APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Mar. 1990, p. 614–622
0099-2240/90/030614-09$02.00/0
Copyright © 1990, American Society for Microbiology
Vol. 56, No. 3

Nitrogen Fixation Dynamics of Two Diazotrophic Communities in Mono Lake, California†

RONALD S. OREMLAND

U.S. Geological Survey, ms 465, 345 Middlefield Road, Menlo Park, California 94025

Received 6 October 1989/Accepted 5 December 1989

Two types of diazotrophic microbial communities were found in the littoral zone of alkaline hypersaline Mono Lake, California. One consisted of anaerobic bacteria inhabiting the flocculent surface layers of sediments. Nitrogen fixation (acetylene reduction) by flocculent surface layers occurred under anaerobic conditions, was not stimulated by light or by additions of organic substrates, and was inhibited by O_2, nitrate, and ammonia. The second community consisted of a ball-shaped association of a filamentous chlorophyte (*Ctenocladus circinnatus*) with diazotrophic, nonheterocystous cyanobacteria, as well as anaerobic bacteria (*Ctenocladus* balls). Nitrogen fixation by *Ctenocladus* balls was usually, but not always, stimulated by light. Rates of anaerobic dark fixation equaled those in the light under air. Fixation in the light was stimulated by 3-(3,4-dichlorophenyl)-1,1-dimethylurea and by propanil [*N*-(3,4-dichlorophenyl)propanamide]. 3-(3,4-Dichlorophenyl)-1,1-dimethyl urea-elicited nitrogenase activity was inhibited by ammonia (96%) and nitrate (65%). Fixation was greatest when *Ctenocladus* balls were incubated anaerobically in the light with sulfide. Dark anaerobic fixation was not stimulated by organic substrates in short-term (4-h) incubations, but was in long-term (67-h) ones. Areal estimates of benthic N_2 fixation were measured seasonally, using chambers. Highest rates (~29.3 μmol of C_2H_4 m^{-2} h^{-1}) occurred under normal diel regimens of light and dark. These estimates indicate that benthic N_2 fixation has the potential to be a significant nitrogen source in Mono Lake.

Nitrogen fixation represents one mechanism by which biologically available nitrogen enters aquatic ecosystems. The contribution this process makes to the sustenance of phytoplankton or macrophyte primary productivity can vary substantially. Thus, N_2 fixation is of considerable importance in the rhizosphere of seagrasses (5, 7) and affects the nitrogen balance of Clear Lake, California (17). However, in some oligotrophic lakes (37) and in the oceans (6), photic zone nitrogen fixation is insignificant when compared with other N sources. One important N source is derived from the recycling of nitrogen by microbial mineralization of sinking dead organic matter. Thus, water column mixing is critical to the resupply of this mineralized nitrogen to the surface phytoplankton communities. When mixing is eliminated, fixation can be important in sustaining productivity (8). In permanently stratified water bodies such as meromictic lakes, nitrogen fixation occurring in mixolimnion waters or in the shallow littoral zone provides a supply of combined nitrogen to the lake's ecosystem (22).

Mono Lake (Fig. 1), California, is an alkaline (pH 9.8), hypersaline (salinity, 90‰), closed-basin lake which became meromictic in 1983 (24). Its ecosystem has received national attention because of potentially threatening water diversion policies (21). The pelagic mixolimnia or monimolimnia waters of Mono Lake and chemically similar Big Soda Lake, Nevada, do not have detectable nitrogenase activity (24; R. Oremland, unpublished data), although substantial planktonic cyanobacterial fixation occurs in the less saline (5.3‰) and less alkaline (pH 9.2) Pyramid Lake, Nevada (16). The littoral zone of Mono Lake differs from that of Big Soda Lake in that the former supports no macrophytes which harbor dense diazotrophic cyanobacterial (*Anabaena* sp.) epiphytes (22). Presumably, this is due to the higher salinity of Mono Lake compared with that of Big Soda Lake (27‰). However, significant nitrogenase activity (NA) occurs in the littoral zone of Mono Lake. This paper characterizes two diazotrophic communities found in the littoral zone: (i) a flocculent surface sediment layer (FSL) comprising diazotrophic anaerobic bacteria, and (ii) a ball-shaped aggregate (*Ctenocladus* balls [CB]) composed of a eucaryotic alga, a nonheterocystous diazotrophic cyanobacterium, and diazotrophic anaerobic bacteria. Contributions to CB fixation by the two latter components were temporally (or spatially) variable, but always O_2 sensitive. Areal seasonal estimates of FSL-associated benthic annual NA has the potential to satisfy 10 to 17% of Mono Lake's N requirement for primary productivity.

MATERIALS AND METHODS

Site description and sampling. Sediments and algal material were collected from the littoral regions of Mono Lake seasonally during 1987 and 1988 and in August 1989. FSL was collected from the upper ~1 cm of sediment surface by suction, using a 60-ml Plastipak syringe (widened bore), and collected samples were pooled in a plastic Zip-lok bag. This excluded the collection of CBs. However, in a "quasi"-in situ experiment, the total surface layer was "scooped up" in a flask, which resulted in the capture of some CBs (both healthy and decomposing) as well as the FSL. Sediments from beneath the FSL layer were sampled by coring with 60-ml syringes with hub ends removed. The upper ~8 cm was collected in this fashion, and after the upper 2-cm FSL layer was discarded, the 2- to 8-cm vertical sections were retained. This experiment was repeated with 10-ml syringes (hub ends removed) to sample the upper 6 cm of sediment (the upper 0.5-cm interval was discarded when extruded into assay flasks). Syringes were plugged with rubber stoppers (no. 1) after sampling to minimize exposure to air. Sloughed-off periphyton was collected from the rocky near-shore region of the northwestern basin, while sediments and CB samples were taken from the mud flats of the northeastern

† This paper is dedicated to the memory of my mother, Rosalie Oremland.

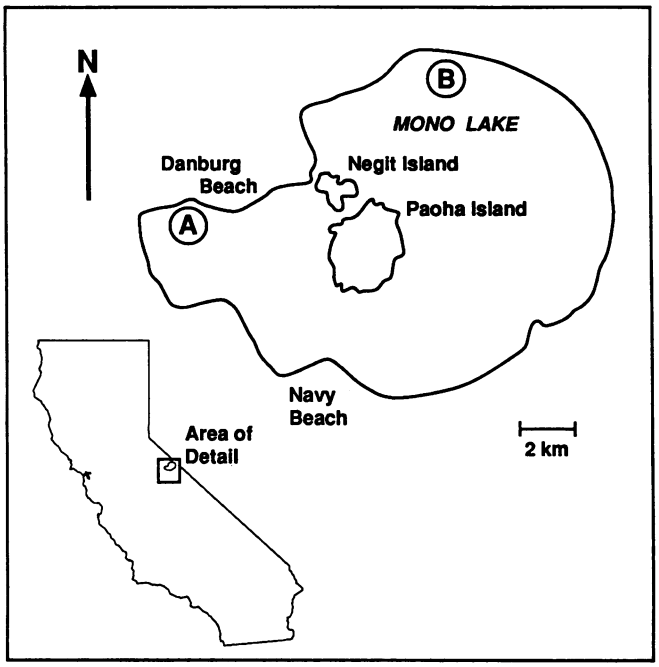

FIG. 1. Map of Mono Lake, California, indicating sampling stations. A, Northwestern basin; B, northeastern basin.

basin, except in August 1989, when CBs were only observed at the rocky northwestern basin site (Fig. 1). In situ chamber incubations (see below) were conducted at the northeastern basin site. Periphyton and CB samples were identified as attached and free-living stages of the green alga *Ctenocladus circinnatus* (3; D. B. Herbst, Ph.D. dissertation, Oregon State University, Corvallis, 1986; D. B. Herbst, personal communication). CBs were green to olive drab and usually 0.5 to 1.0 cm in diameter (Fig. 2A). Microscopic examination revealed the presence of nonheterocystous cyanobacterial filaments interwoven in the green algal matrix (Fig. 2B), but relatively low bacterial densities. No such filaments or bacteria were observed in the periphyton samples, which represented an earlier stage in the *C. circinnatus* life cycle (Herbst, Ph.D. dissertation). CBs were either positively or negatively buoyant, depending on the amount of photosynthetic O_2 entrapped in their fibrous matrices. Free-floating CBs occur in the pelagic region of Mono Lake and are transported to shore by wind action. Divers have observed high benthic densities (~2 CBs cm^{-2}) in 10-m water depth 1 km from the northern shore (D. Herbst and L. Miller, personal communication). The FSL consisted of decomposing pieces of CBs, sediment, and a surface organic film. Microscopic examination of FSL samples revealed that they contained pennate diatoms and green algal filaments (from decomposing CBs), as well as dense bacterial populations.

NA in CBs and sediments. N_2 fixation was measured with the acetylene reduction assay (15, 35) conducted on freshly collected materials at a field laboratory located near Mono Lake. CB incubations were conducted in conical flasks (volume, 58 ml) or serum bottles (volume, 60 ml) containing 20 ml of Mono Lake water from the site and 6 to 12 freshly collected CBs per bottle. FSL samples were suspended to uniform density in the Zip-lok collection bag by pumping action of the collection syringe and then dispensed (20 ml) into the above-mentioned flasks or bottles. Deeper anoxic core sections (2 to 8 cm) of the sulfidic (they had a strong sulfide odor and high sulfate reduction rates [~5.5 mmol $liter^{-1}$ day^{-1}] in the upper 8 cm; Oremland, unpublished data) sediments were pooled, mixed (1:1) with surface water to form a slurry, dispensed (25 ml) into 60-ml serum bottles, and then capped and flushed with Ar. Because this procedure may have impaired diazotrophic fastidious anaerobes, the experiment was repeated by extruding 9 cm^3 of sediment into serum bottles (volume, 60 ml) containing 10 ml of surface water (top 0.5 cm excluded). The bottles were capped (black butyl rubber stoppers), crimp-sealed, and flushed for 5 min with N_2. Amendments of soluble substrates or inhibitors (volume, 0.25 to 0.5 ml) were made from stock solutions made up in Mono Lake water. 3-(3,4-Dichlorophenyl)-1,1-dimethylurea (DCMU) was dissolved in ethanol (5 mM) and added (0.3 ml) to the samples, as was propanil [*N*-(3,4-dichlorophenyl) propanamide] (stock ethanol, 18 mM). Final concentrations of amendments are given in the text. Quasi-in situ experiments were conducted by placing 50 ml of scooped surface material plus 50 ml of surface water in 300-ml conical flasks with 30 ml of C_2H_2. These flasks were incubated in the littoral zone over a 6.5-h incubation period, during which time they were shaken at samplings. Incubation vessels were sealed with sleeved (conical flasks) or butyl (serum bottles) rubber stoppers and, when anaerobic conditions were required, flushed for 5 min with either N_2 or Ar. Dark-incubated samples were wrapped in two layers of Al foil. Acetylene was generated by reaction of water with CaC_2 and injected (6 ml) into the sealed samples (8 cm^3 for deep sediment sample experiments; all ~15% of gas phase). Samples were incubated with constant reciprocal shaking (~200 rpm) at ambient sunlight on cloudless days (40 to 60 einsteins m^{-2} day^{-1}) at temperatures ranging between 23 and 30°C. Hydrocarbon gases in bottle headspaces were sampled by syringe and analyzed by flame ionization gas chromatography (22), and N_2O was analyzed by electron capture gas chromatography (26). After the experiments were terminated, samples were collected on filter paper (ashless; Whatman), rinsed with deionized water, and dried to constant weight. No NA was detected in controls consisting of only lake water, and no ethylene was produced in samples incubated without acetylene or in inhibitor controls having only ethanol additions (data not shown).

Chamber experiments. To assess the areal rates of benthic N_2 fixation, light and dark Plexiglas chambers were deployed seasonally in the northeastern littoral zone. The chamber design, deployment, and sampling protocols have been described previously (22). Briefly, circular chambers (volume, 4 liters) covered a 0.28-m^2 area and were fitted with a smaller upper compartment which contained a gas phase. Upper-compartment gases were circulated through the bottom compartment liquid phase by action of a hand pump attached to a gas dispersion tube. The initial gas phase consisted of 360 ml of C_2H_2 plus 360 ml of air plus 10 ml of propane (internal standard). After equilibration (acetylene solublization), the gas phase was reduced to ~450 ml. Chambers were sampled several times over the course of incubations lasting up to ~24 h. At each sampling, the gas phase was hand pumped vigorously through the liquid phase to promote equilibration. Chambers were deployed in the morning (~10 a.m.) on cloudless days. After equilibration, ethylene in the gas phase of chambers was calculated to be about 96% of the total ethylene, with the remainder dissolved in the liquid phase (11).

FIG. 2. Photograph of CBs (A) and photomicrograph of CB matrix (B) indicating *C. circinnatus* filaments (CC) and nonheterocystous cyanobacteria (NHC). Bar, 10 μm.

RESULTS

Experiments with surficial sediments (FSL). Aerobically incubated FSL samples displayed NA (Fig. 3A), and rates in the light ($\bar{x}$ = 63.9 nmol of C_2H_4 g^{-1} h^{-1}) were equivalent to those in the dark ($\bar{x}$ = 64 nmol of C_2H_4 g^{-1} h^{-1}). However, dark anaerobically incubated samples had about fourfold more activity than aerobically incubated ones, and anaerobic activity was inhibited (~80%) with nitrate (Fig. 3B) or by ~90% with 5 mM NH_4Cl (not shown). No noticeable stimulation of dark anaerobic activity occurred with addition of either glucose or succinate (Fig. 3C). No significant levels of N_2O were present in any of the flasks at any time during the incubations (<0.5 nmol g^{-1}). In the quasi-in situ experiment with FSL incubated in the light or dark under air atmospheres (only shaken upon sampling), activity was detected under both conditions. Triplicate sets of samples gave similar values for light and dark rates (mean ± 1 standard deviation): light, 57 ± 10 nmol of C_2H_4 g^{-1} h^{-1}; dark, 75 ± 28 nmol of C_2H_4 g^{-1} h^{-1}.

NA was confined to the surface layer. After 6 h of incubation, triplicate FSL samples produced an average of 218 nmol of C_2H_4 g^{-1} (standard deviation of 58), while the deeper sediments (2 to 8 cm) formed only 0.75 nmol of C_2H_4 g^{-1} (standard deviation of 0.43). Because this procedure may have exposed fastidious diaztrophic anaerobes to toxic levels of oxygen, the experiment was repeated with individual 10-ml syringe subcores (sampled 0.5- to 6-cm depth interval). Methane increases were used as an index of anaerobiosis in the absence of added acetylene, an inhibitor of methanogenesis (25). Methane levels were initially 27 ± 15 nmol g^{-1} and increased steadily to 126 ± 17 nmol g^{-1} after 22 h of incubation (mean of three samples ± 1 standard deviation). By contrast, samples with acetylene initially had 19 ± 5 nmol of methane g^{-1} and <0.055 nmol of ethylene g^{-1}. By 22 h of incubation, methane levels were 30 ± 10 nmol g^{-1} and ethylene levels increased slightly to 0.29 ± 0.12 nmol g^{-1}. Incubation of sediments with 10 mM sodium lactate (to stimulate sulfate reduction) did not enhance

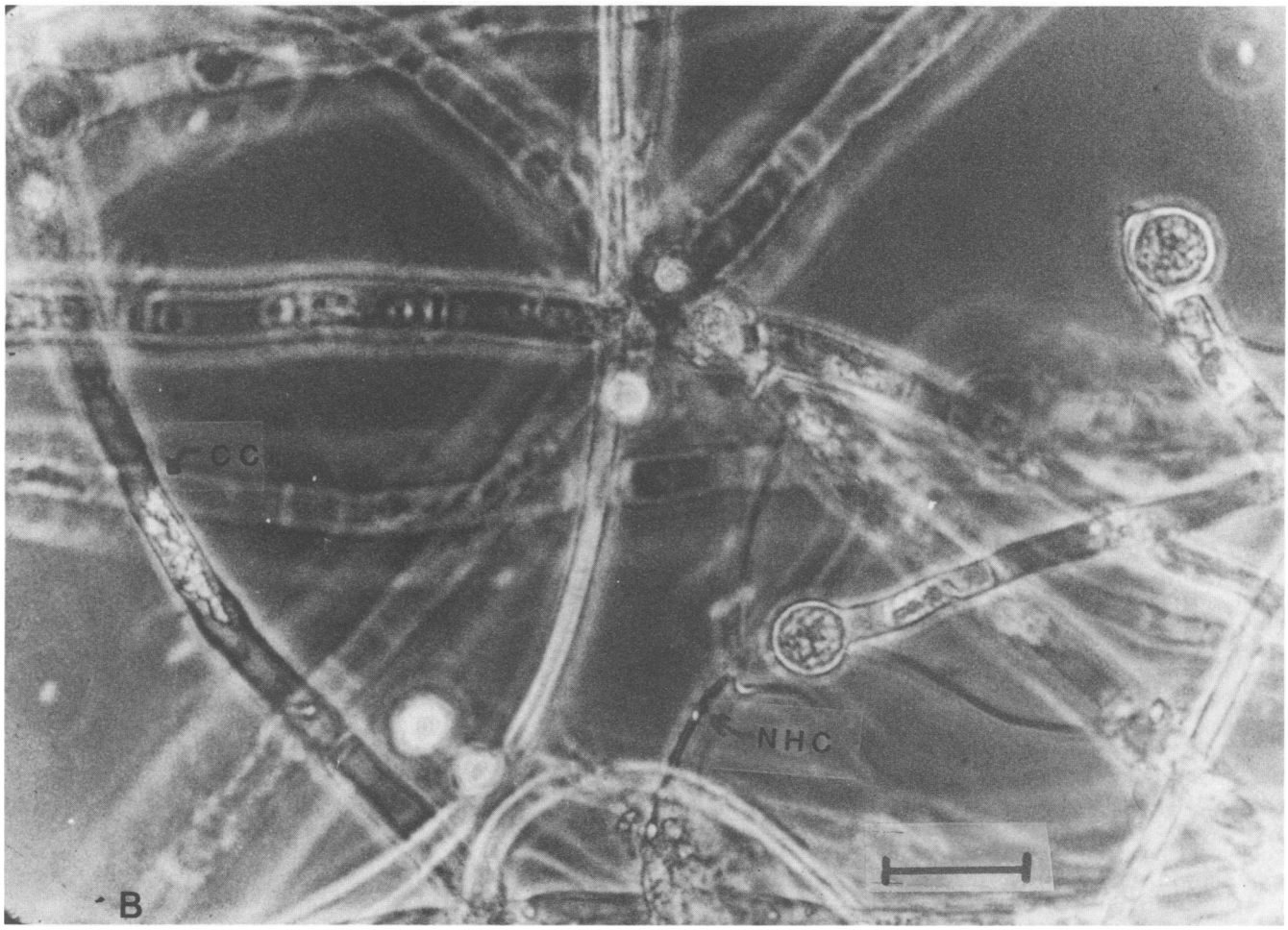

FIG. 2—*Continued.*

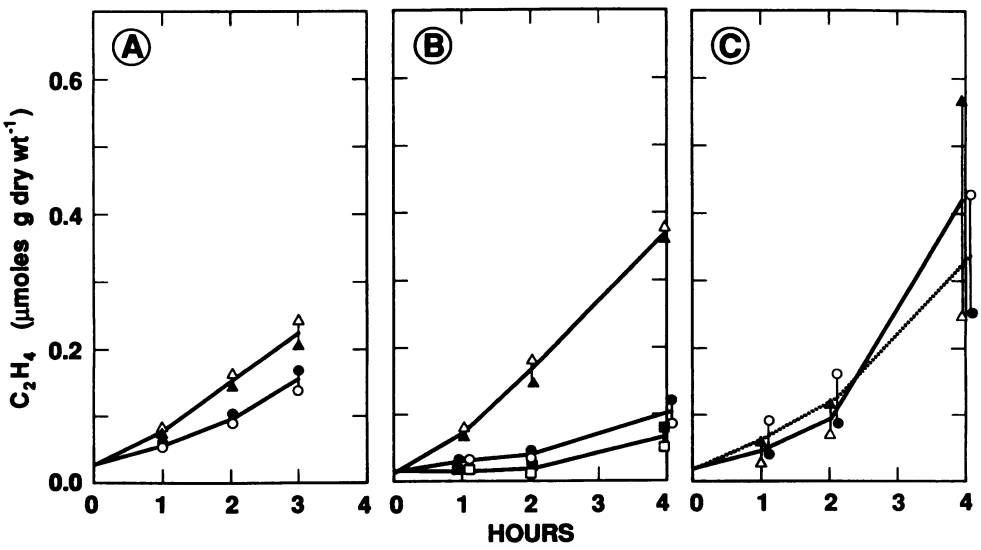

FIG. 3. NA in duplicate FSL samples incubated as follows: (A) aerobically in the light (△, ▲) and dark (○, ●); (B) in the dark under N_2 (△, ▲), under air (○, ●), or under N_2 with 1 mM $NaNO_3$ (□, ■); (C) under N_2 with 1 mM glucose (○, ●) or 1 mM succinate (▲, △). Experiments were conducted during October 1987 with material collected from the northeastern basin.

TABLE 1. Ethylene production of *C. circinnatus* sloughed periphyton collected from the northwestern corner of Mono Lake and incubated aerobically

Conditions	Ethylene (nmol ml of gas phase^{-1})	
	0 h	3 h
Light	0.30	0.23
Dark	0.30	0.30
Dark + sodium succinate (1 mM)	0.22	0.20
Dark + sodium acetate (1 mM)	0.33	0.34

ethylene production over unamended controls (C_2H_4, 0.27 ± 0.03 nmol g^{-1} at 22 h).

Experiments with periphyton. Recently sloughed periphyton samples collected off the northwestern basin shoreline did not display NA. After 3 h of incubation, no increases in headspace C_2H_4 levels were noted when material was aerobically incubated in the light or dark or when dark samples were amended with 1 mM either succinate or glucose (Table 1).

Experiments with CBs. Aerobically incubated CBs had about sixfold-higher activity in the light ($\bar{x}$ = 271 nmol of C_2H_4 g^{-1} h^{-1}) than in the dark ($\bar{x}$ = 43 nmol of C_2H_4 g^{-1} h^{-1}) (Fig. 4A). Light/aerobic activity was inhibited (~50%) by nitrate (Fig. 4B). However, CBs incubated under dark anaerobic conditions had about threefold more activity than dark aerobic samples (Fig. 5A), and this anaerobic activity ($\bar{x}$ = 207 nmol of C_2H_4 g^{-1} h^{-1}) was equivalent to rates observed in the light under air (Fig. 4A). No noticeable stimulation of dark/anaerobic nitrogenase activity occurred with glucose amendments (Fig. 5B) or with amendments of equivalent concentrations (1 mM) of acetate or succinate (not shown). No inhibition of dark/anaerobic activity occurred with addition of nitrate (Fig. 5B), and no significant levels of N_2O were detected during the incubation (≪0.5 nmol of N_2O g^{-1}).

During the first 3 h of illuminated anaerobic incubation,

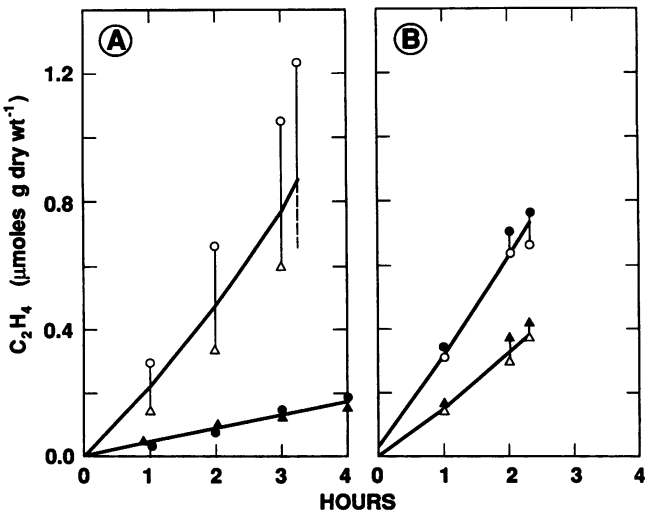

FIG. 4. NA in duplicate CBs samples incubated as follows: (A) aerobically in the light (△, ○) or dark (▲, ●); (B) aerobically in the light (○, ●) or in the light plus 1 mM $NaNO_3$ (△, ▲). Experiments were collected during October 1987 with material collected from the northeastern basin. Replicate of light sample (panel A, △) was lost at the last sampling.

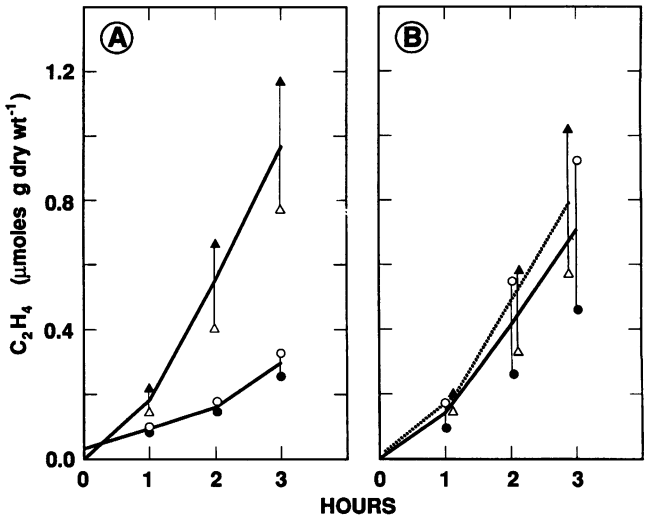

FIG. 5. NA in duplicate CB samples incubated as follows: (A) in the dark under N_2 (△, ▲) or under air (○, ●); (B) in the dark under N_2 with 1 mM $NaNO_3$ (△, ▲) or 1 mM glucose (○, ●). Experiments were conducted during October 1987 with material collected from the northeastern basin.

CBs formed more ethylene in the presence of sulfide than in its absence (Fig. 6). Rates of C_2H_4 formation in the light under aerobic conditions were equivalent to those measured in the dark under anaerobic conditions with sulfide. However, activity in these samples was severalfold lower than that observed under anaerobic conditions in the light (with or without sulfide).

In an initial experiment conducted in August 1988, addition of DCMU to CBs incubated aerobically in the light markedly stimulated C_2H_4 formation compared with samples incubated without DCMU (Table 2). When the experiment was repeated during August 1989, no activity was observed under aerobic conditions in the light. However, NA was elicited by incubation with DCMU, and this activity was inhibited (96%) by ammonia, but only partially (65%) by nitrate (Table 2). Addition of 10 μM propanil, a herbicide (1, 14), also promoted NA. Duplicate samples with propanil had 92 and 163 nmol of C_2H_4 g^{-1} after 2 h of incubation, while duplicates having DCMU and propanil had 209 and 220 nmol of C_2H_4 after 2 h. This lack of light/aerobic activity in the 1989 CBs and its reconstitution with DCMU was repeated in several experiments, and, in addition, dark anaerobic activity was present. For example, in a 2-h experiment, increases of C_2H_4 (in nanomoles per gram) were as follows (duplicate samples when indicated): light plus air, 3 and 9; light plus N_2, 29 and 34; dark plus N_2, 203; light plus air plus DCMU, 731 and 807.

The influence of glucose and nitrate on prolonged dark, anaerobic NA of these August 1989 CBs was followed (Table 3). NA was present in the unamended samples for the first 18.5 h (data point not shown), after which time further NA ceased. Glucose-amended samples, however, displayed NA for the entire incubation, eventually exceeding ethylene levels (64%) in the unamended samples. Nitrate caused a 66% inhibition of NA (Table 3), as did nitrate plus glucose. Exposure of an unamended sample to a 3-h period of sunlight after 67 h of dark incubation did not elicit further ethylene production, nor was ethylene evolved when this sample was returned to the dark for 2 h (data not shown).

Results of chamber experiments. When activity was

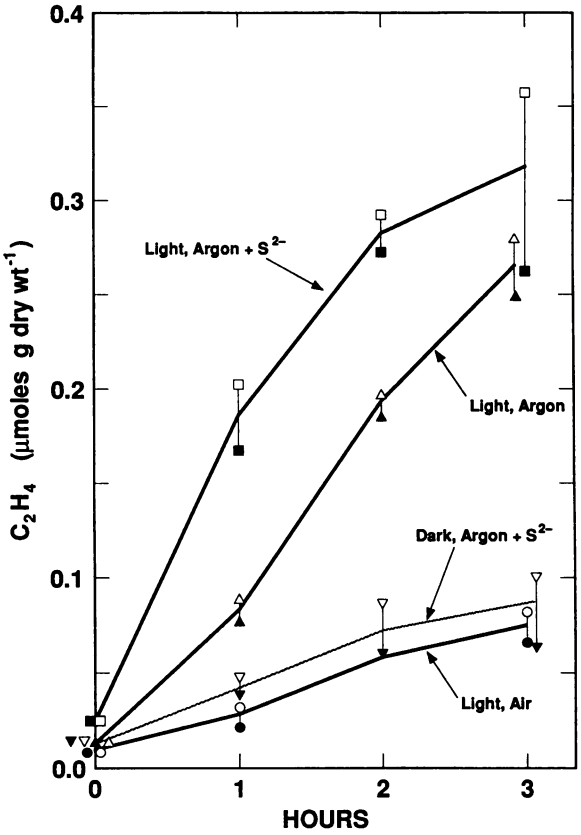

FIG. 6. NA in duplicate CB samples incubated under air in the light (○, ●), under Ar in the dark plus 1 mM Na_2S (▽, ▼), under Ar in the light (△, ▲), and under Ar in the light plus 1 mM Na_2S (□, ■). Experiments were conducted during May 1988 with material collected from the northeastern basin.

present, increased levels of C_2H_4 (well above the zero-time sampling) were detected by the first sampling (2 to 4 h). Subsequent ethylene production rates were generally linear. Chambers were deployed during February, May, and August of 1988. No activity was detected during the single deployment made during February 1988 (Table 4), and this lack of activity combined with the generally denuded characteristics of the sediments (no CBs and sparse FSL), precluded further deployments. Activity was evident during the warmer months of May and August. On both occasions, light-incubated chambers displayed two- to fourfold-higher activity than dark chambers. However, "light" chambers were actually exposed to normal diel periods of light and dark (about 15 h of light/9 h of dark), while dark chambers were always kept in the dark.

TABLE 3. Effect of prolonged incubation on NA in anaerobically incubated CBs with and without glucose or nitrate amendments[a]

Addition(s)	Ethylene (nmol g [dry wt]$^{-1}$)[b]		
	3 h	27 h	67 h
None	926	6,033	5,488
None	633	3,589	3,782
Glucose (10 mM)	545	4,705	7,657
Glucose (10 mM)	662	5,255	8,150
Nitrate (10 mM)	367	1,456	1,593
Nitrate (10 mM)	471	2,129	1,578
Nitrate + glucose (10 mM each)	274	862	1,740

[a] Samples were incubated under N_2 with 1 mM Na_2S added as a reducing agent.
[b] Initial ethylene levels are subtracted out.

DISCUSSION

NA in the FSL was primarily attributable to heterotrophic anaerobic bacteria, with possibly some contribution from aerobic or microaerophilic bacteria. Evidence for this was as follows: (i) highest activity occurred in the dark under N_2 (Fig. 3B); (ii) air did not completely eliminate activity (Fig. 3A and B); and (iii) no stimulation of NA occurred in the light (Fig. 3A). In addition, light did not stimulate NA in surficial material incubated in situ (see Results). No noticeable stimulation of NA in the FSL occurred with either glucose or succinate amendments (Fig. 3C). However, this does not preclude heterotrophic linked NA, but indicates that it was not carbon limited. NA was inhibited by nitrate (Fig. 3B) and ammonia (not shown), both of which inhibit nitrogenase synthesis (10). However, the rapidity of the nitrate inhibition suggests a very active assimilatory (or dissimilatory) nitrate reductase coupled with an ammonia "switch off" reaction (13, 18, 20, 36). Similar observations were reported for salt marsh sediments with regard to the sensitivity of NA to nitrate and ammonium (9).

The confinement of NA to the sediment surface was

TABLE 2. Influence of DCMU on nitrogen fixation by CBs incubated aerobically in the light

Addition(s)	Ethylene (nmol g [dry wt]$^{-1}$)[a]			
	1 h	2 h	3 h	4 h
August 1988 expt (northeast basin)				
None	23	82	95	97
None	33	77	ND[b]	ND
DCMU[c]	163	183	2,064	2,354
DCMU	188	363	ND	ND
August 1989 expt (northwest basin)[d]				
None	1.5 (2.6)	1.0 (2.9)	0.8 (2.1)	0.1 (4.1)
DCMU	160 (26)	431 (41)	838 (92)	1,582 (98)
DCMU + NH_4^+	9 (4)	27 (9)	48 (6)	65 (17)
DCMU + NO_3^-	92 (44)	238 (108)	479 (266)	548 (407)

[a] Zero-time C_2H_4 levels subtracted out.
[b] ND, Not determined.
[c] Concentration of DCMU, 75 μM.
[d] Represents mean of three samples, with values in parentheses indicating 1 standard deviation. Concentration of NH_4Cl and $NaNO_3$, 10 mM.

TABLE 4. Ethylene production in light and dark chambers deployed over three seasons in the littoral zone of the northeastern basin of Mono Lake[a]

Date	Condition	Temp range (°C)	Time (h)[b]	C_2H_4 (μmol m^{-2} h^{-1})
2/17/88	Light	6–10	4	0
5/20/88	Light	19–29	4	8.7
	Dark	19–29	4	9.1
5/21/88	Light	19–29	24	17.9
	Dark	19–29	24	7.9
5/22/88	Light	19–29	24	15.4
	Dark	19–29	24	7.0
8/17/88	Light	20–30	8	31.0
	Light	20–30	8	32.0
	Dark	20–30	4	6.0
8/18/88	Light	20–30	24.5	24.1
	Light	20–30	24.5	24.4
	Dark	20–30	24.5	3.9
8/19/88	Light	20–30	23	35.2
	Dark	20–30	23	11.4

[a] Summary 5/88: light, 14 ± 4.8; dark, 7.7 ± 1.2 μmol m^{-2} h^{-1}. Summary 8/88: light, 29.3 ± 4.9; dark, 7.1 ± 3.9 μmol m^{-2} h^{-1} (mean ± standard deviation).

[b] Total duration of deployment.

probably due to the presence of interstitial ammonia. We detected ammonia (~30 μM) at depths below 4 cm in these sediments (L. Miller, personal communication). Clearly, the lack of NA at depths below the FSL was not due to the absence of anaerobic incubation conditions because production of methane occurred in the acetylene-free controls (see Results). In addition, amendment of the sediments with lactate (a readily metabolized substrate for diazotrophic sulfate reducers) did not stimulate NA, indicating that they were not limited by electron donor availability. The elimination of these possibilities, coupled with the likelihood of higher interstitial ammonia than was observed in one core, suggests a restriction of NA in deeper sediments by ammonia.

Assignment of the observed NA in the CBs to a particular physiological group(s) of microbes is more difficult. A photosynthetic contribution was evident because NA was greater in the light than in dark under aerobic conditions (Fig. 4A), thereby indicating involvement of cyanobacteria. However, the extent of light/aerobic NA was highly variable (e.g., Fig. 4A and 6), and on one occasion (August 1989) no light/aerobic activity was noted (Table 2). These observations suggest a temporally (and/or spatially) variable contribution of a cyanobacterial type of NA ranging from significant, to minor, to a total absence. The evidence points to a varying degree of contributions to NA by nonheterocystous cyanobacteria as well as by diazotrophic anaerobic bacteria (see below). However, these results also illustrate the limitations of using physiological experiments with mixed flora when attempting to assign responsibility of NA to a particular microbial group.

A complicating feature was the observation that dark/anaerobic NA (Fig. 5A) was equal to light/aerobic activity (Fig. 4A). There are three possible explanations for dark NA: (i) diazotrophic, heterotrophic, anaerobic bacteria; (ii) a dark physiological response of the NA of nonheterocystous cyanobacteria such as *Oscillatoria* sp. (31, 33, 34); or (iii) both groups combined. No short-term enhancement of dark/anaerobic NA was observed with glucose (Fig. 5B) or with acetate or succinate (not shown), but this does not eliminate the possibility of NA by heterotrophic anaerobes because they may not have been electron donor limited. Indeed, the fact that dark anaerobic NA was stimulated by glucose in long-term (67-h) incubations suggests that this was the case (Table 3).

The NA results with DCMU and sulfide reinforced the requirement for reducing conditions and indicated a cyanobacterial involvement. A stimulation of light/aerobic NA was observed when DCMU was added to CBs (Table 2, August 1988). Enhanced NA with DCMU was noted for benthic cyanobacterial mats (2, 30) and with *Oscillatoria* sp. (32, 34). In these cases, the cessation of O_2 production by the inhibition of photosystem II resulted in greater NA by the O_2-sensitive nitrogenase. Further support of an important contribution from nonheterocystous cyanobacterial NA was the stimulation of NA by anaerobic conditions in the light (Fig. 6). Sulfide, which acts as an O_2-scavenging reductant for photosynthetically formed O_2, further enhanced this activity, but did not stimulate dark/anaerobic NA over light/aerobic NA. This indicates that *Oscillatoria*-like NA is a major component of the observed activity in the CBs and that anoxic microsites exist which allow nitrogenase to function under outwardly aerobic conditions. Microelectrode investigations have demonstrated the importance of such microsites for the expression of NA in marine cyanobacterium-bacterium aggregates (27, 28). The nonheterocystous cyanobacteria in the CBs displayed NA in both the light and the dark and may therefore be adapted to diel patterns of light as occurs in *Oscillatoria* sp. (33, 34).

However, the absence of light/aerobic activity in the August 1989 samples indicated that an *Oscillatoria*-type NA was not present at that sampling (Table 2). The expression of NA in the presence of DCMU underscored the necessity for reducing conditions, a fact supported by the detection of ethylene production in the dark under N_2 and of limited production in the light under N_2 (see Results). That this activity was strongly inhibited by ammonia and partially by nitrate points to the involvement of a switch off reaction rather than repression of nitrogenase synthesis. A wide diversity of diazotrophs possess this switch off reaction, including cyanobacteria, photosynthetic bacteria, and various aerobic and anaerobic heterotrophs (e.g., see reference 13), which makes these results of limited use in assignment of NA to physiological types. Propanil also elicited light/aerobic NA (see Results). However, this compound inhibits the Hill reaction in plants and algae lacking aryl acylamidases (1, 14) and therefore probably reduced cyanobacterial or *Ctenocladus* O_2 production or both rather than that of cyanobacterial NA. Indeed, NA with DCMU plus propanil was even greater, suggesting that a total inhibition of algal O_2 evolution resulted in greater NA. Because neither DCMU nor propanil inhibits bacteria, these results strongly implicate them as the source of the observed NA. In addition, the fact that NA ceased after ~24 h of dark anaerobic incubation (Table 3) and could not be reconstituted upon reexposure to light further suggests that this NA was due to diazotrophic anaerobic bacteria rather than nonheterocystous cyanobacteria. Although *Oscillatoria* sp. can fix nitrogen for up to 24 h under dark anaerobic conditions by fermentative energy metabolism (L. J. Stahl, Ph.D. dissertation, University of Groningen, Groningen, The Netherlands, 1985), this would not preclude the expression of NA under light/aerobic conditions or of light/dark periodicity of NA. In summary, these observations point out that, at times (or locations), there is exclusive involvement of anaerobic heterotrophic bacteria in the observed NA of the CBs.

No N_2O production occurred when CBs or FSLs were incubated under N_2 with acetylene and nitrate. This indicates that they do not harbor active populations of denitrifiers (38) and represent net sources of N to the lake. The question of how and why *C. circinnatus* forms CBs is of interest, but as yet is unexplained. When it was in the attached or recently sloughed form of periphyton, *C. circinnatus* did not display NA (Table 1). These detached vegetative cells are the precursors of the free-floating CBs (3; Herbst, Ph.D. dissertation). Apparently, at stages in the development of the ball structure, diazotrophs are incorporated, thereby conferring ability to carry out NA under a diversity of conditions (light or dark; aerobic or anaerobic). Cyanobacterium-bacterium aggregates having O_2-deficient microzones and NA have been described in oceanic surface waters (28); however, the CBs of Mono Lake represent the first description of such activity centered around a floating chlorophyte matrix.

Chamber incubations indicated that, when benthic NA was present, highest activity occurred under diel regimes of light and dark (~15 h of light/9 h of dark). Although these results would fit the pattern expected for NA displayed by CBs, few were present and the community primarily consisted of the FSL. These results are perplexing because NA in isolated FSLs did not show significant enhancement in the light (Fig. 3A), and static in situ incubated FSLs had higher dark NA (see Results). Therefore, some photosynthetic component was present in the chamber experiments which was not expressed in the bottle experiments. It is possible that diazotrophic heterotrophs in the FSL community were under a substrate limitation (from a lack of photosynthate release) in the dark chambers, but not in the light chambers. Although no such carbon limitation was evident in the bottle experiments (Fig. 3C), they were generally of much shorter duration than the chamber incubations.

Benthic NA was not present in the winter, but it was present in both the spring and summer (Table 4) as well as the fall (the quasi-in situ experiments were conducted in October 1987). Highest observed activity (~29.3 μmol of C_2H_4 m^{-2} h^{-1}) was 2- to 20-fold lower than that reported for marine benthic cyanobacterial mats (2, 30) and about 10-fold lower than near-shore NA in Big Soda Lake (22), but comparable to the rates displayed by many other benthic marine communities (4). It is of interest to be able to consider the estimates of areal N_2 fixation with respect to the fixation of carbon in the water column of the lake, recognizing that there are numerous constraints and caveats in such a comparison. The average of the light chamber incubations was ~22 μmol of C_2H_4 m^{-2} day^{-1}, which, using a 4:1 ratio (29), equals about 6 μmol of N_2 fixed m^{-2} day^{-1}, or about 26 mmol of N_2 fixed m^{-2} $year^{-1}$, assuming that this activity persists for about 6 months. Water column primary production in Mono Lake has been estimated to be 28 to 45 mmol of C m^{-2} $year^{-1}$ (19). If this productivity extends over 150 km^2 of the lake, this extrapolates to 4.2×10^6 to 6.8×10^6 mol of C $year^{-1}$. If the benthic N_2 fixation of Mono Lake's eastern basin extends 0.25 km offshore for about half the lake's circumference (~16 km), then annual benthic N_2 fixation would equal $\sim 0.10 \times 10^6$ mol $year^{-1}$. Assuming a 7:1 Redfield ratio of C/N for the live phytoplankton, this potential benthic N_2 fixation could satisfy 10 to 17% of the total nitrogen requirement for primary productivity. These estimates ignore any contribution of the CBs. Nonetheless, it appears that nitrogen fixation by benthic FSL communities may be a significant N source for Mono Lake.

ACKNOWLEDGMENTS

I am grateful to D. Herbst for algal identification and to L. Miller and C. Culbertson for technical assistance. I thank D. Capone, H. Paerl, and N. Steinberg for constructive criticisms and manuscript reviews.

LITERATURE CITED

1. **Bartha, R., and D. Pramer.** 1970. Metabolism of acylanilide herbicides. Adv. Appl. Microbiol. **13**:317–341.
2. **Bebout, B. M., H. W. Paerl, K. M. Crocker, and L. E. Prufert.** 1987. Diel interactions of oxygenic photosynthesis and N_2 fixation (acetylene reduction) in a marine microbial mat community. Appl. Environ. Microbiol. **53**:2353–2362.
3. **Blinn, D. W.** 1971. Autoecology of a filamentous alga, *Ctenocladus circinnatus* (Chlorophyceae), in saline environments. Can. J. Bot. **49**:735–743.
4. **Capone, D. G.** 1983. Benthic nitrogen fixation, p. 105–138. *In* E. J. Carpenter and D. G. Capone (ed.), Nitrogen cycling in the marine environment. Academic Press, Inc., New York.
5. **Capone, D. G.** 1988. Benthic nitrogen fixation, p. 85–123. *In* T. H. Blackburn and J. Sorensen (ed.), Nitrogen cycling in coastal marine environments. J. Wiley & Sons, Inc., New York.
6. **Capone, D. G., and E. J. Carpenter.** 1982. Nitrogen fixation in the marine environment. Science **217**:1140–1142.
7. **Capone, D. G., P. A. Penhale, R. S. Oremland, and B. F. Taylor.** 1979. Relationship between productivity and N_2 (C_2H_2) fixation in a *Thalassia testudinum* community. Limnol. Oceanogr. **24**: 117–125.
8. **Carpenter, E. J.** 1983. Nitrogen fixation by marine *Oscillatoria* (*Trichodesmium*) in the world's oceans, p. 65–104. *In* E. J. Carpenter and D. G. Capone (ed.), Nitrogen in the marine environment. Academic Press, Inc., New York.
9. **Dicker, H. J., and D. W. Smith.** 1980. Physiological ecology of acetylene reduction (nitrogen fixation) in a Delaware salt marsh. Microb. Ecol. **6**:161–171.
10. **Drozd, J. W., R. S. Tubb, and J. R. Postgate.** 1972. A chemostat study of the effect of fixed nitrogen sources on nitrogen fixation, membranes and free amino acids in *Azotobacter chroococcum*. J. Gen. Microbiol. **73**:221–232.
11. **Flett, R. J., R. D. Hamilton, and N. E. R. Campbell.** 1976. Aquatic acetylene-reduction techniques: solutions to several problems. Can. J. Microbiol. **22**:43–51.
12. **Fu, H., and R. H. Burris.** 1989. Ammonium inhibition of nitrogenase activity in *Herbaspirillum seropedicae*. J. Bacteriol. **171**:3168–3175.
13. **Gordon, J. K., V. K. Shah, and W. J. Brill.** 1981. Feedback inhibition of nitrogenase. J. Bacteriol. **148**:884–888.
14. **Habte, M., and M. Alexander.** 1980. Nitrogen fixation by photosynthetic bacteria in lowland rice culture. Appl. Environ. Microbiol. **39**:342–347.
15. **Hardy, R. W. F., R. D. Holsten, E. K. Jackson, and R. C. Burns.** 1968. The acetylene-ethylene assay for N_2 fixation: laboratory and field evaluation. Plant Physiol. **43**:1158–1207.
16. **Horne, A. J., and D. L. Galat.** 1985. Nitrogen fixation in an oligotrophic, saline desert lake: Pyramid Lake, Nevada. Limnol. Oceanogr. **30**:1229–1239.
17. **Horne, A. J., and C. R. Goldman.** 1972. Nitrogen fixation in Clear Lake, California. I. Seasonal variation and the role of heterocysts. Limnol. Oceanogr. **17**:678–692.
18. **Howard, K. S., B. J. Hales, and M. D. Socolofsky.** 1983. Nitrogen fixation and ammonia switch-off in the photosynthetic bacterium *Rhodopseudomonas viridis*. J. Bacteriol. **155**:107–112.
19. **Jellison, R., and J. M. Melack.** 1988. Photosynthetic activity of phytoplankton and its relation to environmental factors in hypersaline Mono Lake, California. Hydrobiologia **158**:69–88.
20. **Klugkist, J., and H. Haaker.** 1984. Inhibition of nitrogenase activity by ammonium chloride in *Azotobacter vinlandii*. J. Bacteriol. **157**:148–151.
21. **National Academy of Sciences.** 1987. The Mono Basin ecosystem: effect of changing lake level. National Academy Press, Washington, D.C.
22. **Oremland, R. S.** 1983. Hydrogen metabolism by decomposing

cyanobacterial aggregates in Big Soda Lake, Nevada. Appl. Environ. Microbiol. **45**:1519–1525.

23. **Oremland, R. S., J. E. Cloern, Z. Sofer, R. L. Smith, C. W. Culbertson, J. Zehr, L. Miller, B. Cole, R. Harvey, N. Iversen, M. Klug, D. J. Des Marais, and G. Rau.** 1988. Microbial and biogeochemical processes in Big Soda Lake, Nevada, p. 59–75. *In* A. J. Fleet, K. Kelt, and M. R. Talbot (ed.), Lacustrine petroleum source rocks. Geological Society Special Publication no. 40. Geological Society, London.
24. **Oremland, R. S., L. G. Miller, and M. J. Whiticar.** 1987. Sources and flux of natural gases in Mono Lake, California. Geochim. Cosmochim. Acta **51**:2915–2929.
25. **Oremland, R. S., and B. F. Taylor.** 1975. Inhibition of methanogenesis in marine sediments by acetylene and ethylene: validity of the acetylene reduction assay for anaerobic microcosms. Appl. Microbiol. **30**:707–709.
26. **Oremland, R. S., C. Umberger, C. W. Culbertson, and R. L. Smith.** 1984. Denitrification in San Francisco Bay intertidal sediments. Appl. Environ. Microbiol. **47**:1106–1112.
27. **Paerl, H. W., and R. G. Carlton.** 1988. Control of nitrogen fixation by oxygen depletion in surface-associated microzones. Nature (London) **332**:260–262.
28. **Paerl, H. W., and L. E. Prufert.** 1987. Oxygen-poor microzones as potential sites of microbial N_2 fixation in nitrogen-depleted aerobic marine waters. Appl. Environ. Microbiol. **53**:1078–1087.
29. **Postgate, J. R.** 1982. The fundamentals of nitrogen fixation. Cambridge University Press, London.
30. **Stahl, L. J., S. Grossberger, and W. E. Krumbein.** 1984. Nitrogen fixation associated with the cyanobacterial mat of a marine laminated microbial ecosystem. Marine Biol. **82**:217–224.
31. **Stahl, L. J., and H. Heyer.** 1987. Dark anaerobic nitrogen fixation (acetylene reduction) in the cyanobacterium *Oscillatoria* sp. FEMS Microbiol. Ecol. **45**:227–232.
32. **Stahl, L. J., and W. E. Krumbein.** 1981. Aerobic nitrogen fixation in pure cultures of a benthic marine *Oscillatoria* (cyanobacteria). FEMS Microbiol. Lett. **11**:295–298.
33. **Stahl, L. J., and W. E. Krumbein.** 1985. Nitrogenase activity in the non-heterocystous cyanobacterium *Oscillatoria* sp. grown under alternating light-dark cycles. Arch. Microbiol. **143**:67–71.
34. **Stahl, L. J., and W. E. Krumbein.** 1985. Oxygen protection of nitrogenase in the aerobically nitrogen fixing, non-heterocystous cyanobacterium *Oscillatoria* sp. Arch. Microbiol. **143**: 72–76.
35. **Stewart, W. D. P., N. G. P. Fitzgerald, and R. H. Burris.** 1967. In situ studies on N_2 fixation using the acetylene reduction technique. Proc. Natl. Acad. Sci. USA **58**:2071–2078.
36. **Sweet, W. J., and R. H. Burris.** 1981. Inhibition of nitrogenase activity by NH_4^+ in *Rhodospirillum rubrum*. J. Bacteriol. **145**: 824–831.
37. **Tison, D. L., F. E. Palmer, and J. T. Staley.** 1977. Nitrogen fixation in lakes of the Washington drainage basin. Water Res. **11**:843–847.
38. **Triska, F. J., and R. S. Oremland.** 1981. Denitrification associated with periphyton communities. Appl. Environ. Microbiol. **42**:745–748.

ERRATUM

Nitrogen Fixation Dynamics of Two Diazotrophic Communities in Mono Lake, California

RONALD S. OREMLAND

U.S. Geological Survey, ms 465, 345 Middlefield Road, Menlo Park, California 94025

Volume 56, no. 3, p. 614, abstract: the last sentence should be deleted.
Page 614, column 2, line 12: "10 to 17%" should read "0.010 to 0.017%."
Page 621, column 1, line 49: "~22 μmol of C_2H_4 m^{-2} day^{-1}" should read "~22 μmol of C_2H_4 m^{-2} h^{-1}."
Line 50: "6 μmol of N_2 fixed m^{-2} day^{-1}" should read "6 μmol of N_2 fixed m^{-2} h^{-1}."
Line 53: "28 to 45 mmol" should read "28 to 45 mol."
Line 55: "4.2×10^6 to 6.8×10^6 mol" should read "4.2×10^9 to 6.8×10^9 mol."
Line 61: "10 to 17%" should read "0.010 to 0.017%."