Giver[1,2]

[1]*Ecosystem Science and Technology Branch, Mail Stop 239-20, NASA/Ames Research Center, Moffett Field, CA 94035-1000, USA*
e-mail: lrothschild@mail.arc.nasa.gov
[2]*Codexis, Inc., 515 Galveston Drive, Redwood City, CA 94063*
e-mail: lori.giver@codexis.com

Abstract: The discovery of subsurface communities has encouraged speculation that such communities might be present on planetary bodies exposed to harsh surface conditions, including the early Earth. While the astrobiology community has focused on the deep subsurface, near-subsurface environments are unique in that they provide some protection while allowing partial access to photosynthetically active radiation. Previously we identified near-surface microbial communities based on photosynthesis. Here we assess the productivity of such an ecosystem by measuring *in situ* carbon fixation rates in an intertidal marine beach through a diurnal cycle, and find them surprisingly productive. Gross fixation along a transect (99×1 m) perpendicular to the shore was highly variable and depended on factors such as moisture and mat type, with a mean of $\sim$41 mg C fixed m^{-2} day^{-1}. In contrast, an adjacent well-established cyanobacterial mat dominated by *Lyngbya aestuarii* was $\sim$12 times as productive ($\sim$500 mg C fixed m^{-2} day^{-1}). Measurements made of the *Lyngbya* mat at several times per year revealed a correlation between total hours of daylight and gross daily production. From these data, annual gross fixation was estimated for the *Lyngbya* mat and yielded a value of $\sim 1.3 \times 10^5$ g m^{-2} yr^{-1}. An analysis of pulse-chase data obtained in the study in conjunction with published literature on similar ecosystems suggests that subsurface interstitial mats may be an overlooked endogenous source of organic carbon, mostly in the form of excreted fixed carbon.

Accepted 15 November 2002

Key words: carbon fixation, cyanobacteria, intertidal, *Lyngbya aestuarii*, microbial mat, photosynthesis, subsurface.

Introduction

Solar radiation is such a powerful and widely available source of energy for the terrestrial biosphere that until recently it was thought that all life on Earth ultimately depended on oxygenic photosynthesis for organic carbon. However, the surface of Earth has its own perils, including exposure to ultraviolet radiation. Microbial ecosystems are now known to live as much as kilometres beneath the surface of the Earth (e.g. Stevens & McKinley 1995; Onstott *et al*. 2000) and hundreds of metres below the bottom of the ocean (D'Hondt *et al*. 2002). Subseafloor prokaryotes alone may constitute one-tenth to one-third of the Earth's biomass (D'Hondt *et al*. 2002).

Many (but not all, Chapelle *et al*. 2002) of the subsurface communities may ultimately rely on organic carbon from photosynthesis or oxidized products produced from biogenic oxygen. Thus, even though they live beneath the surface, they depend on products produced by organisms with access to solar radiation. These discoveries have been of particular interest to the astrobiological community as they provide a model for potential ecosystems on early Earth when surficial fluxes of ultraviolet were likely to have been high (Rothschild 1999; Cockell & Horneck 2001), and extraterrestrial bodies such as Mars (Weiss *et al*. 2000), Europa (Chyba 2000) and comets, locations where the surface may be sterile but where subsurface communities are likely to have access to liquid water and the chemicals necessary for life. Particularly exciting in this regard are the Mars Odyssey results suggesting large quantities of water ice, a seemingly necessary ingredient for life, in the subsurface of Mars (Mitrofanov *et al*. 2002; Boynton *et al*. 2002).

There is, however, an intermediate type of ecosystem inhabiting the near subsurface. In this locale, organisms may have access to sufficient solar radiation to drive photosynthesis but at least partial protection from ultraviolet radiation, desiccation and surface predators. Examples of such ecosystems include freshwater (Pennak 1989), marine (Amos 1965; Rothschild 1995) and hotspring (Rothschild 1995) beaches, which contain an interstitial microbial community. The community composition may be taxonomically diverse, including cyanobacteria and eukaryotes such as diatoms, dinoflagellates, ciliates and small invertebrates (Ganapati *et al*. 1959; Fenchel 1987; Carey 1992; Faust

1995). Interstitial pigmented microbial communities have been previously described (Warming 1904) as 'Farbstreifan-Sandwatt', or 'coloured striped sand' (Schulz 1937; Hoffmann 1942, 1949; Flint 1955).

We identified such a community on the northeastern shore of Laguna Ojo de Liebre, Baja California Sur, Mexico (28° N, 114° W). In some locations, its structure was comparable to a laminated microbial sediment ecosystem, thought to be a young microbial mat (Stal *et al.* 1985). Because photosynthetic microbial mats and benthic microalgae can be an important source of fixed carbon and critical to inorganic nutrient cycling (Varela & Penas 1985; Fielding *et al.* 1988; Christensen *et al.* 1990; Moncreiff *et al.* 1992; Pinckney & Zingmark 1993), we hypothesized that cryptic interstitial communities also might play a key role in biogeochemical cycles. Locally, the role might include providing a source of organic carbon for the heterotrophic component of the intertidal community. On a more global scale, interstitial communities are an overlooked carbon sink of unknown magnitude and could possibly provide a vibrant ecosystem on other bodies with harsh surface conditions, including the early Earth.

To compare the carbon fixation capacity of the subsurface intertidal mat with a well-established intertidal mat, similar carbon fixation experiments were performed the same week on a nearby established mat. The established mat was ~2–5 mm in thickness and was composed primarily of the filamentous cyanobacterium *Lyngbya aestuarii* Agardh (a mat described in Rothschild & Mancinelli 1990).

Materials and methods

Assessment of primary production

To assess the capacity of the beach to fix carbon, a transect was made from a dirt road used <1 time per day (0 m) to the shore, ~99 m. The beach community structure was sampled by examining a vertical core taken every metre along the transect. From the transect four representative, but morphologically distinct, sites were chosen for further study. These sites were chosen because they represented the range of morphologies of the 99 cores, and thus these sites could be used as analogues of the unstudied sites in determining carbon fixation.

At each sand site, carbon fixation was determined on 2–3 June 1991 by the acid-stable incorporation of $H^{14}CO_3^-$, essentially as described by Rothschild (1991). Glass 125 ml serum bottles without bottoms were inserted into each site, and 6 ml lagoon water augmented with $NaH^{14}CO_3^-$ (DIC = 2 mM; final concentration $^{14}C = 2$ μCi ml^{-1}) added to the incubation vials. Incubations were for 20 min, except when time courses were performed. Dark controls were preincubated in vials wrapped in opaque foil for 1 h prior to the addition of the label (0830–0930 h), and incubated in the dark. 'Cold controls' were not exposed to radioactivity. After incubation, all samples were transferred to sterile Whirlpak® bags and immediately frozen on dry ice in the dark. Experimental samples were stored under these conditions until processed in the laboratory. Upon return to the laboratory, samples were defrosted and sonicated on ice. Two aliquots from each of the duplicate samples were removed to separate scintillation vials where they were acidified to below pH 4 with acetic acid and dried under a stream of warm water. The dried samples were resuspended in 200 μl warm water and 4 ml scintillation fluid (Ecolume, ICN Biomedicals, Inc.) added. Samples were recounted with internal standards to adjust for quenching. Carbon fixation rates were calculated by determining total acid-stable counts in the duplicate aliquots from each of the duplicate experimental samples. Carbon fixation rates were plotted against time of day. Total daily carbon fixation was determined graphically from these plots.

Carbon fixation in the *Lyngbya* mat was determined the same way as for the sand sites with the following exceptions. The final concentration of $NaH^{14}CO_3$ in the lagoon water was 1 μCi ml^{-1}. Incubations were for 15 min. The samples were homogenized in a small food processor prior to sonication. Counts were accumulated by scintillation spectroscopy for duplicate aliquots from each of the triplicate experimental samples. In analysing the data, the highest and lowest count from each time point was ignored.

Irradiance, organic carbon, pigment and protein measurements

Photosynthetic photon flux (400–700 nm) was determined using a LiCor model LI-185B quantum radiometer photometer with an atmospheric/underwater quantum sensor (LiCor, Lincoln, NE). The organic content of the mat was determined by combustion (Nelson & Sommers 1982). Replicate samples ($n = 2$–4) were taken from the top 5 mm of the mat. The samples were dried at 100 °C overnight, weighed, heated to 500 °C overnight and reweighed. This method may slightly overestimate organic carbon because some inorganic compounds are thermal-labile at this temperature (Nelson & Sommers 1982). To determine chlorophyll *a* and pheophytin *a* content, replicate cores ($n = 3$–8) were taken from each study site, immediately frozen on dry ice in the dark, and returned to the laboratory in that state for analysis. Upon defrosting, the samples were sonicated in 50 ml of buffer (100 mM Tris, pH 8.0; 5 mM EDTA). The protein content was determined using the bicinchoninic acid (BCA) protein assay reagent (Pierce Chemical Company, Rockford, IL), which we found was not affected by concentrations of NaCl ⩽5 M. Chlorophyll *a* and pheophytin *a* content were measured as described by Stal *et al.* (1984).

Pulse-chase experiments

Incubation bottles were set up on 2 June at sand sites 2–4 as for the carbon fixation experiments, except the incubation mix consisted of a final concentration of $^{14}C = 15$ μCi ml^{-1}. Samples were incubated ('pulsed') for 60 min (1300–1400 h) to obtain sufficient counts. The incubation bottles were then removed, and the samples rinsed several times with washes of 'cold' lagoon water. Samples remained *in situ* ('chased' with non-radioactive water), and harvested during the next day.

https://doi.org/10.1017/S1473550403001320 Published online by Cambridge University Press

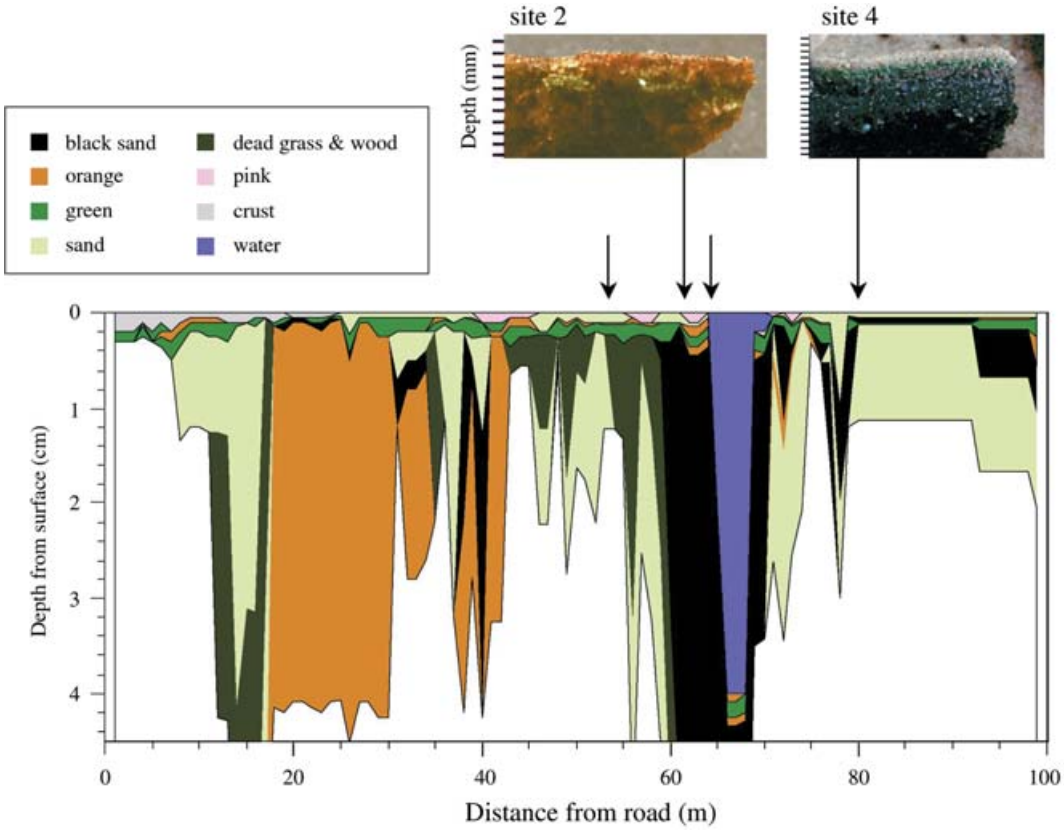

Fig. 1. Cross-section of transect. The top few centimetres were sampled every metre throughout the transect, and the resulting map was used to determine assignments of unstudied cores to the four study sites. The layers are coloured for clarity and correspond roughly to the actual colours. The water in the tidepool is represented as blue. White indicates that data were not recorded for that depth. Photographs are shown of the subsurface community at sites 2 and 4. The scales on the photographs are in millimetres.

Incubations were stopped and samples returned to the laboratory as for the carbon fixation experiments.

Carbon fixation was determined as described above. Fixed carbon that was incorporated into DNA was measured as follows. Upon return to the laboratory, samples were ground in lysis buffer (100 mM TRIS, pH 8; 5 mM EDTA) and 400 μL of the sample mixed with an equal volume of CTAB isolation buffer (4% [w/v] hexadecyltrimethyl-ammonium bromide, 2.8 M NaCl, 0.4% [v/v] 2-mercaptoethanol, 40 mM EDTA, 200 mM TRIS, pH 8) and incubated at 60 °C for 30 min with occasional swirling. The sample was extracted once with an equal volume of chloroform (24:1 [v/v] chloroform:isoamyl alcohol). The DNA was precipitated in isopropanol, washed in ethanol, dried and resuspended in TE (10 mM TRIS, pH 8; 1 mM EDTA) and incubated at 75 °C for 10 min to inactivate DNases. The size of the DNA and potential RNA contamination was assessed by agarose gel electrophoresis. DNA was quantified spectrophotometrically, and the results determined using a nomograph based on the data of Warburg & Christian (1942), which subtracts the protein component. Counts were obtained as described above. The relative amounts of fixed carbon present as DNA was calculated as specific activity, that is, radioactivity per unit DNA (cpm (μg DNA)$^{-1}$).

Results

The beach community structure was assessed by examining a vertical core every metre along the transect (Fig. 1). The top 5 mm usually contained green, orange or pink layers, often $\leqslant$1 mm thick. Microscopic examination showed that the community was numerically dominated by cyanobacteria, including unicellular species and *Lyngbya aestuarii* Agardh (M.R. Klovstad, personal communication). Along the transect was a small tide pool present throughout the first week of June 1991. The tide pool contained a thick, exposed crop of filamentous cyanobacteria, suggesting that it had been there for some time. This community was included for comparison with the interstitial sand community.

From the transect line, four representative but morphologically distinct sites were chosen for further study (Fig. 1). Site 1 (54 m) was dry to the touch on the surface, but moist underneath. A layer of sand was visible from the surface, followed by a 0.25 mm orange layer, 1 mm of green mat and sand mixed with decaying grass. The surface of site 2 (62 m) was covered by a pink mat that was dry to the touch. Beneath the surface was a 1 mm layer of orange, then 1 mm of green, another millimetre of orange, followed by dark black mud. Site 3 was in the tidepool under 2 cm of water. The surface of

https://doi.org/10.1017/S1473550403001320 Published online by Cambridge University Press

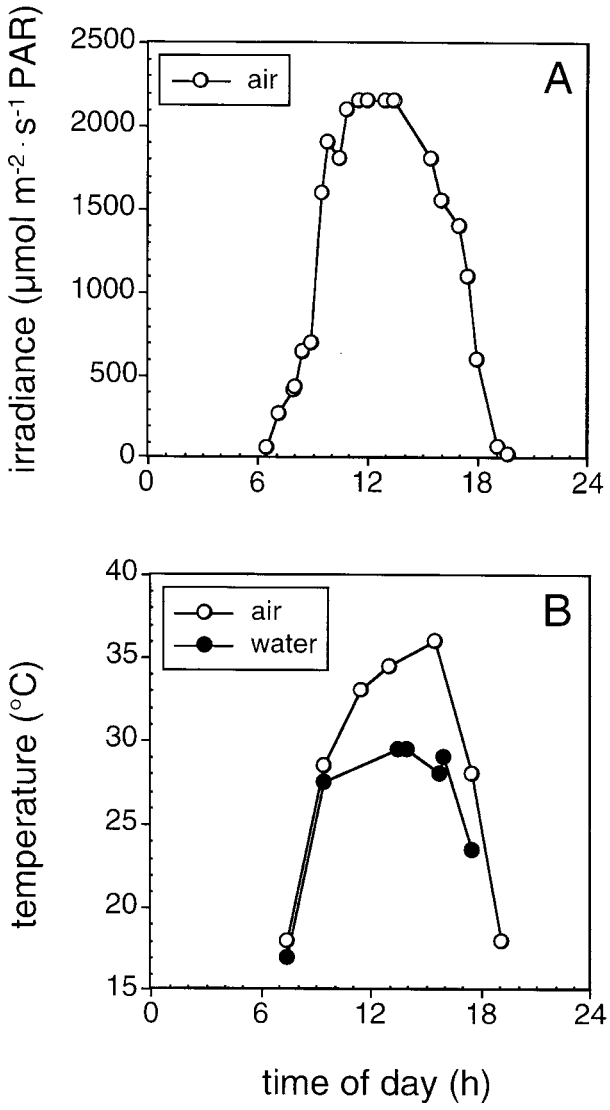

Fig. 2. Diurnal variation in light and temperature at the four study sites. These data are provided to show the correlation between irradiance and carbon fixation, and thus, the importance of assessing diurnal changes in carbon fixation. (a) Irradiance as PAR was determined on the surface of the mat at site 2. Because site 3 was under 2 cm of water, the irradiance was slightly lower than shown. (b) Temperature was measured on the surface of the mat inside an incubation bottle at sites 2 (bottle exposed to air) and 3 (bottle in tidepool).

the algal community was covered by a 1 mm orange layer, then 1.5 mm dark green filamentous mat, 1 mm dark orange grass and then black silt. Site 4 (80 m) was not submerged on the days of the experiments, but was water-logged. The ripples on the surface indicate that it had recently been submerged. The top 0.25 mm of grey sand of site 4 were followed by a 0.25 mm orange layer, 1.5 mm of a loosely packed green layer, then 1 cm each of grey, black and then grey sand. The black layers suggest anaerobic conditions.

Carbon fixation was measured several times during a 24 h period in order that diurnal changes in environmental parameters (e.g. irradiance and temperature; Fig. 2) and metabolic activities that affect diel variation in carbon fixation would be assessed (Rothschild 1991; Cockell & Rothschild 1999). First, a time course was performed to find the linear portion of the uptake curve. The time that acid-stable carbon production is linear should represent fixation in the absence of remineralization, i.e. gross fixation. After that time, the apparent production rate decreases because some portion of the fixed carbon is being remineralized, and net rather than gross production is measured. A representative time course for acid-stable carbon production in *Lyngbya* is presented in Fig. 3, and shows clearly that a 20 min incubation is well within the linear portion of the curve. Next, diurnal patterns of carbon fixation were determined by measuring carbon fixation periodically during the day. The counts from the controls to which no radioactive carbon was added ('cold controls') were found to be indistinguishable from counts obtained with scintillation fluid and water ($\sim$75 cpm). For this reason, we concluded that the sample itself was not responsible for background counts. The average standard deviation in the data was 28% with most of the deviation occurring at the ends of the day when fixation was lowest. There was $<$8% standard deviation associated with $\sim$65% of the total daily fixation. Carbon fixation rates were plotted against time of day in order to obtain total carbon fixed per day (Fig. 4). Total daily carbon fixation was approximately 16.5, 53.4, 170.4 and 48.5 mg C fixed m^{-2} day^{-1} for sites 1–4, respectively.

Gross carbon fixation along the transect was estimated by assigning each sample to one of the study sites on the basis of the mat structure. 45 other samples were assigned to site 1, 17 to site 2, five to site 3 and 25 to site 4. Only four cores (15, 17, 20, 21 m) did not contain green, orange or pink layers. Because the pigmented layers are primarily responsible for the carbon fixation in the community (see below), these four sites were assumed to fix negligible amounts of carbon. When all other samples along the transect were assigned to a study site, total diurnal carbon fixation along the transect was calculated as 4.041 g C, for an average rate of 41 mg C fixed m^{-2} day^{-1}. If the cores that were not essentially identical to the study sites are excluded, the estimated total fixation dropped by $\sim$5%.

Organic matter in the top 5 mm, which included the photosynthetic layer(s), was 614, 677, 433 and 289 g m^{-2} in sites 1–4, respectively, with an average error in the data of 17%. Following Fenchel & Finlay (1983), we assume that 1 g C $\approx$ 2 g dry weight $\approx$ 14 g wet weight. Gross fixation for the four sites was 16.5, 53.4, 170.4 and 48.5 mg C m^{-2} day^{-1}, or approximately 231, 748, 2386 and 679 mg wet weight biomass, respectively. Thus, we calculate organic matter produced per day as a percentage of the total organic matter as 0.04%, 0.11%, 0.55% and 0.23% for sites 1–4. The amount of organic matter produced that was lost from the mat owing to respiration and grazing were not measured directly.

Diurnal carbon fixation was expressed in terms of protein, chlorophyll *a* and pheophytin (Table 1). Graphical analysis (CA Cricket Graph III, version 1.5.3, Computer Associates

https://doi.org/10.1017/S1473550403001320 Published online by Cambridge University Press

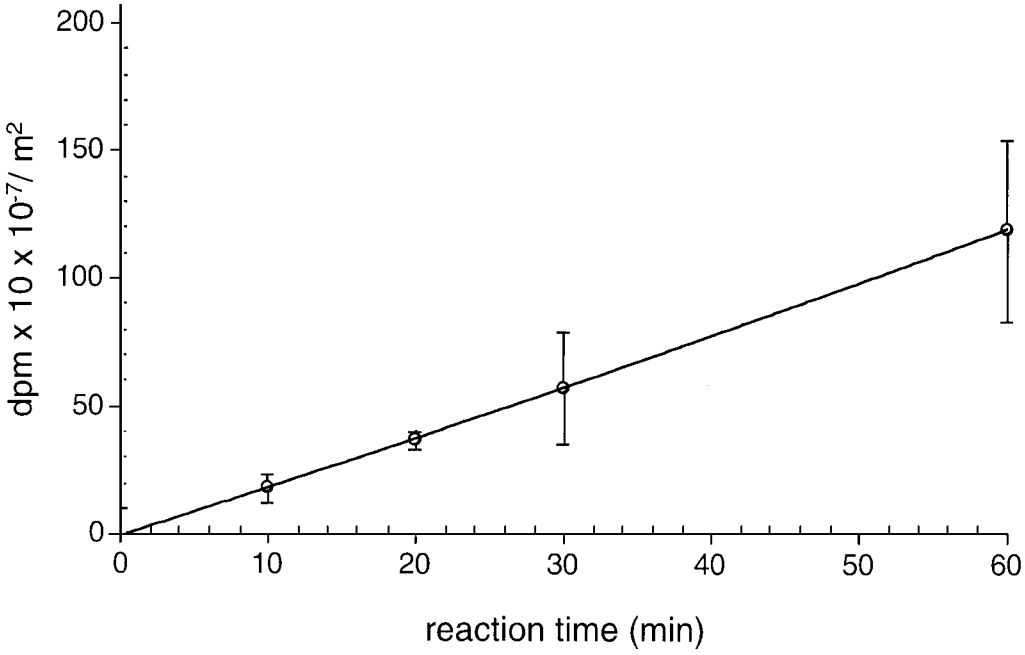

Fig. 3. Production of fixed carbon as a function of incubation time. A time course was performed on the *Lyngbya* mat on 7 February, 1989 near noon to determine the linear range of acid-stable ^{14}C production. Each data point is the mean of six data points obtained from triplicate experimental incubations, with each incubation sampled twice. The error bars represent the standard deviation.

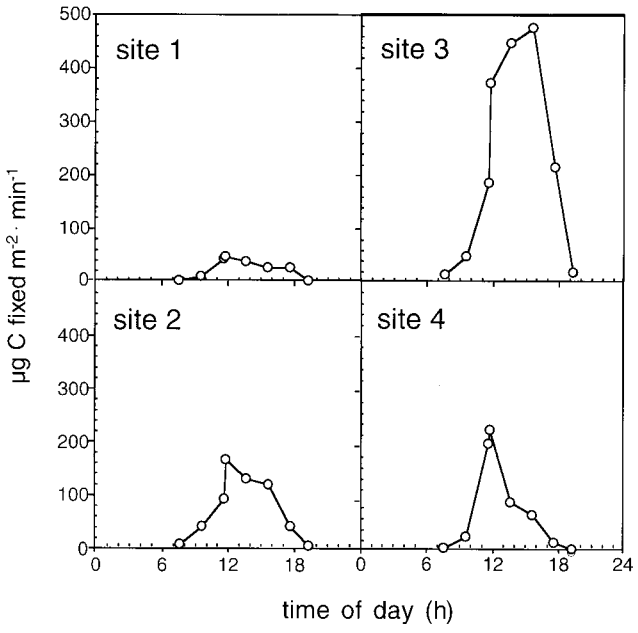

Fig. 4. Diurnal patterns of carbon fixation in the four representative sites of the interstitial community, 2–3 June, 1991. For each time point, two independent experiments were run and two subsamples were analysed from each experimental bottle. The lines represent mean values.

International, Inc., Islandia, NY) revealed a statistically significant correlation between total diurnal carbon fixation and the chlorophyll *a* to protein ratio ($r^2 = 0.97$ for a second-order polynomial fit). Graphical analysis also suggested that >0.8 mg chlorophyll *a* per g protein was needed for detectable carbon fixation to occur.

Gross daily carbon fixation was determined in a similar manner for the established *Lyngbya aestuarii* mat. From the diurnal pattern of carbon fixation (Fig. 5), we calculated gross carbon fixation as 494.4 mg C fixed m^{-2} day^{-1}, ~ 12 times the average rate for the subsurface mat. It should be noted that in both cases carbon fixation was assessed while the mats were submerged. Parts of both intertidal mats are continuously water-logged. Those parts that are at least sometimes immersed are likely to have higher fixation rates when submerged. Carbon fixation in submerged *Lyngbya* mats is approximately an order of magnitude higher than moist mats exposed to the air (Rothschild & Mancinelli 1990). Similarly, while slight desiccation (4–8%) enhances photosynthesis in the intertidal brown alga *Fucus spiralis*, desiccation in general inhibits net photosynthesis with photosynthesis stopping at a water content of $\sim 15\%$ (Madsen & Maberly 1990). Thus, the total daily fixation given here may be higher than the actual rates.

Dark controls were performed at every mat site and for the *Lyngbya*. Carbon fixation was never detected above the background. Therefore, we conclude that carbon fixation in the absence of sunlight, while probably present as anaplerotic fixation by phosphoenolpyruvate carboxylase to form oxaloacetate, is negligible, and that the vast majority of carbon fixation at these sites is the result of photosynthesis.

To assess seasonal variability and estimate total annual gross fixation, gross daily carbon fixation was measured for the *Lyngbya* mats at two additional times of the year. The total daily fixation was 380 mg C fixed m^{-2} day^{-1} on 24 March 1990 and 218 mg C fixed m^{-2} day^{-1} on 2 November 1989. While carbon fixation is related to many factors such as temperature, light level and nutrient status, to a first-order

https://doi.org/10.1017/S1473550403001320 Published online by Cambridge University Press

Table 1. *Carbon fixation as a function of protein and pigment content*

Site	g protein/m² mat	mg chl. *a*/m² mat	ratio chl. *a* to pheophytin *a*	mg C fixed g protein^{-1} day^{-1}	mg C fix mg chl. *a*/day
1	26.6±27%	28.13±19%	0.6	0.62	0.587
2	53.7±14%	80.33±13%	0.9	0.99	0.665
3	47.9±15%	118.90±25%	0.5	10.33	1.433
4	14.7±11%	24.94±50%	1.4	3.32	1.944

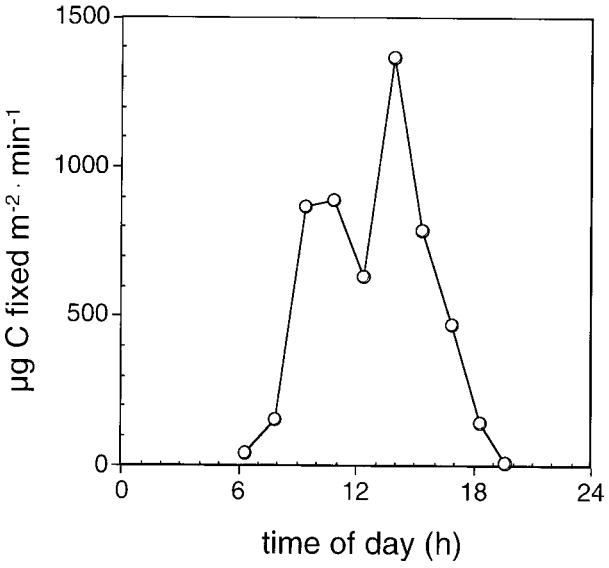

Fig. 5. Diurnal pattern of carbon fixation in *Lyngbya* mat, 31 May–1 June, 1991. The experimental protocol was as for Fig. 4.

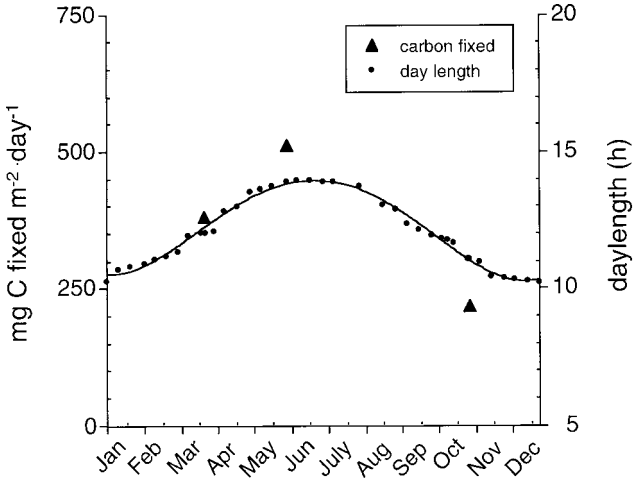

Fig. 6. Gross daily carbon fixed by *Lyngbya* mats in relation to day length. Day length in Guerrero Negro through a calendar year is similar in pattern to gross daily carbon fixation.

approximation the total daily gross fixation for the *Lyngbya* mats was correlated with day length (Fig. 6).

Using the correlation between total daily gross fixation and day length, the total annual carbon fixation for the *Lyngbya* mat was calculated in two ways. First, we took the day length for each of the days that carbon fixation measurements were obtained and calculated the percentage of the total annual daylight that it represented. We then assumed that the carbon fixed each day was the same percentage of the annual carbon fixed. These calculations yielded a total annual fixation of 130088 g m^{-2}. Secondly, we used the correlation to estimate the total daily fixation for several day lengths. At the field site, 95 days have a day length between 10 and 11 h, 70 between 11 and 12, 70 between 12 and 13, and 130 between 13 and 14. For each category, we calculated the total carbon fixed for a day length at the midpoint of the range (i.e. 10.5 for the 10–11 h range). Summing these calculations yielded a total annual fixation of 129562 mg m^{-2}, a result very similar to that obtained by the first method. Both methods indicate that 0.39% of the annual fixation occurred during the June experiment. Assuming that this correlation pertains to the sand mat, the daily fixation results obtained in June suggest a total annual gross fixation of 10.5 g m^{-2}, or 4.2, 13.7, 43.7 and 12.4 g m^{-2} for sites 1–4, respectively.

To begin to assess the fate of the fixed carbon, sites 2, 3 and 4 were 'pulsed' with $H^{14}CO_3^-$, rinsed in lagoon water to remove as much unincorporated label as practical without disturbing the mat, and 'chased' under ambient conditions, in analogy with 'pulse-chase' subcellular biochemical experiments. Thus, the fate of the carbon fixed during the initial 'pulse' could be studied. Over a 21 h period, total fixed carbon in the system declined at all three sites, with the greatest decline occurring at the driest of the three, site 2 (Fig. 7a). In contrast, there was an initial increase in fixed carbon partitioned into DNA over the first 5 h of the experiment, with a subsequent decline in labelled DNA between 5 and 21 h (Fig. 7b).

Discussion

The rates of carbon fixation presented here for the subsurface and the *Lyngbya* mats are comparable to published rates for algae when a similar method of assessing diurnal changes in carbon fixation was used to calculate total daily carbon fixation. Because photosynthetic carbon fixation changes constantly throughout the day (e.g. Figs 4 and 5), realistic measurements cannot be extrapolated from data obtained at a single time point. Unfortunately, many studies do not monitor the entire diurnal cycle *in situ*. Directly comparable data are available for a benthic microbial mat community at ESSA. Carbon fixation by a benthic hypersaline cyanobacterial (photosynthetic component primarily *Microcoleus chthonoplastes* Desmazieres) mat was 139.5 mg C fixed m^{-2} day^{-1} at the end of October 1989 (salinity = 7.63%

https://doi.org/10.1017/S1473550403001320 Published online by Cambridge University Press

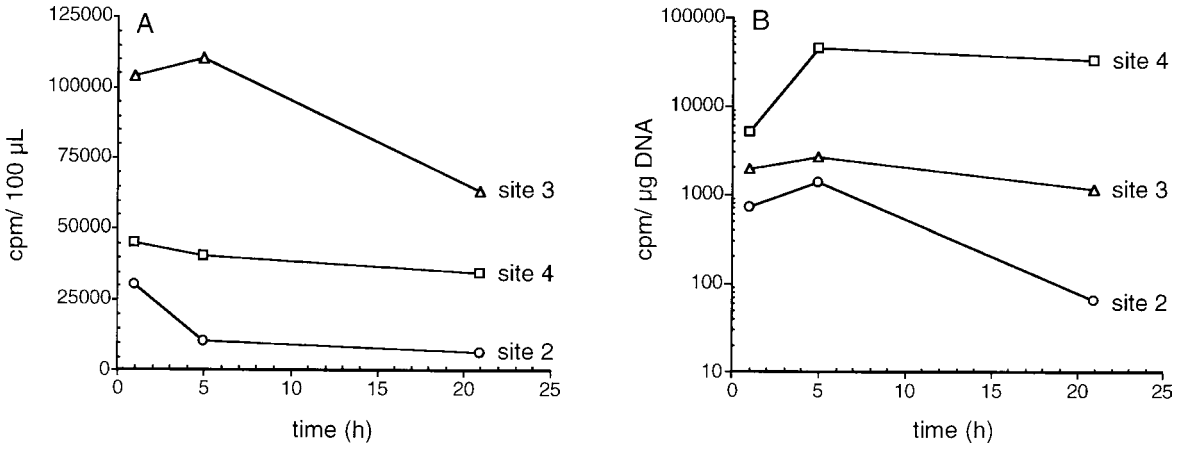

Fig. 7. Presence of fixed carbon and fixed carbon partitioned into DNA over time. The sites were pulsed with $NaH^{14}CO_3$ and chased over a 21 h period. Total fixed ^{14}C is shown in (a), while ^{14}C incorporated in to DNA is shown in (b).

NaCl) and 63.6 in March 1990 (salinity = 9.91 % NaCl) (Rothschild 1991). Similarly, carbon fixation by a cyanobacterial (primarily *Oscillatoria submembranacea* Vaucher) mat from a freshwater stream in Alabama ranged from 432 to 907 mg C fixed m^{-2} day^{-1} (Stock & Ward 1991). Stock & Ward's data are essentially comparable to those of this study, although long (3 h) incubation periods were used. Long incubations underestimate gross primary productivity because some of the fixed ^{14}C carbon may be remineralized during this period (see Fig. 2). In another study (Bebout *et al.* 1987), samples of a cyanobacterial microbial mat from Shackleford Banks, North Carolina were incubated in a tub with $H^{14}CO_3$ in a manner comparable to the protocol used in this study. If the published data are replotted using the same graphical interpolation program that was used here, the total daily fixation is 90 mg C m^{-2} day^{-1} for the Shackleford Banks mat. Similarly, Hoffman & Dawes (1980) obtained daily carbon fixation rates from diurnal fixation data in two macrophytic red algae, *Gracilaria verrucosa* and *Bostrychia* sp., in the range of 141–216 mg C fixed m^{-2} day^{-1}. The red algal data are lower than previous reports, and Hoffman & Dawes attributed the discrepancy to the fact that previous workers had not calculated total daily fixation from diurnal data. We believe that this methodological difference contributes to the discrepancy between the total daily fixation calculated for area 5 and the Shackleford Banks mat, and the total daily fixation of 10 g C fixed m^{-2} day^{-1} reported by Krumbein *et al.* (1977) for a cyanobacterial mat of Solar Lake, Sinai.

We have shown that on this beach primary production rates vary both temporally and spatially. Diurnal patterns of primary production and total daily gross production are closely linked to the availability of photosynthetically active radiation. Spatial variation depends on the quality of the mat, specifically the chlorophyl *a* to protein ratio, an indication of the proportion of primary producers. In other studies on microalgal production in the rocky intertidal, production is significantly correlated with the concentration of nutrients (nitrite, nitrate, phosphate and silicate) in the intertidal water (Bustamante *et al.* 1995). However, nutrients in the pore water cannot account for the different rates of primary production along our sand site transect as the incubation mix was from a single source. It should be noted that carbon fixation in this interstitial mat is carbon-limited (Rothschild 1995).

The ecological ramifications of the results of the primary productivity data is of great interest. Is the fixed carbon produced by the interstitial mat important energetically to the intertidal community? Exogenous inputs such as organic input from the lagoon water were not measured. But previous work on intertidal communities combined with the 'pulse-chase' data provide a starting point for answering this question.

There are several potential fates for the fixed carbon. It can stay in the system by remaining in the autotrophs as fixed carbon, the autotrophs can be eaten by consumers and the fixed carbon not remineralized by the consumers, it can be remineralized by the autotrophs and refixed, it can be excreted as fixed carbon and remain as dissolved organic carbon, or the excreted carbon can be taken up by heterotrophs. Alternatively, it can be lost to the system by remineralization (either by the autotrophs themselves or after consumption), or the fixed carbon can migrate out of the system as excreted fixed carbon or by movement of consumers (Fig. 8).

Indirect experiments were performed to assess the turnover rate of the fixed carbon. After pulsing the samples with $H^{14}CO_3^-$, the samples were analysed for total fixed carbon as well as fixed carbon that was partitioned into DNA (Fig. 7). The production of fixed carbon was higher per unit area in sites 3 and 4 than in site 2, a result consistent with the diurnal data. Most interesting was that the loss from the system from site 2 was fastest, 78 % loss in 21 h, compared with 40 % from site 3 and 24 % from site 4. As the other two sites were either submerged (site 3) or water-logged (site 4) and site 2 was not, it is likely that remineralization as opposed to diffusion of excreted carbon accounts for the loss in site 2. While vertical migration of members of interstitial mat communities has been documented (Flint 1955; Ganapati *et al.* 1959), the autotrophs are unlikely to move significant lateral distances.

https://doi.org/10.1017/S1473550403001320 Published online by Cambridge University Press

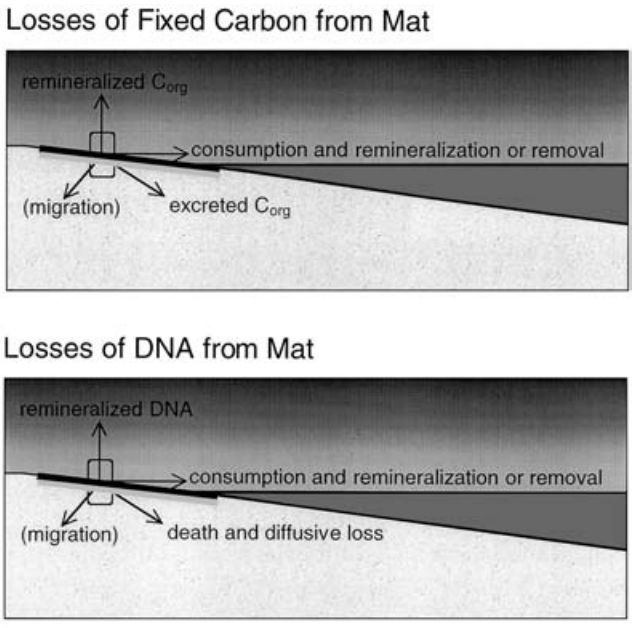

Fig. 8. Potential fate of carbon fixed by an interstitial mat. Various ways that all forms of fixed carbon, and fixed carbon subsequently incorporated into DNA, can be lost from the system are shown.

There was no significant bioturbation noted in the mat, so heavy predation by macroscopic organisms seems unlikely.

The fate of the DNA in the community was also studied. If one assumes that living organisms do not remineralize their own DNA once it has been synthesized[1], this experiment should give an indication of what the turnover rate of the autotrophs themselves might be. The labelled DNA could remain in the system by remaining in the organisms in which it was synthesized, these organisms could die but the DNA remains intact and in place, or the photosynthetic organisms could be consumed and the DNA either not degraded or the labelled nucleotides incorporated into consumer DNA. The labelled DNA could be lost from the system for a variety of reasons similar to those for loss of other forms of fixed carbon. It can be lost to the system by remineralization after consumption, or the DNA can migrate out of the system as free molecules after cell death or by movement of consumers (Fig. 8).

In contrast with total ^{14}C-labelled organic carbon, total labelled DNA increased for 5 h from the end of exposure to $H^{14}CO_3^-$ at all three sites. This is probably owing to the continued conversion of labelled organic carbon to labelled DNA. After that time, there was a loss of DNA from the sites ranging from a low of 28% from site 4 to 57% from site 3 to 95% from site 2. Because bioturbation does not appear to be heavy at site 2 and this site was by far the most water-stressed of the three, we suggest that the rapid loss of labelled DNA from site 2 was the result of cell death and subsequent remineralization of the DNA.

The pulse-chase data taken together suggest the following scenario. Site 2, the site furthest from the lagoon, had the lowest carbon fixation rates coupled with the highest rate of loss of fixed carbon and labelled DNA and no obvious bioturbation. This suggests a relatively rapid turnover of cells in the mat with a heterotrophic component that rapidly remineralizes the labelled DNA. Site 3, which was submerged in a tidal pool, had the greatest amount of carbon fixed per unit area. However, by the end of 21 h almost half of the fixed carbon and more than half of the DNA had been lost to the system. Because more of the DNA than fixed carbon had been lost, we speculate that while there was significant remineralization of fixed carbon, at least some of the remineralized carbon was re-fixed. A similar scenario is suggested for site 4. Still, in all three sites there was a net loss of labelled fixed carbon and labelled DNA, so the possibility that these mats are supporting consumers cannot yet be ruled out.

What theoretical level of heterotrophic activity might be supported by the organic carbon produced by an interstitial mat? If the mat biomass is to increase, net primary production (gross production − respiration) must be between 0 and the gross production. Gross daily production in the interstitial mat studied here was $\sim$40 mg C m^{-2}. If we assume that 1 g C $\approx$ 2 g dry weight $\approx$ 14 g wet weight (Fenchel & Finlay 1983), daily net primary production ranged from 0 to 560 mg wet weight of biomass m^{-2}. The efficiency with which a heterotroph can convert food consumed into new tissue depends on such factors as the organism, its physiological state and the nutritional quality of the food consumed. Bacteria, protozoa and poikilotherm metazoa convert input nutrients to new protoplasm with an average efficiency of $\sim$0.6 (Fenchel & Finlay 1983; Calow 1977). This implies that the interstitial sand mat could at best theoretically support a biomass of herbivores with a net daily production of 336 mg wet weight of biomass m^{-2} if gross fixation equals net fixation (e.g. none of the fixed carbon was utilized by the primary producers). However, net fixation is probably substantially lower than gross fixation because in strict autotrophs a certain percentage of photosynthate must be used to meet the metabolic needs of the autotroph.

What consumers are likely to utilize the fixed carbon produced by the interstitial mat? Bacteria are likely to consume organic carbon released into the system by excretion or cell lysis, a process that is well documented in other microbial mats. Although there are methodological difficulties with estimating release of photosynthate, estimates are from 2–12% for benthic algal mats in thermal springs (Belly *et al.* 1973; Bauld & Brock 1974). Skyring & Bauld (1990) reported that 2–3% of photosynthate was excreted by *Lyngbya aestuarii* mats over common environmental salinities. Bartley & Rothschild (unpublished observations) found that in laboratory-raised *Lyngbya aestuarii* mats not exposed to macrofauna, $\sim$14% of the fixed carbon was excreted, 77% remineralized and only 6% was retained in the particulate fraction.

[1] The known exception is the remineralization of DNA during the process of excision repair of damaged DNA. While there is preliminary data suggesting that the amount of DNA remineralized by excision repair may be significant in nature (L.J. Rothschild & C.S. Cockell, unpublished data), there has been no measure of excision repair in the *Lyngbya* or interstitial mat.

https://doi.org/10.1017/S1473550403001320 Published online by Cambridge University Press

Fauna could also utilize dissolved organic carbon, as well as graze directly on the organisms. The composition of the insterstitial fauna (or 'mesopsammon') depends strongly on the size of the interstitial spaces (Fenchel 1987). In the finest sediments, burrowing microfauna (e.g. nematodes) dominate, and other fauna are confined to the surface. Intermediate-sized sand grains (0.12–0.25 mm) are rich in ciliates, whereas in coarser sediments the interstitial fauna is dominated by metazoa (e.g. turbellarians, gastrotrichs, harpacticoid copepods and archannelids). Other phagotrophic protists of the interstitial include dinoflagellates, euglenoids, bodonids and amoebae. The distribution of the consumers can vary diurnally, influenced by such factors as the location of the oxic/anoxic boundary. Thus, consumption at a particular location in the sand mat may vary diurnally because of changes in taxonomic composition as well as diurnal changes in physiological state. And, even if a herbivore is present, it may consume resources brought in by the tide. For example, sheltered bays can collect significant quantities of drifting fronds of phaeophyta (brown) algae, and these fronds my account for 60–80% of limpet diet (Bustamante *et al.* 1994). There is a strong positive correlation between average grazer biomass and rates of primary production, especially if the herbivore biomass is not subject to predation (Bustamante *et al.* 1995). In contrast, the biomass of filter-feeders is more strongly influenced by coastal productivity as filter-feeders filter nearshore water that is washed into the intertidal (Bustamante *et al.* 1995).

Epstein *et al.* (1992) studied ingestion rates of benthic ciliates in a temperate zone sandy tidal flat community. While most species were herbivorous, and an estimated 10% of the total daily primary productivity was consumed by the ciliates, the percentage consumption varied among algal taxa. Cyanobacteria were seen infrequently in food vacuoles, and bacterivory by ciliates was low (Epstein *et al.* 1992). Since that time, Epstein (personal communication) has more recently routinely seen cyanobacteria inside protozoan digestive vacuoles and metazoan guts. Because no quantitative data are yet available, it is difficult to estimate the importance of cyanobacteria to such communities. Macrofauna are also possible, although they did not appear to be numerous and one would predict bioturbation of the mat (Lopez & Levinton 1987), which was not observed.

Subsurface intertidal mats thus may be of energetic importance to intertidal ecosystems. This result suggests an overlooked base for intertidal food chains without exogenous input. Yet, the finely laminated structure of the interstitial mat suggests minimal disturbance, so the organic input would probably be to either very small heterotrophs or in the form of excreted organic carbon.

We also analysed our data on carbon fixation in subsurface sand mats to see whether such mats might be relevant to balancing the global carbon budget. Estimates of global carbon fluxes suggest a net imbalance of sources over sinks of $\sim 1.6 \pm 1.4 \times 10^{15}$ g C yr^{-1} (Edmonds 1992; Sundquist 1993). Coastal ecosystems are thought to be an important carbon sink (Twilley *et al.* 1992) and may account for some of the missing carbon. If coastal areas ($\sim$419 800 km) were responsible for the missing carbon, it would require a daily net fixation rate of 1.0×10^4 g C m^{-2} coastline, an unlikely rate for interstitial mats alone given the data presented here. If all of the world's coastlines contained subsurface mats with a net fixation equivalent to the gross fixation presented here, subsurface mats would only account for 0.04% of the missing carbon. The mats described here are a potential coastal carbon sink that, while small on a global scale, have not been accounted for previously.

The implications of this work for astrobiology range in time and space, wherever harsh surficial conditions inhibit life. Early in Earth's history the atmosphere contained little if any molecular oxygen, which means that the resulting fluxes of solar UVB and UVC radiation on the Earth's surface were likely to have substantially higher than those of today (Rothschild 1999; Cockell & Horneck 2001). Thus, there would have been a great advantage to organisms that could access photosynthetically active radiation while having as much shading from UV radiation as possible. A subsurface mat community, such as described here, would be ideally suited in this respect. A similar argument can be made for the emergence of such communities on any other body where high UV radiation fluxes might be present, such as Mars. Thus, we suggest that looking for such communities in both the fossil record of Earth and on Mars would be a useful search strategy. As shown here, the contribution of such communities to the organic carbon input of such a location could be significant.

Acknowledgements

We wish to thank Exportadora de Sal for access to the mats, and R. Mancinelli, D. Peterson and J. Livingston for a critical review of early drafts. Valuable references were provided by M. Bertness, G. Branch, S. Epstein, I. Friedmann and L. Stal. This work was supported by a grant from NASA's Exobiology Program to LJR.

References

Amos, W.H. (1965). The living sand. *National Geographic* **127**, 820–833.

Bebout, B.M., Paerl, H.W., Crocker, K.M. & Prufert, L.E. (1987). Diel interactions of oxygenic photosynthesis and N_2 fixation (acetylene reduction) in a marine microbial mat community. *Appl. Environ. Microbiol.* **53**, 2353–2362.

Bauld, J. & Brock, T.D. (1974) Algal excretion and bacterial assimilation in hot spring algal mats. *J. Phycol.* **10**, 101–106.

Belly, R.T., Tansey, M.R. & Brock, T.D. (1973). Algal excetion of ^{14}C-labeled compounds and microbial interactions in *Cyanidium caldarium* mats. *J. Phycol.* **9**, 123–127.

Boynton, W.V. *et al.* (2002). Distribution of hydrogen in the near surface of Mars: evidence for subsurface ice deposits. *Science* **297**, 81–85.

Bustamante, R.H., Getz, W.M. & Branch, G.M. (1994). Analysis of a limpet fishery using a metaphysiological stand-growth model. *Natural Resource Model.* **8**, 139–161.

Bustamante, R.H. *et al.* (1995). Gradients of intertidal primary productivity around the coast of South Africa and their relationships with consumer biomass. *Oecologia* **102**, 189–201.

Calow, P. (1977). Conversion efficiencies in heterotrophic organisms. *Biol. Rev*. **52**, 385–409.

Carey, P.G. (1992). *Marine Interstitial Ciliates*. Chapman and Hall, New York.

Chapelle, F.H., O'Neill, K., Bradley, P.M., Methe, B.A., Ciufo, S.A., Knobel, L.L. & Lovley, D.R. (2002). A hydrogen-based subsurface microbial community dominated by methanogens. *Nature* **415**, 312–315.

Christensen, P.B., Nielsen, L.P., Sorensen, J. & Revsbech, N.P. (1990). Denitrification in nitrate-rich streams: diurnal and seasonal variation related to benthic oxygen metabolism. *Limnol. Oceanogr*. **35**, 640–651.

Chyba, C.F. (2000). Energy for microbial life on Europa. *Nature* **403**, 38–2.

Cockell, C.S. & Horneck, G. (2001). The history of the UV radiation climate of the Earth – theoretical and space-based observations. *Photochem. Photobiol*. **73**, 447–451.

Cockell, C.S. & Rothschild, L.J. (1999). The effects of ultraviolet radiation A and B on diurnal variation in photosynthesis in three taxonomically and ecologically diverse microbial mats. *Photochem. Photobiol*. **69**, 203–210.

D'Hondt, S., Rutherford, S. & Spivack, A.J. (2002). Metabolic activity of subsurface life in deep-sea sediments. *Science* **295**, 2067–2070.

Edmonds, J. (1992). Why understanding the natural sinks and sources of CO_2 is important: a policy analysis perspective. *Water Air Soil Pollution* **64**, 11–21.

Epstein, S.S., Burkovsky, I.V. & Shiaris, M.P. (1992). Ciliate grazing on bacteria, flagellates, and microalgae in a temperate zone sandy tidal flat: ingestion rates and food niche partitioning. *J. Exper. Marine Biol. Ecol*. **165**, 103–123.

Faust, M.A. (1995). Observation of sand-dwelling toxic dinoflagellates (Dinophyceae) from widely differing sites, including two new species. *J. Phycol*. **31**, 996–1003.

Fenchel, T. (1987). Ecology of Protozoa, Ch. 8.3. Science Tech Publishers, Madison, WI.

Fenchel, T. & Finlay, B.J. (1983). Respiration rates in heterotrophic, free-living protozoa. *Microbial Ecol*. **9**, 99–122.

Fielding, P.J., Damstra, K.S.J. & Branch, G.M. (1988). Benthic diatom biomass production and sediment chlorophyll in Langebaan Lagoon, South Africa. *Estuarine Coastal Shelf Sci*. **27**, 413–426.

Flint, L.H. (1955). A green sand from the gulf of Mexico. *Proc. Louisiana Acad. Sci*. **18**, 22–24.

Ganapati, P.N., Lakshmano, M.V. & Subba, D.V. (1959). Tidal rhythms of some diatoms and dinoflagellates inhabiting the intertidal sands of the Visakhapatnam beach. *Current Sci*. **28**, 450–451.

Hoffman, W.E. & Dawes, C.J. (1980). Photosynthetic rates and primary production by two Florida benthic red algal species from a salt marsh and a mangrove community. *Bull. Marine Sci*. **30**, 358–364.

Hoffmann, C. (1942). Beiträge zur Vegetation des Farbstreifen-Sandwattes. *Kieler Meeresforschungen* **4**, 85–108.

Hoffmann, C. (1949). Über die Durchlässigkeit dünner Sandschichten für Licht. *Planta* **37**, 48–56.

Krumbein, W.E., Cohen, Y. & Shilo, M. (1977). Solar Lake (Sinai). 4. Stromatolitic cyanobacterial mats. *Limnol. Oceanogr*. **22**, 635–656.

Lopez, G.R. & Levinton, J.S. (1987). Ecology of deposit-feeding animals in marine sediments. *Q. Rev. Biol*. **62**, 235–260.

Madsen, T.V. & Maberly, S.C. (1990). A comparison of air and water as environments for photosynthesis by the intertidal alga Fucus spiralis (Phaeophyta). *J. Phycol*. **26**, 24–30.

Mitrofanov, I. *et al*. (2002). Maps of subsurface hydrogen from the high energy neutron detector, Mars Odyssey. *Science* **297**, 78–81.

Moncreiff, C.A., Sullivan, M.J. & Daehnick, A.E. (1992). Primary production dynamics in seagrass beds of Mississippi Sound. The contributions of seagrass, epiphytic algae, sand microflora and phytoplankton. *Marine Ecology Progress Series* **87**, 161–171.

Nelson, D.W. & Sommers, L.E. (1982). Total carbon, organic carbon, and organic matter. In *Methods of Soil Analysis, Part 2*, ed. Page, A.L., pp. 539–579. American Society of Agronomy, Inc., Soil Science Society of America, Inc., Madison, WI.

Onstott, R.C., Phelps, T.J., Kieft, T., Colwell, F.S., Balkwill, D.L., Fredrickson, J.K. & Brockman, F.J. (2000). A global perspective on the microbial abundance and activity in the deep subsurface. In *Enigmatic Microorganisms and Life in Extreme Environments*, ed. Seckbach, J., pp. 487–500. Kluwer, Dordrecht.

Pennak, R.W. (1989). *Fresh-Water Invertebrates of the United States*, 3rd edn, p. 23. Wiley, New York.

Pinckney, J.L. & Zingmark, R.G. (1993). Modeling the annual production of intertidal benthic microalgae in estuarine ecosystems. *J. Phycol*. **29**, 396–407.

Rothschild, L.J. (1991). A model for diurnal patterns of carbon fixation in a Precambrian microbial mat based on a modern analog. *BioSystems* **25**, 13–23.

Rothschild, L.J. (1995). A 'cryptic' microbial mat: a new model ecosystem for extant life on Mars. *Advan. Space Res*. **15**, 223–228.

Rothschild, L.J. (1999). The Influence of UV Radiation on protistan evolution. *J. Euk. Microbiol*. **46**, 548–555.

Rothschild, L.J. & Mancinelli, R.L. (1990). Model of carbon fixation in microbial mats from 3,500 Myr ago to the present. *Nature* **345**, 710–712.

Schulz, E. (1937). Das Farbstreifen – Sandwatt und seine Fauna, Eine ökologisch-biozönotische Untersuchung and der Nordsee. *Kieler Meeresforschungen* **1**, 359–378.

Skyring, G.W. & Bauld, J. (1990). Microbial mats in Australian coastal environments. *Advances in Microbial Ecology*, Vol. 11, ed. Marshall, K.C., pp. 461–498. Plenum, New York.

Stal, L.J., van Gemerden, H. & Krumbein, W.E. (1984). The simultaneous assay of chlorophyll and bacteriochlorophyll in natural microbial communities. *J. Microbiol. Methods* **2**, 295–306.

Stal, L.J., van Gemerden, H. & Krumbein, W.E. (1985). Structure and development of a benthic marine microbial mat. *FEMS Micro. Ecol*. **31**, 111–125.

Stevens, T.O. & McKinley, J.P. (1995). Lithoautotrophic microbial ecosystems in deep basalt aquifers. *Science* **270**, 450–454.

Stock, M.S. & Ward, A.K. (1991). Blue–green algal mats in a small stream. *J. Phycol*. **27**, 692–698.

Sundquist, E.T. (1993). The global carbon dioxide budget. *Science* **259**, 934–941.

Twilley, R.R., Chen, R.H. & Hargis, T. (1992). Carbon sinks in mangroves and their implications to carbon budget of tropical coastal ecosystems. *Water Air Soil Pollution* **64**, 265–288.

Varela, M. & Penas, E. (1985). Primary production of benthic microalgae in an intertidal sand flat of the Ria-de-Arosa, northwest Spain. *Marine Ecology Progress Series* **25**, 111–120.

Warburg, O. & Christian, W. (1942). Isolierung und Kristallisation des Gärungsferments Enolase. *Biochem. Zeit*. **310**, 384–421.

Warming, E. (1904). Bidrag til Vadernes, Sandernes og Marskens Naturhistorie. *Kongelige Danske Videnskabernes Selskabets Skrifter* **7 RII**, 1–56.

Weiss, B.R., Yung, Y.L. & Nealson, K.H. (2000). Atmospheric energy for subsurface life on Mars? *Proc. Natl. Acad. Sci., USA* **97**, 1395–1399.