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Nitrogenase activity and estimates of nitrogen fixation by freshwater benthic blue-green algae

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The sandy substrate of Lake Taharoa (west coast, North Island, New Zealand – 35°50'S, 173°41'E) is covered by communities of filamentous algae that extend from the exposed beach down to 21 m depth. The algae bind the sand to form crusts and mats which may break off as discrete plates. The dominant species are the blue-greens *Microcoleus, Nostoc, Phormidium, Lyngbya, Oscillatoria, Scytonema, Stigonema, Shizothrix, Calothrix, Dichothrix, Tolypothrix,* and *Anabaena,* with occasional high concentrations of the desmid *Cylindrocystis.* Nitrogenase activity, measured by acetylene reduction, showed a wide range of rates (4–150 μmol C_2H_4 $m^{-2}h^{-1}$). Estimates of annual rates of nitrogen fixation by the Taharoa communities are comparable with those for periphytic blue-green algae-dominated systems reported elsewhere.

INTRODUCTION

Blue-green algal nitrogen fixation is commonly associated with bloom-forming planktonic species characteristic of eutrophic waters (Whitton 1973). By contrast, few reports have considered the nitrogen-fixing capabilities of benthic blue-green algae, which are of widespread marine and freshwater distribution. Horne & Fogg (1970) indirectly estimated that benthic blue-greens in Lake Windermere may fix 0.8 t of nitrogen annually. Fixation rates of 42–320 mg N $m^{-2}y^{-1}$ have been measured for gelatinous *Nostoc* colonies living at the bottom of certain Californian streams (Horne & Carmiggelt (1975). Higher rates have been recorded by Stewart (1967) for *Calothrix* in a rocky marine area in Scotland. All such reports, however, are restricted to periphytic forms localised above rather than within the bottom substrate. Although the soil- and sand-binding properties of blue-green algae have been well documented for several terrestrial and aquatic situations (e.g., Golubic 1973) we know of no previous attempts to measure nitrogenase activity in freshwater benthic substrates. This paper describes the distribution and nitrogen-fixing capability of an unusual heterocystous blue-green algal community living in the sandy substrate of an oligotrophic dune lake.

STUDY SITE

Lake Taharoa (35°50'S, 173°41'E) is situated 2 km inland from the west coast of the North Island, New Zealand. The lake and two smaller adjoining lakes (Kai-iwi and Waikare) constituted a dammed valley in old, consolidated sand dunes at 70 m a.s.l. Taharoa has an area of 210 ha and a small catchment with no permanent river inflows or outflows. The lake has an extensive, gently sloping margin that drops steeply to a maximum depth of 37 m (Irwin 1971). An annual fluctuation of 1.5 m in lake level uncovers large areas of sandy shore during summer.

MATERIALS AND METHODS

Core samples (30 cm^2 and 50 cm^2 × 1 cm) of surface or subsurface sand were collected in midsummer (January/February 1974) and late spring (November 1974) along transects traversing the gently sloping beach and underwater using SCUBA to a depth of 21 m. Duplicate samples from each depth were immediately preserved with Lugol's iodine for species identification. Other samples were placed in 500 ml jars for acetylene reduction assays. The jars were closed with a gas-tight lid and serum stopper and incubated with 10% acetylene. The samples were incubated for 3–5 h either *in situ* or under fluorescent lights (2000 lux, 20°c) in the laboratory. Ethylene production was measured by gas chromatography using a Carle 9500 gas chromatograph fitted with a 160 cm Porapak T column.

A surface water sample collected at mid lake in the January sampling was analysed immediately on receipt by the Auckland Regional Authority's water

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laboratory using standard techniques (American Public Health Association 1971). Phytoplankton samples from the same station were concentrated by sedimentation and then enumerated with a haemocytometer cell.

The nitrogen content of sand and algal layers was determined by Kjeldahl analysis of 10 cm^2 × 1 cm core samples. Total protein estimates were derived by multiplying the resulting nitrogen levels by 6.25.

RESULTS

In both late spring and midsummer, the exposed shore and littoral zone of Taharoa were covered with a community of mainly filamentous algae dominated by blue-greens. The community was variable in species composition, but normally consisted of a 2–10-mm-thick layer binding the sand to form a compact, continuous mat. A total of 13 genera of blue-green algae, 16 of green algae, and several of diatoms were recorded from within these mats (Tables 1 and 2). A similar species composition was recorded from benthic samples taken at seven depths in adjacent Lake Kai-iwi (Table 3).

Blue-green algae in the Taharoa and Kai-iwi mats comprised a surface layer of tuft-forming species (*Dichothrix, Tolypothrix,* and *Scytonema*) overlying a central region of *Nostoc, Anabaena,* and *Calothrix,* which in turn overlaid the bottom layer containing fine filaments of *Oscillatoria, Microcoleus,* and *Phormidium.* Each layer was highly variable in thickness, however, and often two of the layers were greatly reduced, resulting in overall dominance of the mat community by species of the third. From the samples collected in January/February 1974 five major communities could be delineated in terms of position on the shore and dominant algal species as follows.

1. DRY BEACH COMMUNITY. Extensive areas of the exposed shore contained algae in sand moistened by capillary water 10 mm or more below the dry sand surface. The algal layer was 2–5 mm thick, and was dominated by *Nostoc* and *Oscillatoria.*

2. WET BEACH COMMUNITY. In wetter areas at or near the water table a 1-mm-thick, brown, mucilaginous layer of algae and silt covered the sand. This surface assemblage was dominated by desmids (particularly *Cylindrocystis*) and *Anabaena.* Beneath this was a 1–2-mm layer of sand free of algae which covered a 3–5-mm layer of *Microcoleus, Stigonema,* and *Nostoc.*

3. BURIED COMMUNITY. At the lake edge one or more algal mats were buried by up to 200 mm of sand. The mats retained their blue-green colour and remained structurally intact. Several mats which were

Table 1. Generalised distribution of benthic algae in different habitats along beach margin of L. Taharoa, 30 Jan 1974 (D, dominant; A, abundant; C, common; R, rare; — absent).

	Habitat				
	1 Dry beach	2 Wet beach	3 Buried	4 Sub-merged	5 Plates
CYANOPHYCEAE					
Anabaena	R	C	—	—	—
Aphanothece	—	—	—	R	—
Dichothrix	—	—	R	C	C
Lyngbya	R	C	C	R	—
Microcoleus	—	D	R	D	—
Nostoc	D	A	A	R	A
Oscillatoria	A	—	A	—	D
Phormidium	—	C	D	—	D
Scytonema	—	—	—	—	R
Stigonema	—	A	—	R	—
Tolypothrix	—	R	—	C	R
CHLOROPHYCEAE					
Chlorella	—	C	—	—	—
Cosmarium	R	C	—	R	R
Cylindrocystis	R	A	R	R	—
Microspora	—	C	—	R	—
Scenedesmus	—	C	—	—	—
Staurastrum	—	C	—	C	—
Zygnema	R	C	—	R	—
BACILLARIOPHYCEAE	—	C	C	C	C

Table 2. Generic composition of submerged benthic algal communities in L. Taharoa (30 Jan 1974) (+ present).

	Depth (m)										
	0.1	0.2	2	3	5	6	7	8	10	15	21
CYANOPHYCEAE											
Aphanothece							+	+			
Dichothrix	+	+	+	+	+	+					
Microcoleus			+	+	+	+	+	+			
Nostoc	+	+									
Oscillatoria	+	+								+	+
Phormidium	+	+									
Scytonema		+		+	+	+					
Tolypothrix	+	+	+			+		+			
CHLOROPHYCEAE											
Actinastrum						+				+	
Bulbochaete					+				+	+	+
Chara								+			
Closterium										+	
Cosmarium			+	+		+	+		+		
Euastrum				+			+			+	+
Mougeotia									+		
Netrium				+							
Nitella										+	
Pleurotaenium				+							
Scenedesmus										+	
Staurastrum			+	+		+			+	+	+
Zygnema				+							
DINOPHYCEAE											
Peridinium		+	+	+		+		+	+	+	
BACILLARIOPHYCEAE			+	+	+	+					

piled one on top of the other were observed in certain sand profiles of this region. This community was dominated by the non-heterocystous genera *Lyngbya* and *Phormidium*.

4. CONTINUOUSLY SUBMERGED COMMUNITY. This occurred down to a lakewater depth of 21 m, and consisted of an algal layer 3–5 mm thick buried by up to 20 mm of loose sand. Species composition was highly variable. The only consistent trend with depth was an increase in the proportion of greens versus blue-greens. Heterocystous blue-green algae were absent from samples below 8.5 m (Table 2).

5. DETACHED PLATE COMMUNITY. Plates of algae broken up by wave action were a common underwater feature near the lake edge. These were dominated by *Phormidium, Oscillatoria,* and *Nostoc*.

Triplicate mid-lake surface samples were collected for phytoplankton enumeration on 31 January 1974. At this time of year total cell densities were extremely low (5.75×10^3 cells per litre), and comprised 38% greens (*Bulbochaete, Chlorella, Cosmarium, Desmidium, Euastrum, Oedogonium, Staurasastrum, Xanthidium,* and *Zygnema*), 58% dinoflagellates (*Peridinium* and *Ceratium*), and 4% the chrysophycean *Dinobryon*. Analysis of a mid-lake surface water sample collected on the same date (Table 4) confirmed the low nutrient status of the water suggested by the low algal counts. Both nitrate and soluble reactive phosphate were present in concentrations typical of highly oligotrophic waters.

Algal mat samples from the five communities were tested for nitrogenase activity by incubation with acetylene in transparent jars submerged 0.25 m below the surface of the lake. Highest activities were recorded in wet beach samples (Table 5). Dry beach nitrogenase activity was less than 25% of that in the wet beach community. Buried community mats exhibited no nitrogen-fixing ability, whereas detached plates reduced acetylene as rapidly as dry beach samples. The continuously submerged samples exhibited decreasing nitrogenase activity with increasing depth of collection (Table 6); no activity was detected in samples from below 8.5 m.

To determine whether nitrogen fixation could be associated with blue-green algae rather than with bacterial constituents of the mats, a series of experiments was conducted to examine the effect of light on nitrogenase activity. Algal mats from three communities were incubated with acetylene in both normal orientation (heterocystous community exposed to light) and inverted (non-heterocystous community exposed to light).

For all samples, inversion of the mats resulted in a significant ($P < 0.05$) depression of nitrogenase activity (Table 7). The buried algal layer (community 3), although intensely blue-green, had no nitrogenase activity over the 5-h incubation in the light immediately after uncovering. After 24 h of incubation in the light, nitrogenase activity was detectable.

Further experiments were conducted in the laboratory with detached plate samples transported there in lake water in an ice chest. These were grown in a temperature-controlled room (20°c, 2000 lux fluorescent light) for 1 week in a nutrient solution (ASM – Gorham *et al.* 1964) with and without a combined nitrogen source, and in distilled water. At the end of this period nitrogenase activity was measured during 3-h incubations in the light (2000 lux) and in darkness. In all samples nitrogenase activity was markedly stimulated in the light (Table 8). Activity was greatest in samples grown in the nutrient solution without nitrate. Addition of nitrate reduced nitrogenase activity to barely detectable levels.

To horizontally localise the regions of maximum

Table 3. Generic composition of benthic algal communities in L. Kai-iwi, 2 Feb. 1974 (+ present).

	Depth (m)						
	0.5	1.0	1.5	2.0	3.0	5.0	5.5
CYANOPHYCEAE							
Dichothrix	+						
Microcoleus	+						
Nostoc				+	+	+	
Oscillatoria		+	+			+	+
Scytonema				+	+	+	
Stigonema				+			
Tolypothrix				+			
CHLOROPHYCEAE							
Bulbochaete				+	+		
Cosmarium		+					
Oedogonium				+	+		
Pediastrum				+	+		
Pleurotaenium						+	
Scenedesmus				+		+	
Spirogyra				+	+		
Staurastrum			+		+	+	+
DINOPHYCEAE							
Peridinium				+	+	+	+
BACILLARIOPHYCEAE	+			+		+	

Table 4. Physical and chemical data for L. Taharoa at a mid-lake station, 31 Jan 1974.

Secchi depth	8 m
Thermocline depth	20 m
pH	6.7
Phosphate-P (total)	3 $mg.m^{-3}$
Phosphate-P (soluble reactive)	2 $mg.m^{-3}$
Silicate	<10 $mg.m^{-3}$
Nitrate-N	1.0 $mg.m^{-3}$
Nitrite-N	<1.0 $mg.m^{-3}$
Ammonium-N	<1.0 $mg.m^{-3}$
Alkalinity ($CaCO_3$)	5 $g.m^{-3}$
Total hardness ($CaCO_3$)	21 $g.m^{-3}$
Iron (total)	20 $mg.m^{-3}$
Manganese (total)	1 $mg.m^{-3}$
Sulphate	4.8 $g.m^{-3}$

Table 5. Nitrogenase activity of mats from L. Taharoa blue-green algal communities, 30 Jan 1974; each figure is the mean of c. 8 replicates ± S.E.

	Community	μmol ethylene $m^{-2}h^{-1}$
1.	Dry beach	15.6 ± 2.4
2.	Wet beach	68.1 ± 10.8
3.	Buried	0
4.	Continuously submerged	12.9 ± 3.0
5.	Detached plate	11.8 ± 1.2

Table 6. Nitrogenase activity of L. Taharoa algal mats from 2 m to 12.5 m depth, 1 Feb 1974; single samples collected from each depth and incubated in full sunlight for 2 h.

Depth (m)	Nitrogenase activity (μmol ethylene $m^{-2}h^{-1}$)
2	17.0
3	18.1
4.5	18.7
6.5	16.0
8.5	8.0
10.5	0
12.5	0

Table 7. Effect on nitrogenase activity of inverting algal mats from 3 L. Taharoa communities. Equivalent mat areas were immersed in lake water and incubated in full sunlight. In all instances inverted mat is significantly ($P < 0.05$) lower in activity than mat in normal orientation (each figure the mean of triplicates).

Community	Orientation	Nitrogenase activity (μmol ethylene $m^{-2}h^{-1}$)
2. Wet beach	normal	76.0
	inverted	26.0
4. Continuously submerged	normal	8.6
	inverted	4.5
5. Detached plates	normal	13.0
	inverted	7.2

Table 8. Nitrogenase activity in detached algal pates taken from L. Taharoa 2 Feb. 1974 and laboratory-grown in distilled water or nutrient media (ASM) with and without nitrate-N. Mats then incubated for 3 h in light (2000 lux) or dark. Each figure the mean of triplicates ± SE.

Medium	Incubation	Nitrogenase (μmol ethylene $m^{-2}h^{-1}$)
Dist. water	light	23.0 ± 1.4
Dist. water	dark	7.2 ± 0.6
ASM + N	light	1.3 ± 0.8
ASM + N	dark	0
ASM – N	light	77.3 ± 20.5
ASM – N	dark	44.0 ± 14.1

nitrogen fixation, samples were taken in November 1974 at 1-m intervals along a transect 0–24 m from the lake edge, and were incubated *in situ* with acetylene. Nitrogenase activity was measurable at all beach sites (Fig. 1), and was at a maximum in the wet sand region (4–9 m). Further core samples across the transect were analysed for total nitrogen. These values (Fig. 1) were highly correlated with rates of nitrogen fixation ($r = 0.8356$, $P < 0.01$). When expressed on a protein basis, the nitrogenase activity over this transect ranged from 1.8 to 462.0 nmol ethylene per mg protein per hour.

Conversion of these rates of ethylene production to nitrogen fixed involves a calibration of the acetylene reduction assay with $^{15}N_2$. Several calibrations of this type with blue-green algal nitrogen-fixing systems in our laboratory have given a ratio of acetylene reduced to nitrogen fixed of 3.2:1.0. Using this conversion factor, rates of nitrogen fixation ranged from 0.01 mg N $m^{-2}h^{-1}$ (dry plate at 22 m) to 1.36 mg N $m^{-2}h^{-1}$ (wet beach at 6 m). Across the transect the mean rate of nitrogen fixation was 0.31 mg N $m^{-2}h^{-1}$. Samples taken from the submerged region of the lake on the same date as the transect and incubated *in situ* yielded an average value of 8.5 μmol of acetylene reduced per m^2 per h; this converts to 0.074 mg N per m^2 per h.

DISCUSSION

Previous descriptions of blue-green algal mats associated with aquatic sediments have mainly been confined to marine systems. It is perhaps of significance that the Taharoa communities described here bear strong resemblance to those found in the intertidal zone. In both habitats algal mats experience periodic flooding and exposure to air, and must adjust to severe gradients in water supply. Particularly common in such marine communities are *Microcoleus, Shizothrix, Scytonema, Lyngbya,* and *Phormidium* (Golubic 1973); these genera were common constituents of the Taharoa and Kai-iwi algal mats. The multi-layered buried community seems strikingly similar to the tidally laminated *Shizothrix* mats studied by Gebelein & Hoffman (1970) in Florida Bay. Within such marine mats the algal assemblage tends to be differentiated both floristically and physiologically with depth (e.g., Fischer & Golubic, in Golubic 1973). A similar degree of vertical differentiation was observed in Taharoa samples.

The Taharoa communities differ from described marine systems in containing a wider range of heterocystous—and hence potentially nitrogen-fixing (Stewart 1966)—species that are localised at the top of the algal mats. The mat inversion experiment supports the contention that these species are the dominant nitrogen-fixers in Taharoa mats. The stimula-

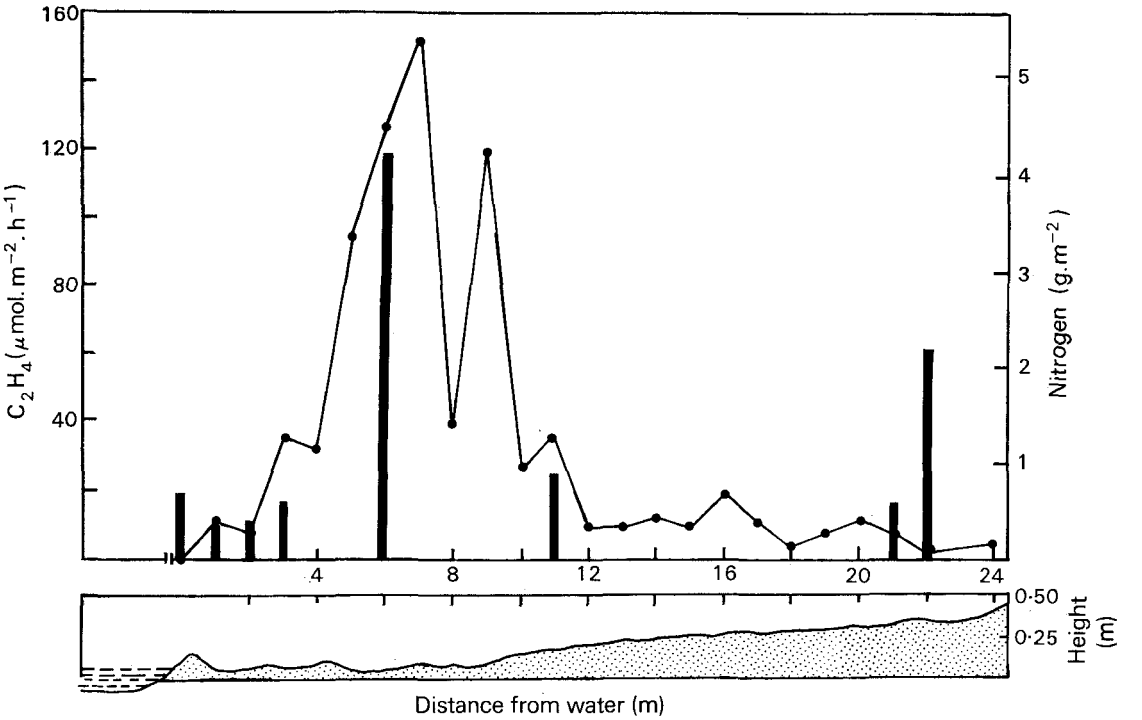

Fig. 1. Acetylene reduction assays across a horizontal transect of beach at L. Taharoa, 26 Nov 1974. *Upper profile,* acetylene reduction (bars – nitrogen content of sand); *lower profile,* topography.

tion by light of nitrogen fixation in the laboratory is strong evidence that much of the nitrogenase activity is associated with algae rather than bacteria in these samples. The lack of nitrogenase activity in the deeply buried mat community is also consistent with this hypothesis. In this region penetration by light must be negligible, yet there is abundant organic material which might fuel bacterial metabolism.

Much further research must precede any definitive statements regarding the contribution of blue-green algae to the nitrogen economy of Lake Taharoa. However, the transect data recorded here allow for a very approximate estimate of nitrogen input by fixation. The average acetylene reduction rates across the beach and the shallow-water zone represent fixation rates of 0.309 and 0.074 mg N per m^2 per h respectively. Assuming that this rate is maintained 200 days of the year (10 h of light per day), the mean annual rates of nitrogen input in this region by fixation are 0.618 g m^{-2} (shore) and 0.148 g m^{-2} (shallows). These figures are comparable with those for soil and benthic algal systems reported elsewhere (Table 9). The exposed shore area of the lake is 20 ha, and the submerged shallows down to the limits of nitrogenase activity (8.5 m) occupy 24 ha. The total annual nitrogen input from fixation in the Taharoa ecosystem is therefore 160 kg, made up of 134 kg from the exposed beach and 36 kg from the submerged region. This estimate necessarily neglects seasonality, spatial variation, and the effect of a rising and falling water level on blue-green algal metabolism. However, even as an order of magnitude estimate it represents a substantial quantity of nitrogen in relation to the amount of dissolved inorganic nitrogen that was present (at 1 mg.m^{-3} the lake contained 420 kg) at a time of year when phytoplankton densities—and hence particulate

Table 9. Estimated nitrogen fixation rates in various soil and benthic systems attributable to blue-green algae.

Ecosystem	g m^{-2}y^{-1}	Source
Exposed beach	0.62	This study
Submerged beach	0.15	This study
California stream	0.04–0.36	Horne & Carmiggelt 1975
Scottish coast	2.5	Stewart 1967
Oslofjord shore	1.1	Warmling 1973
Salt marsh	0.43	Jones 1974
Desert algae	1.1	Mayland *et al.* 1966
Wheatfields	1.5–5.1	Henriksson 1971
Cornfields	0.2	Jahnke 1967
Rice paddy	1.5–4.8	De & Mandal 1956

nitrogen concentration—were very low. The extremely low dissolved inorganic N:P ratio measured (0.5:1.0, cf. a ratio of 5:1 below which algae respond to nitrogen additions – Chiaudani & Vighi 1976) suggests that nitrogen may be a primary limiting nutrient in this system. Input of nitrogen from the surrounding littoral community of blue-green algae might therefore exert an important control on primary production in Lake Taharoa.

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