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Evaluation of nutrient limitation of CO_2 and N_2 fixation in marine microbial mats

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ABSTRACT: Photosynthetic CO_2 fixation and N_2 fixation are fundamentally important in mediating production dynamics of intertidal and subtidal marine microbial mat communities. We examined nutrient [N, P, Fe, Mo, dissolved organic carbon (DOC)] limitation of CO_2 and N_2 fixation in geographically and physiologically diverse mats. Nitrogen enrichment (as NO_3^-) infrequently stimulated CO_2 fixation. Phosphorus, Fe and Mo enrichment generally failed to stimulate CO_2 fixation. The frequent absence of N limitation appears linked to the ability of mat microbial communities to fix N_2 and effectively recycle fixed N. Nitrogen fixation was enhanced by DOC, while no P, Fe or trace element stimulation was observed. The lack of nutrient stimulation of CO_2 fixation appeared related to low net growth rates of some mats. Slow growing mats, including hypersaline, stromatolitic (Storrs Lake, Bahamas) and certain hypersaline, lagoonal mats (Guerrero Negro, Baja California, Mexico) exhibited virtually no nutrient stimulation of either CO_2 or N2 fixation. More productive coastal (North Carolina, USA) and estuarine (Tomales Bay, California, USA) mats showed higher frequencies of nutrient limitation of either process. Seasonally, N and DOC stimulation were most profound during periods of maximum growth. Mats are able to minimize C and N limitation by metabolically coupling CO_2 and N_2 fixation as sources of 'new' C and N inputs respectively. Phototrophic-heterotrophic microbial consortia appear to mediate coupling, which minimizes losses of fixed C and N to overlying waters.

INTRODUCTION

Laminated benthic microbial mats are geographically widespread, ubiquitous and often highly productive components of estuarine and marine inter- and subtidal environments (Whitton & Potts 1982, Cohen et al. 1984, Cohen & Rosenberg 1989). Recent studies have shown mats to be far more common and important from production and biogeochemical cycling perspectives than previously assumed (Gebelein 1976, Krumbein & Cohen 1977, Bauld et al. 1979, Paerl et al. 1981, Whitton & Potts 1982, Canfield & Des Marais 1993). Mats vary widely in appearance, ranging from barely perceptible mucilagenous coatings on sand, mud and organic debris to well-developed, accreted, multilayered 'leathery' carpets dominating lagoonal, reef, mud and sandflat as well as saltmarsh ecosystems. Perhaps the most striking 'permanent' form of mats are stromatolites, highly laminated mounds preserved through calcification associated with mat-building cyanobacteria and bacteria (Gebelein 1976, Golubic 1976, Monty 1976, Walter 1976, Cohen & Rosenberg 1989).

The ability of mats to accumulate, either as calcified or leathery laminae, has in part been related to the physical stability of the habitats in which they are found (Cohen & Rosenberg 1989). From a biochemical perspective, however, it is remarkable how mats can fluorish and accrete in nutrient depleted, oligotrophic waters (Whitton & Potts 1982, Cohen & Rosenberg 1989). Striking examples are tropical and subtropical reefs and sand flats, where mats appear to thrive despite a 'desert-like' void in ambient planktonic, macroalgal and macrophyte production (Webb et al. 1975, Wiebe et al. 1975). Work by Webb et al. (1975), Wiebe et al. (1975) and more recently by others (Bauld et al. 1979, Bautista & Paerl 1985, Stal & Krumbein 1985, Bebout et al. 1987) has identified diazotrophy (N_2

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fixation) as a process circumventing chronic N-limitation, a condition characterizing marine ecosystems in which mats are frequently found. N_2 fixation is important in meeting mat and ecosystem (in the case of reefs, for example; see Webb et al. 1975) N demands, while in other cases N_2 fixation serves as a supplementary as opposed to exclusive source of 'new' nitrogen (Bebout 1992, Joye & Paerl 1993a). Both situations indicate that environmental factors regulating N_2 fixation may play important roles in controlling mat primary production. Specifically, nutrient supply may control N_2 and CO_2 fixation potentials.

During the past 5 yr, we have investigated nutrient-N_2 fixation-production interactions among geographically and physiologically diverse mat systems, including seasonal impacts of nutrient limitation on CO_2 and N_2 fixation under natural irradiance and temperature conditions. Here, we evaluate results from nutrient addition bioassays conducted on mats representative of temperate, subtropical and tropical intertidal and subtidal marine habitats.

METHODS AND MATERIALS

Research sites. ***North Carolina Atlantic barrier island habitats:*** North Carolina's (USA) Atlantic Ocean coast is characterized by a barrier island system (i.e. Outer Banks). The barrier islands are composed of intertidal lagoon, salt marsh and mudflat regions supporting mats. Mats are largely comprised of filamentous, non-heterocystous (*Lyngbya* spp., *Oscillatoria* spp., *Microcoleus* spp.) and single celled (*Synechocystis* sp., *Synechococcus* sp.) cyanobacteria and diatoms as dominant oxygenic phototrophs (Polimeni 1976, Bebout et al. 1987). In subsurface layers, anoxic phototrophs, including *Chromatium* sp., *Chlorobium* sp., and *Rhodospeudomonas* sp., have been observed. In addition, a broad range of oxic and anoxic heterotrophic, chemoorganotrophic and chemolithotrophic bacteria resides in these mats.

Mats at 2 locations near the Institute of Marine Sciences (IMS; Morehead City, NC) were chosen for study: the western lagoonal sandspit of Shackleford Banks (SB; approximately 6 km from IMS), and a lagoonal region of Carrot Island, a component of the Rachel Carson National Estuarine Research Reserve (RCNERR), located near Beaufort, NC (approximately 5 km from IMS). Mats at both locations were frequently sampled (in the case of RCNERR, every 2 to 3 wk), facilitating seasonal examinations of nutrient limitation.

Storrs Lake, San Salvador, Bahamas: Storrs Lake, located on San Salvador Island, Bahamas, is a small (ca 100 ha), shallow (3 m maximum depth), hypersaline (60 to ca 100 ppt), highly turbid lake, dominated by extensive littoral submerged and partially emerged mats dominated by the heterocystous N_2 fixer *Scytonema*. The deeper, central portions of the lake support actively, albeit very slowly, growing calcifying stromatolites (Neumann et al. 1990, Bebout 1992). Stromatolites are covered by mats, comprized of a mixture of non-heterocystous cyanobacteria (*Phormidium*, *Oscillatoria*, *Synechocystis*, etc.). Both littoral *Scytonema* and submersed stromatolitic mats are photosynthetically active and capable of N_2 fixation (acetylene reduction) at relatively low rates (Bebout 1992). Low growth rates were evidenced by dye marker (Bebout 1992) and ^{14}C dating methods (Paull et al. 1993).

Guerrero Negro, Baja California, Mexico: Permanently submersed mats were examined in hypersaline ponds, which are part of a large salt evaporation concern (Exportadora de Sal, S.A.) located at Guerrero Negro, on the Pacific side of Baja California, approximately half-way down the Baja California peninsula. Mat community composition varies according to salinity regimes, which are closely controlled by the salt company. We examined a hypersaline (ca 60 ppt) mat community dominated by the non-heterocystous, aggregated filamentous cyanobacterial N_2 fixer *Microcoleus* spp. This mat also contained photosynthetic bacteria, and a diverse community of microheterotrophic and chemolithotrophic bacteria. The mats have been thoroughly documented by Javor & Castenholz (1981) and Des Marais and coworkers (Des Marais et al. 1989, Canfield & Des Marais 1993).

In addition, we examined an intertidal, slightly hypersaline (ca 40 ppt) lagoonal mat adjacent to the salt evaporation pond system. This mat is regularly covered by seawater originating from the Ojo de Liebre lagoon, from which the salt company draws its water. This mat was dominated by the non-heterocystous N_2 fixer *Lyngbya* spp. Photosynthetic bacteria can be found in patches on the underside of the leathery *Lyngbya* surface mats. In general, rates of CO_2 fixation [as well as DIC (dissolved inorganic carbon) utilization], O_2 production and N_2 fixation are approximately 10 times higher in the *Lyngbya* as opposed to *Microcoleus*-dominated mats (Bebout 1992, Canfield & Des Marais 1993).

Tomales Bay, California: Tomales Bay is a shallow, narrow and long (20+ km) estuary formed by the San Andreas fault as it transits from coastal northern California into the Pacific Ocean. Extensive mudflats comprise 60 to 70% of the bay's intertidal region. These mudflats are generally covered by highly productive, laminated mats (Joye & Paerl 1993b). Cyanobacteria and diatoms are the dominant primary producers, but diverse anoxygenic phototrophs (photosynthetic bac-

teria), heterotrophs, and chemolithotrophs also form an important fraction of the microbial biomass. Dominant cyanobacteria include filamentous, non-heterocystous genera: *Lyngbya* sp., *Microcoleus* sp., and *Oscillatoria* sp. On occasions, colonial coccoid cyanobacteria, including *Merismopedia* sp., *Synechocystis* sp. and *Gleothece* sp., were important contributors to the phototrophic biomass.

Two representative intertidal mat communities were examined, one at the southern end of the bay, near the largest freshwater inflow (Lagunitas Creek), the other on a creek discharge delta (Walker Creek Delta), near the bay's mouth. Salinities ranged from 20 to 28 ppt at the Lagunitas site and 20 to 32 ppt at the Walker Creek site. The 2 sites are 20 km apart.

Sampling and bioassay procedures. Mats were easily removed from respective substrates by cutting a 4 to 5 cm thick, 200 to 400 cm^2 surface area section with a scalpel, carefully inserting a plastic or wooden plate underneath the cut section (making sure to include all the vertical biogeochemical zonations) and transporting the mat in plastic dish pans to a nearby outdoor location for further dissection and incubation. Care was taken to keep mats moist with ambient seawater during transport. In most instances, mat pieces were immediately prepared for bioassays. In approximately 20% of cases, overnight storage (12 to 16 h) in either circulating or freshly added ambient water was required.

Previous studies (Bautista & Paerl 1985, Bebout et al. 1987) indicated that it was imperative to examine mat community photosynthetic and diazotrophic responses to environmental factors within a day of sampling and to monitor these responses within a period of 2 to 4 d. These constraints were established because mat microbial community composition and structural changes were evident after enclosure (either in running or stagnant seawater) over extensive periods (>3 d). Accordingly, bioassays were conducted for no longer than 72 h.

Bioassays were conducted on 25 cm^2 mat subsamples. Triplicate or quadruplicate (for each treatment) subsamples were cut by scalpel and placed in cleaned (0.1 N HCl, followed by 2 rinses of distilled, deionized water) 20 × 20 cm square, 500 ml polyethylene containers (i.e. Tupperware), to which 200 or 300 ml of ambient seawater was added. Care was taken to include all biologically active vertical zones when sectioning mats. Nutrients were then added with a cleaned plastic pipette and gently mixed into seawater. The choice of nutrients was based on their potential roles as limiting factors for marine photosynthetic CO_2 and N_2 fixation (Smith 1984, Paerl et al. 1987, Howarth et al. 1988). Nutrient concentrations added were based on previously established concentrations known to stimulate CO_2 and N_2 fixation in coastal and estuarine waters of North Carolina (Paerl et al. 1987, 1990, Paerl & Prufert 1987). Nutrients added included: dissolved inorganic nitrogen as NO_3^- ($NaNO_3$ at 20 to 200 µM), dissolved inorganic phosphorus as PO_4^{3-} (NaH_2PO_4 at 20 to 100 µM), dissolved molybdenum (Na_2MoO_4 at 1 to 10 µM), dissolved iron ($FeCl_3$ in equimolar EDTA at 1 to 20 µM), a trace metal mixture (including 2 µM Fe, 1 µM Mo, Co, Cu, Mn, Zn and B), and dissolved organic carbon (mannitol, D-maltose and D-glucose, at 5 to 10 mM). All organic nutrient stock solutions were sterilized by autoclaving prior to use.

Except for specified (see below) experiments on RCNERR mats, all mat types were incubated under natural sunlight and temperature conditions. When intense sunlight (>1500 µE m^{-2} s^{-1}) prevailed, a layer of neutral density fiberglas screening was placed over the bioassay containers to prevent potential photoinhibition and photorespiration in mat surface layers. This reduced incident irradiance by approximately 25%.

In one set of experiments, we tested the potential for molybdenum limitation of N_2 fixation, a possibility recently proposed for marine environments by Howarth & Cole (1985). These experiments were performed on RCNERR mats placed in a greenhouse several days prior to nutrient additions. Molybdenum (Na_2MoO_4 at 1 and 10 µM) was added alone and in combination with 5 mM D-glucose. Greenhouse-incubated mats were exposed to warmer than ambient temperatures, in order to optimize production and N_2 fixation potentials. Nutrients were added at least 24 h prior to assays.

All bioassays were gently agitated by swirling twice daily to ensure mixing and diffusion of nutrients and gases. Treatments were subsampled daily, starting 16 to 24 h after nutrient additions. Pieces of 1 cm^2 were cut with a scalpel. Triplicate pieces were cut for N_2 fixation (acetylene reduction) and primary productivity ($^{14}CO_2$ fixation) assays on controls and various treatments.

For acetylene reduction assays, 1 cm^2 subsamples were placed in either 15 or 30 ml serum bottles to which 10 or 20 ml water was added from respective treatments. Serum bottles were stoppered and 4 or 7 ml of CaC_2-generated acetylene was injected into the water phase. Serum bottles were then placed, with mat surfaces facing up, into a flowing water bath exposed to natural irradiance. Triplicate light and dark bottles were run for each treatment and controls. Samples were agitated every 30 min to minimize formation of diffusional gradients. Incubations lasted from 2 to 4 h near midday. They were terminated by shaking bottles for 30 s and withdrawing 9 ml headspace samples, transferring the gas to 9 ml evacuated serum bottles for transport to the laboratory. There, 0.3 ml was with-

drawn and assayed for ethylene production using either Shimadzu GC 9 or GC 14 gas chromatographs equipped with flame ionization detectors at 200°C. A 2 m long Poropak T column, heated to 80°C, separated ethylene and acetylene. Nitrogen was the carrier gas. Ethylene production was quantified against a standard curve derived from known quantities of UHP ethylene (Matheson).

Primary production was estimated by photosynthetic $^{14}CO_2$ incorporation (Bebout et al. 1987, Paerl et al. 1987). Triplicate light-treatment and single dark-treatment 1 cm^2 mat pieces were placed in pre-cleaned 22 ml scintillation vials having polypropylene-lined caps. Twenty ml of bioassay water and 0.2 ml ^{14}C-$NaHCO_3$ (2.9 µCi total activity; 58 mCi $mmol^{-1}$ specific activity; ICN, Inc.) were added. Vials were then sealed, gently mixed and placed on their sides, with mat surface facing up, in the same water bath used for acetylene reduction assays. Vials were agitated to ensure uniform distribution of isotope, nutrients and gases. DIC was determined by infrared analyses (Beckman 865 IR analyzer) of acidified bioassay water (Paerl 1987).

Incubations were terminated by removing mat pieces from vials, and placing them face up in a fuming HCl atmosphere for 30 min. This step inactivated photosynthesis and volatilized abiotically precipitated ^{14}C (Paerl 1987). Mat pieces were then air dried and processed for liquid scintillation spectrometry, using Cytoscint cocktail (ICN, Inc.). Quenching was corrected for by developing a quench curve with unlabeled mat pieces to which known amounts of calibrated ^{14}C-hexadecane (New England Nuclear, Inc.) were added.

Daily photosynthetically active radiation (PAR; 400 to 700 nm) flux was monitored over 10 min intervals with a LiCor 2π quantum sensor, coupled to a LI-1000 data logger. Ambient temperatures and meteorological conditions were routinely monitored.

RESULTS AND DISCUSSION

Nutrient limitation of primary production

Some general and specific (for mat types) patterns of nutrient limitation of primary production emerged. Inorganic nitrogen additions, as NO_3^-, stimulated primary production (CO_2 fixation) relative to controls occasionally in Tomales Bay (Table 1), and far less frequently in RCNERR mats (Table 2). When added in parallel at equimolar (to NO_3^-) concentrations, NH_4^+ enrichments yielded similar stimulatory (or lack thereof) effects on primary production (Table 2). When NO_3^- stimulation occurred, it proved effective over a wide range of concentrations; even additions as small as 20 µM elicited a positive response. Stimulation was evident within 24 h, indicating a rapid ability to utilize and biochemically reduce NO_3^-. Hence, periodic fluxes of dissolved inorganic nitrogen (DIN)-enriched water over the mats are likely to be assimilated rapidly, and serve to support accelerated mat growth. Rapid utilization of NO_3^- fluxes by dissimilatory processes, including denitrification, has also been documented (Joye & Paerl 1993a, b).

Table 1 CO_2 fixation bioassays, Tomales Bay, CA (values in mg C m^{-2} h^{-1}). Ctr.: control; N: 100 µm NO_3; P: 50 µM PO_4; M: 10 mM mannitol; TM: trace metal mixture; WCD: Walker Creek Delta; LAG: Lagunitas Creek; nd: not determined

Month	Site	Ctr.	+N	+P	+M	+N&P	+M&P	+TM
Nov 1990	WCD	2.8	5.9*	3.7	3.4	4.3	3.2	nd
	LAG	2.2	13.3*	1.97	3.3	2.1	2.4	nd
Mar 1991	WCD	12.1	16.7	9.7	10.4	19.8*	13.3	15.6
	LAG	7.2	7.9	7.5	7.1	7.9	8.6	7.9
Jul 1991	WCD	6.6	7.3	5.4	5.8	9.5	4.3	4.9
	LAG	5.2	4.7	5.4	2.8	7.3	4.0	5.3
Nov 1991	WCD	14.7	21.4	18.6	17.0	24.3*	21.5[a]	22.4
	LAG	5.9	5.8	7.0	6.2	2.6	7.2	4.9
Mar 1992	WCD	8.3	8.8	6.6	6.0	9.4	6.1	4.5
	LAG	16.4	15.8	17.9	9.6	19.7	6.9	10.2

[a] On this date 10 mM glucose was substituted for mannitol and phosphate at the WCD site

*Denotes where nutrient additions yielded significant ($p < 0.05$; 1-way ANOVA) stimulation relative to controls

Table 2. CO_2 fixation bioassays, Rachel Carson National Estuarine Research Reserve (RCNERR) site, Beaufort, NC (values in mg C m^{-2} h^{-1}). Ctr.: control; P: 2 µM PO_4; N: 10 µM NO_3; NH_4: 10 µM NH_4; Fe: 5 µM Fe-EDTA; M: 5 mM mannitol; nd: not determined

Month	Ctr.	+P	+N	+NH_4	+Fe	+M
Aug 1990	53.5	81.2*	56.3	nd	52.7	49.9
Sep 1990	30.8	61.5*	59.5*	47.8	nd	90.4*
Oct 1990	12.1	9.6	13.5	12.2	nd	10.9
Jan 1991	6.9	6.5	8.6	12.5	nd	7.6
Mar 1991	12.2	15.6	18.9	12.9	nd	13.2
May 1991	84.8	49.3	71.5	68.8	nd	60.1

*Denotes where nutrient additions yielded significant ($p < 0.05$; 1-way ANOVA) stimulation relative to controls

The above results generally proved true for mats exhibiting relatively high net rates of primary productivity, including those found in Tomales Bay and North Carolina's outer banks (Table 3). Among slower-growing mats, such as the extremely slow-growing calcifying stromatolitic mats in hypersaline Storrs Lake and the hypersaline lagoonal mats of Guerrero Negro (Table 3), nutrient additions (of any type) generally failed to stimulate CO_2 fixation. In interpreting Table 3, it should be noted that primary production ranges presented are based on different techniques, thus making quantitative comparisons difficult. It is likely, for example, that O_2 production (gross production) represents overestimates, while $^{14}CO_2$ fixation (closer to net production, but hampered by diffusional artifacts; Bebout 1992) represents underestimates. Values are therefore approximate indicators of relative growth rates of physiologically diverse mat types. Based on these approximations, it appears that the lack of nutrient stimulation may in part be a methodological problem. In some mats net growth was often so slow and variation between replicates so large during a 24 or 48 h bioassay period that it was not possible to document significant growth enhancement.

The problem with choosing an adequate (for detecting growth responses) and appropriate (in terms of microbial community composition) bioassay response period is a formidable one. On the one hand, it is essential to run bioassays long enough to obtain measurable growth responses, which in the case of slow-growing mats most certainly exceeded the 72 h alloted incubation period. On the other, it is desirable to maintain representative community composition, which in the case of faster-growing mats was noticeably altered after 72 h. In this regard, efforts at determining nutrient and other (light, DIC, diffusion, grazing, etc.) limitations may benefit from parallel longer-term (weeks) incubations of larger field plots (Van Raalte et al. 1976).

Phosphorus additions failed to stimulate CO_2 fixation above controls in all systems, except on 2 occassions, when marginal stimulation was observed in the RCNERR mats (Table 2). Neither Fe, Mo or a trace metal mixture significantly enhanced CO_2 fixation in all mats (Tables 1 & 2). This proved true when metals were added singly or in combination with other nutrients. It appears that P and metal supplies are sufficient for meeting photosynthetic production demands in most mats, even when production is enhanced by N or organic matter.

The addition of dissolved organic carbon, DOC (D-glucose, D-maltose and mannitol), generally did not have a significant impact on primary production in mats, except for one occasion at RCNERR. At this time, DOM (mannitol) stimulated production as did NO_3^- (Table 2). In prior work (Paerl et al. 1993) DOM and dissolved organic nitrogen (DON) compounds (amino acids) were readily assimilated by mat heterotrophs and, more importantly, photoheterotrophs (including dominant primary producers such as diatoms, cyanobacteria and photosynthetic bacteria). Our observed stimulation of CO_2 fixation by DOC may therefore be closely coupled to stimulation of mat phototrophs, perhaps initially via the enhancement of light-mediated DOC assimilation (photoheterotrophy) (Paerl et al. 1993). The underlying mechanism(s) for contemporaneous stimulation of phototrophy and photoheterotrophy by these compounds are currently unknown.

In some cases, reduced rates of primary productivity were observed in response to DOC additions (Table 1). It is suspected that this decline may have been due to an increase in the DIC pool, resulting from metabolic breakdown of added DOC compounds. The overall effect of this would have been decreased specific activity of the ^{14}C-CO_2 pool; this would result in underestimates of CO_2 fixation rates.

The Tomales Bay and RCNERR mats were sampled frequently enough to evaluate seasonal patterns of nutrient limitation. At Tomales Bay, significant ($p < 0.05$) N limitation of CO_2 fixation was observed in the fall of 1990 at both sites (Table 1). Statistically insignifi-

Table 3. Estimated ranges and means of daily primary productivity determined on the microbial mat types assayed for nutrient limitation. Productivity values are based on either net dissolved inorganic carbon (DIC) flux (Des Marais & Canfield 1992, Canfield & Des Marais 1993), $^{14}CO_2$ fixation (light minus dark bottle technique; Paerl 1987, Bebout et al. 1987), or O_2 evolution (gross production; Bebout 1992) techniques

Mat type/Location	Primary prod. (mg C m^{-2} d^{-1}, ×10)		Method
	Range	Mean	
Scytonema mats, Storrs Lake, Bahamas	2.4 – 12	4.5	$^{14}CO_2$ fixation
	18.0 – 34	24.0	O_2 evolution
Microcoleus mat, Guerrero Negro, Mexico	–7.2 – 2.4	~0	DIC flux
Lyngbya mat, Guerrero Negro, Mexico	54	54	DIC flux
	6.0 – 24.0	19	$^{14}CO_2$ fixation
Lyngbya/Microcoleus mat, RCNERR, NC	7.2 – 114.0	35	$^{14}CO_2$ fixation
Lyngbya/Microcoleus mats, Tomales Bay, CA	6.0 – 90.5	57	$^{14}CO_2$ fixation

cant N and N+P stimulation was observed during both spring and fall of 1991 at the Walker Creek site (Table 1). Nitrogen-stimulated CO_2 fixation was also evident in the RCNERR mats, but high variability among triplicates tended to obscure statistical significances between treatments and controls. Generally, maximum N stimulation occurred during the spring and fall periods (Tables 1 & 2). In North Carolina coastal waters, this pattern coincides with periods of most profound N limitation (Paerl et al. 1990, Rudek et al. 1991). Although examined over fewer intervals (quarterly), results from the Tomales Bay mats generally concur with the North Carolina findings. The addition of NO_3^- significantly ($p < 0.05$; 1-way ANOVA) stimulated CO_2 fixation in approximately 20% of both the Lagunitas and Walker Creek samples. NO_3^- plus PO_4^{3-} significantly stimulated 33% of the Walker Creek samples. In Tomales Bay, maximum N stimulation of mat CO_2 fixation coincides with minimum water column DIN concentrations (S. V. Smith & J. T. Hollibaugh pers. comm.).

In all mat systems, stimulation of microalgal production is rapid (within 24 h) and maintained as long as N enrichment continues, in support of longer-term enrichment experiments (Van Raalte et al. 1976). It would therefore appear that in a large number of cases, mat systems are poised to rapidly respond to N enrichment pulses, an observation shared with other N assimilatory and dissimilatory transformation (NO_3^- reduction, denitrification, nitrification) studies (Joye & Paerl 1993a, b).

Table 4. N_2 fixation (acetylene reduction) bioassays, Tomales Bay, CA (values in nmol ethylene cm^{-2} h^{-1}). Ctr.. control; N: 100 µm NO_3; P: 50 µM PO_4; M: 10 mM mannitol; TM: trace metal mixture; WCD: Walker Creek Delta; LAG: Lagunitas Creek; nd: not determined

Month	Site	Ctr.	+N	+P	+M	+N&P	+M&P	+TM
Jul 1990	WCD	8.3	nd	8.4	18.6*	nd	nd	nd
	LAG	22.7	nd	6.7*	50.7*	nd	nd	nd
Nov 1990	WCD	5.5	2.4	9.2*	13.3*	3.6	17.5*	nd
	LAG	7.3	5.0	6.6	18.0*	4.5	8.6	nd
Mar 1991	WCD	25.0	1.2	21.9	85.3*	12.8	114.7*	42.7
	LAG	20.3	36.4	18.7	310.3*	26.3	276.0	12.9
Jul 1991	WCD	47.8	33.1	51.7	60.7	38.9	58.3	64.2
	LAG	5.2	6.5	3.0	23.7*	6.3	13.7*	1.4
Nov 1991	WCD	24.7	13.7	33.7	105.0*	40.3	120.3*[a]	24.7
	LAG	2.7	3.0	10.0	38.3*	27.0*	9.3	16.0*
Mar 1992	WCD	8.3	6.6	8.8	6.0	9.4*	6.1*	4.5
	LAG	nd	0.7	2.0	6.7*	0.7	6.3*	0.3

[a] On this date 10 mM glucose was substituted for mannitol and phosphate at the WCD site

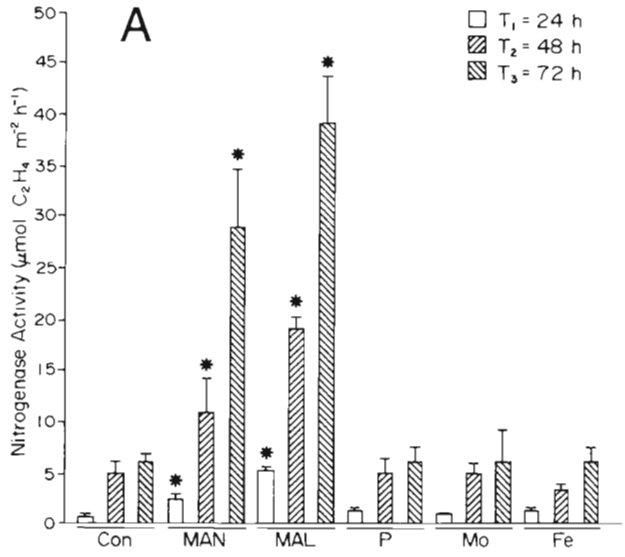
* Denotes where nutrient additions yielded significant ($p < 0.05$; 1-way ANOVA) stimulation relative to controls

Nutrient impacts on mat N_2 fixation

The general observation of N-stimulated mat primary production, together with observed rapid production responses to N enrichment, suggests that potential sources of biologically available 'new' N are both in high demand and readily utilized to support primary production. In this regard, factors controlling 'new' N input, namely N_2 fixation, may be of considerable importance in mediating mat production.

To varying degrees, all mat systems exhibited N_2 fixation; this process contributes significant amounts of 'new' N (ranging from 10 to nearly 100% of mat primary production N demands) (Bebout 1992, Joye & Paerl 1993a, b). By far, the most important and consistent nutrient controlling N_2 fixation (nitrogenase activity, NA) in bioassays was DOC, supplied as either glucose, maltose or mannitol (Table 4, Figs. 1 & 2). In previous work on diverse North Carolina mats, other sugars (sucrose, mannose, xylose, galactose) and organic acids (acetate, malate, lactate) elicited stimulation of NA, although the magnitude of stimulation varied with the amount of C added (Paerl & Prufert 1987). Stimulation of NA by DOC was most profound, persistent (seasonally) and significant ($p < 0.05$) in Tomales Bay (Table 4). The RCNERR and SB mats also exhibited DOC stimulation (Fig. 1).

The slower-growing Guerrero Negro and Storrs Lake mats showed less profound stimulation by DOC additions (Fig. 2). As with CO_2 fixation studies, the relatively slow growth rates of these mat types prevented clear-cut observations of nutrient stimulation of NA. Added to this was the problem of high variability among triplicates. This variability was in large part attributable to the heterogeneity of mats (particularly stromatolitic mats, which were highly convoluted). It should also be pointed out that the Storrs Lake littoral mats were dominated by a heterocystous cyanobacterium (*Scytonema* spp.), in contrast to all other mats, in which non-heterocystous genera prevailed. Previous work has indicated that DOC stimulation of NA is more prevalent in mats dominated by non-heterocystous cyanobacteria (Paerl et al. 1987, Paulsen et al. 1991). The likely reason for this differential effect is that non-heterocystous cyanobacterial N_2 fixation is more sensitive to O_2 inactivation than it is among heterocystous cyanobacteria. Since a well-known effect of DOC enrichment in mats is enhanced O_2



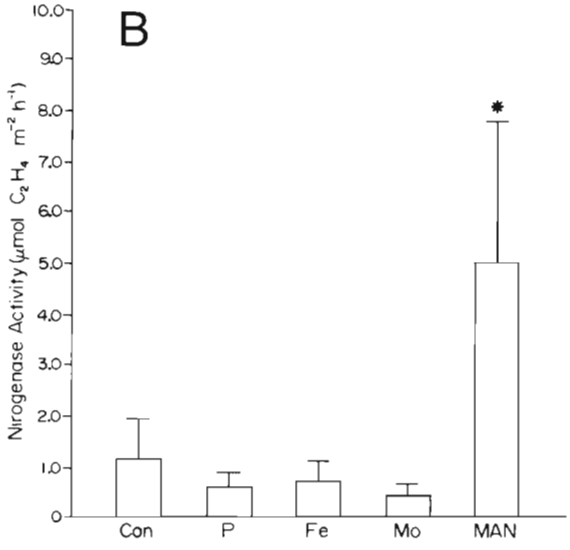


Fig. 1. (A) Nutrient limitation of N_2 fixation (acetylene reduction) in a Shackleford Banks, NC, mat monitored over a 72 h period. Note the consistently significant ($^*p < 0.05$; 1-way ANOVA) stimulation (relative to controls) when dissolved organic matter, either as 5 mM mannitol or maltose, was added. The stimulatory impact increased substantially during the course of this bioassay. (B) Bioassay illustrating the highly significant impact of dissolved organic matter (5 mM mannitol) on N_2 fixation in a *Lyngbya*- and *Microcoleus*-dominated mat located at the RCNERR site, NC. No significant impacts of inorganic nutrient additions were observed in this and other NC mat systems

consumption, this mechanism may provide some protection for N_2 fixation in non-heterocystous mats. Overall, Storrs Lake mats consistently exhibited the lowest rates of primary production and NA among all mat types. Furthermore, NA was partially suppressed by the hypersaline (>60 ppt salinity) conditions characterizing overlying and interstitial waters (Bebout 1992). We suspect that the rather weak signal for nutrient limitation may have in part reflected salt stress and low net growth rates, attributable to rapid light extinction exerted by the mats themselves.

There was agreement that, among all mats and over a range of concentrations, Fe, individual and combined trace metals did not limit NA (Table 4, Figs. 1 & 2). Moreover, when NA was enhanced by DOC addition, further additions of Fe or trace metals failed to additionally stimulate NA (Paerl et al. 1987, Paerl 1990, Paulsen et al. 1991), evidence that existing pools, internal cycling and supply rates of these metals were sufficient for meeting mat production demands.

Molybdate additions, at either 1 or 10 μM, to both oxic (O_2 sparged) and anoxic (N_2 sparged) mats did not alter NA when compared to mats not enriched with Mo

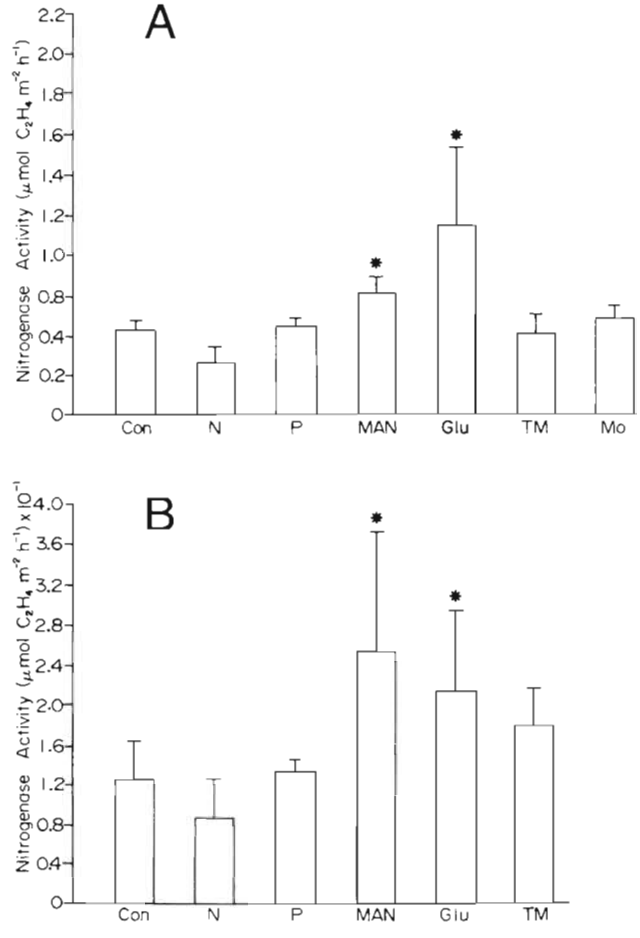


Fig. 2. Effects of a range of nutrient additions on N_2 fixation in the (A) *Lyngbya*- and (B) *Microcoleus*-dominated mats located at Guerrero Negro, Baja California, Mexico. Only dissolved organic matter (D-glucose and mannitol, added at 5 mM each) additions led to significant ($^*p < 0.05$) stimulation of this process

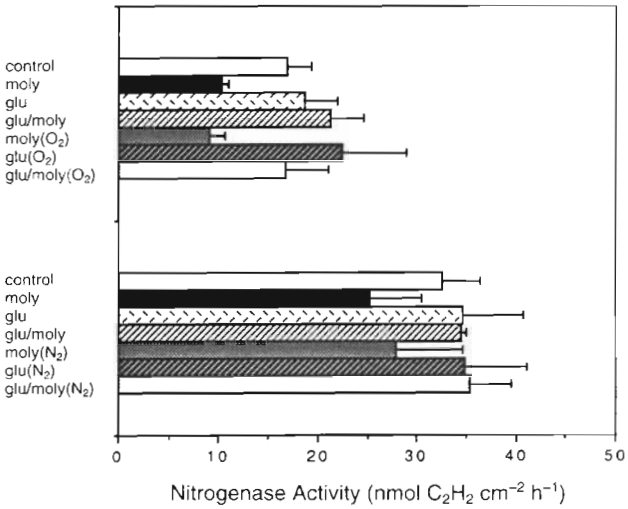


Fig. 3. Impacts of molybdate (moly; at 1 µM), alone and in combination with 5 mM D-glucose (glu), on nitrogenase activity in RCNERR mat incubated under ambient, O_2 sparged (oxic) and N_2 sparged (anoxic) conditions. Oxic and anoxic conditions were confirmed by O_2 microelectrode measurements conducted after sparging. Nitrogenase activity measurements were made during the day following nutrient additions. Responses during oxic conditions were examined during midday (11:00 to 14:00 h) while anoxic conditions were examined at nighttime (00:00 to 05:00 h). Note that nitrogenase activity was generally higher at night than during the day, attributable to daylight photosynthetic O_2 inhibition of nitrogenase, also reported by Bebout et al. (1987) and Paerl et al. (1989) for this mat type

(Fig. 3). On the contrary, Mo-enriched oxic mats revealed significantly ($p < 0.05$) lower rates of NA than did untreated mats (Fig. 3). The lower rates of NA are likely due to inhibition of sulfate reduction, which is known to be carried out by bacteria capable of diazotrophy. Decreases in NA as a result of sulfate reduction inhibition have previously been documented by Bebout (1992). Nitrogenase activity was not affected by Mo enrichment in anoxic mats. Furthermore, glucose additions were instrumental in overriding the inhibitory effect (on NA) of oxygenation, while Mo additions were not.

These results discount the hypothesis put forth by Howarth & Cole (1985) that constraints on molybdenum availability (due to uptake competition by SO_4^{2-}) are broadly responsible for limiting marine N_2 fixation. While SO_4^{2-} uptake competition with the structural analogue MoO_4^{2-} may be taking place in mats (especially under oxic conditions), there appears to be sufficient Mo entering or stored by diazotrophs (Ter Steeg et al. 1986) to satisfy NA demands. It has additionally been argued by Howarth et al. (1988) that DOC additions such as those used here would enhance localized, or 'microzone'-level, deoxygenation, which in turn would enhance the availability of certain metals in mat interstitial waters. While enhanced microzonal deoxygenation certainly results from DOC enrichment (Paerl 1985, Paerl et al. 1987), its positive impact on NA appears to be independent of Mo enrichment.

Nutrient-stimulated NA was observed in bioassays conducted on Lagunitas and Walker Creek mats in Tomales Bay. At the Lagunitas site, both the mannitol and mannitol plus PO_4^{3-} treatments significantly ($p < 0.05$) stimulated 45% of the time. At Walker Creek, mannitol additions stimulated NA 50% of the time, while mannitol plus PO_4^{3-} led to stimulation only 15% of the time. The noticeable reduction of NA stimulation in response to PO_4^{3-} enrichment may have been attributable to other nutrient limitations (e.g. Fe) induced by precipitation with added PO_4^{3-}.

Overall, the most consistent trend which emerged was DOC (mannitol) stimulation of NA. The nature of and hence underlying mechanisms responsible for DOC stimulation of NA remain the subject of current research. At present 2 scenarios seem possible: (1) DOC is directly utilized by bacterial heterotrophs and cyanobacterial or photosynthetic bacterial photoheterotrophs as an energy and carbon source supporting NA. (2) DOC enrichment stimulates respiration among a wide variety of mat heterotrophs; resultant enhanced localized O_2 consumption alleviates O_2 inhibition of NA in mats (Paerl et al. 1987), thereby enhancing rates of NA. Evidence points to both mechanisms operating contemporaneously, with the predominance of one over the other varying spatially and temporally (Paerl 1990).

Ecosystem-level implications

From mat habitat (mudflats, marshes, etc.) and larger ecosystem (estuarine, coastal) perspectives, the spatial and temporal interactions of organic carbon limitation of N_2 fixation and N limitation of primary production can be viewed as a circular feedback or 'chicken or the egg' predicament with respect to ecosystem trophodynamics. On the one hand, adequate N supply is needed to maintain optimal rates of primary production. On the other, the availability of organic carbon, much of it originating from primary production, exerts a potent control on mat N_2 fixation capacity. To a large extent, this apparent paradox can be reconciled if we consider external loadings (import) as well as periodic losses (export) of fixed C and biologically utilizable N. By virtue of the dynamic environments in which they occur, mats are not closed systems merely operating on recycled C and N. They are, however, remarkably adept at sequestering and retaining biologically available organic C and N via a variety of biochemical

mechanisms, including N_2 fixation, heterotrophic and photoheterotrophic 'harvesting' of DOC from the overlying water column (Paerl et al. 1993). Despite being faced with chronic N and organic C limitation of primary production and N_2 fixation respectively, mats optimize C and N sequestering and retention, and hence tend to minimize such limitations. The result is an effective way to accumulate microbial biomass, despite 'desert-like' oligotrophic conditions characterizing ambient waters in which mats are frequently found.

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LITERATURE CITED

Bauld, J., Chambers, L. A., Skyring, G. W. (1979). Primary productivity, sulfate reduction and sulfur isotope fractionation in algal mats and sediments of Hamelin Pool, Shark Bay, W. A. Aust. J. mar. Freshwat. Res. 30: 753–764

Bautista, M. F., Paerl, H. W. (1985). Diel N_2 fixation in an intertidal marine microbial mat community. Mar. Chem. 16: 369–377

Bebout, B. M. (1992). Interactions of nitrogen and carbon cycling in microbial mats and stromatolites. Ph.D. dissertation, Univ. of North Carolina at Chapel Hill

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

Canfield, D. E., Des Marais, D. J. (1993). Biogeochemical cycles of carbon, sulfur and free oxygen in a microbial mat. Geochim. Cosmochim. Acta (in press)

Cohen, Y., Castenholz, R. W., Halvorson, H. O. (eds.) (1984). Microbial mats: stromatolites. Alan R. Liss, New York

Cohen, Y., Rosenberg, E. (eds.) (1989). Microbial mats: physiological ecology of benthic microbial communities. Am. Soc. Microbiol. Publications, Washington, DC

Des Marais, D. J., Cohen, Y., Nguyen, H., Cheatham, M., Cheatham, T., Munoz, E. (1989). Carbon isotopic trends in the hypersaline ponds and microbial mats at Guerrero Negro, Baja California Sur, Mexico: implications for Precambrian stromatolites. In: Cohen, Y., Rosenberg, E. (eds.) Microbial mats: physiological ecology of benthic microbial communities. Am. Soc. Microbiol. Publications, Washington, DC, p. 191–203

Gebelein, C. D. (1976). Open marine subtidal and intertidal stromatolites (Florida, Bahamas and Bermuda). In: Walter, M. R. (ed.) Stromatolites. Elsevier, New York, p. 381–388

Golubic, S. (1976). Organisms that build stromatolites. In: Walter, M. R. (ed.) Stromatolites. Elsevier, New York, p. 21–30

Howarth, R. W., Cole, J. J. (1985). Molybdenum availability, nitrogen limitation, and phytoplankton growth in natural waters. Science 229: 653–655

Howarth, R. W., Marino, R., Lane, J., Cole, J. J. (1988). Nitrogen fixation in freshwater, estuarine, and marine ecosystems. I. Rates and importance. Limnol. Oceanogr. 33: 688–701

Javor, B. J., Castenholz, R. W. (1981). Laminated microbial mats, Laguna Guerrero Negro, Mexico. Geomicrobiol. J. 3: 237–273

Joye, S. B., Paerl, H. W. (1993a). Contemporaneous nitrogen fixation and denitrification in intertidal microbial mats: rapid response to runoff events. Mar. Ecol. Prog. Ser. 94: 267–274

Joye, S. B., Paerl, H. W. (1993b). Nitrogen fixation and denitrification in the intertidal and subtidal environments of Tomales Bay, CA. In: Oremland, R. S. (ed.) The biogeochemistry of global change: radiative trace gases. Blackwell, Oxford, p. 633–653

Krumbein, W. E., Cohen, Y. (1977). Primary production, mat formation and lithification: contribution of oxygenic and facultative anoxygenic cyanobacteria. In: Flugel, E. (ed.) Fossil algae: recent results and developments. Springer-Verlag, Berlin, p. 37–56

Monty, C. L. (1976). The origin and development of cryptalgal fabrics. In: Walter, M. R. (ed.) Stromatolites. Elsevier, New York, p. 193–249

Neumann, A. C., Paerl, H. W., Bebout, B. M., McNeese, L. R., Paull, C. K. (1989). Modern stromatolites and associated mats: San Salvador, Bahamas. In: Gerace, D. T. (ed.) Proceedings of the 4th Symposium on the Geology of the Bahamas. San Salvador, Bahamas. June 1988. Bahamian Field Station Publications, San Salvador, p. 235–251

Paerl, H. W. (1985). Microzone formation: its role in the enhancement of aquatic N_2 fixation. Limnol. Oceanogr. 30: 1246–1252

Paerl, H. W. (1987). Dynamics of blue-green algal (*Microcystis aeruginosa*) blooms in the lower Neuse River, NC: causative factors and potential controls. Univ. of North Carolina Water Resources Research Institute Rep. No. 229, Raleigh

Paerl, H. W. (1990). Physiological ecology and regulation of N_2 fixation in natural waters. Adv. microb. Ecol. 11: 305–344

Paerl, H. W., Bebout, B. M., Joye, S. B., Prufert, L. E., Des Marais, D. J. (1993). Microscale characterization of dissolved organic matter production and uptake in marine microbial mat communities. Limnol. Oceanogr. (in press)

Paerl, H. W., Bebout, B. M., Prufert, L. E., Currin, C. A. (1989). Mediation of aquatic N_2 fixation potentials by O_2-depleted microzones. In: Hattori, T., et al. (eds.) Recent advances in microbial ecology. Japan. Scient. Soc., Tokyo, p. 113–117

Paerl, H. W., Crocker, K. M., Prufert, L. E. (1987). Limitation of N_2 fixation in coastal marine waters: relative importance of molybdenum, iron, phosphorus, and organic matter availability. Limnol. Oceanogr. 32: 525–536

Paerl, H. W., Prufert, L. E. (1987). Oxygen-poor microzones as potential sites of microbial N_2 fixation in nitrogen-depleted waters. Appl. environ. Microbiol. 53: 1078–1087

Paerl, H. W., Rudek, J., Mallin, M. A. (1990). Stimulation of phytoplankton production in coastal waters by natural rainfall inputs: nutritional and trophic implications. Mar. Ecol. 107: 247–254

Paerl, H. W., Webb, K. L., Baker, J., Wiebe, W. J. (1981). Nitrogen fixation in waters. In: Broughton, W. (ed.) Nitrogen fixation, Vol. 1, Ecology. Clarendon Press, Oxford, p. 193–240

Paull, C. K., Neumann, A. C., Bebout, B., Zabielski, V., Showers, W. (1993). Growth rate and stable isotope character

of modern stromatolites from San Salvador, Bahamas. Paleogeogr. Paleoclimatol. Paleoecol. (in press)
Paulsen, D. M., Paerl, H. W., Bishop, P. E. (1991). Evidence that molybdenum-dependent nitrogen fixation is not limited by high sulfate concentrations in marine environments. Limnol. Oceanogr. 36: 1325–1334
Polimeni, C. (1976). Seasonality and life history of a blue-green algal mat on Shackleford Banks, NC. M.Sc. thesis, Univ. of North Carolina at Chapel Hill
Rudek, J., Paerl, H. W., Mallin, M. A., Bates, P. W. (1991). Seasonal and hydrological control of phytoplankton nutrient limitation in the lower Neuse River Estuary, North Carolina. Mar. Ecol. Prog. Ser. 75: 133–142
Smith, S. V. (1984). Phosphorus vs. nitrogen limitation in the marine environment. Limnol. Oceanogr. 29: 1149–1160
Stal, L. J., Grossberger, S., Krumbein, W. E. (1984). Nitrogen fixation associated with the cyanobacterial mat of a marine laminated microbial ecosystem. Mar. Biol. 82: 217–224
Stal, L. J., Krumbein, W. E. (1985). Nitrogenase activity in the non-heterocystous cyanobacterium *Oscillatoria* sp. grown under alternating light-dark cycles. Arch. Microbiol. 143: 67–71
Ter Steeg, P., Hanson, P. J., Paerl, H. W. (1986). Growth-limiting quantities and accumulation of molybdenum in *Anabaena oscillarioides*. Hydrobiologia 40: 143–147
Van Raalte, C. D., Valiela, I., Teal, J. M. (1976). Production of epilithic salt marsh algae: light and nutrient limitation. Limnol. Oceanogr. 21: 862–872
Walter, M. R. (1976). Introduction. In: Walter, M. R. (ed.) Developments in sedimentology, Vol. 20, Stromatolites. Elsevier, New York, p. 1–3
Webb, K. L., Dupaul, W. D., Wiebe, W., Scotile, W., Johannes, R. E. (1975). Enewetak (Eniwetok) Atoll: aspects of the nitrogen cycle on a coral reef. Limnol. Oceanogr. 20: 198–210
Whitton, B. A., Potts, M. (1982). Marine littoral. In: Carr, N. G., Whitton, B. A. (eds.) The biology of Cyanobacteria. Blackwell Sci. Publ., Oxford, p. 515–552
Wiebe, W. J., Johannes, R. E., Webb, K. L. (1975). Nitrogen fixation in a coral reef community. Science 188: 257–259

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