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Nitrogen Fixation Associated with the New Zealand Mangrove (*Avicennia marina* (Forsk.) Vierh. var. *resinifera* (Forst. f.) Bakh.)

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Nitrogenase activity in mangrove forests at two locations in the North Island, New Zealand, was measured by acetylene reduction and $^{15}N_2$ uptake. Nitrogenase activity (C_2H_2 reduction) in surface sediments 0 to 10 mm deep was highly correlated ($r = 0.91$, $n = 17$) with the dry weight of decomposing particulate organic matter in the sediment and was independent of light. The activity was not correlated with the dry weight of roots in the top 10 mm of sediment ($r = -0.01$, $n = 13$). Seasonal and sample variation in acetylene reduction rates ranged from 0.4 to 50.0 μmol of C_2H_4 m^{-2} h^{-1} under air, and acetylene reduction was depressed in anaerobic atmospheres. Nitrogen fixation rates of decomposing leaves from the surface measured by $^{15}N_2$ uptake ranged from 5.1 to 7.8 nmol of N_2 g (dry weight)$^{-1}$ h^{-1}, and the mean molar ratio of acetylene reduced to nitrogen fixed was 4.5:1. Anaerobic conditions depressed the nitrogenase activity in decomposing leaves, which was independent of light. Nitrogenase activity was also found to be associated with pneumatophores. This activity was light dependent and was probably attributable to one or more species of *Calothrix* present as an epiphyte. Rates of activity were generally between 100 and 500 nmol of C_2H_4 pneumatophore^{-1} h^{-1} in summer, but values up to 1,500 nmol of C_2H_4 pneumatophore^{-1} h^{-1} were obtained.

Asymbiotic nitrogen fixation has been demonstrated in many forest ecosystems (17, 22). Although nitrogenase activity is generally low in these environments, over the life of a forest these inputs often more than offset losses due to leaching and denitrification, resulting in long-term accumulations of nitrogen in forests (7, 21). At least three sites of asymbiotic nitrogen fixation have been identified in forest environments: measurable activities have been identified in decaying litter (18), decaying wood (11, 20), and the rhizospheres of some tree species (16, 19). The majority of workers have been unable to detect significant nitrogenase activity in mineral soil (17, 22). Although some workers have implicated anaerobes as the agents of nitrogen fixation (17), recent work indicates that aerobic or microaerophilic organisms are predominant (20).

The mangrove forest ecosystem represents a very special environment, in which sediments are almost entirely anaerobic and loss of nitrogen from denitrification may be very high (12). Acetylene reduction assays have shown nitrogenase activity in the soils of the mangrove species *Avicennia germinans*, *Laguncularia racemosa*, and *Rhizophora mangle* (10, 24). These studies indicated that the measured nitrogenase activity was root associated (24) and was greatest under anaerobic conditions (10, 24).

In addition, Gotto and Taylor (8) measured acetylene reduction in decaying leaves of *R. mangle*, and recently there has been a report of nitrogen-fixing bacteria occurring in warty lenticellate bark of *Brugiera gymnorrhiza* (23).

The present study was initiated to investigate the occurrence of nitrogen fixation in the mangrove forests of New Zealand, the southernmost mangroves in the world. Also investigated were the specific sites of nitrogenase activity in soils and the requirements for oxygen.

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MATERIALS AND METHODS

Study sites. Sediment and plant materials for most experiments were collected from two sites in the Auckland Province (Fig. 1). Site 1 was in the northwestern corner of Whangateau Harbour (36°19′ S, 174°46′ E), 58 km north of Auckland. Site 2 was close to Auckland City (36°52′ S, 174°49′ E), in a tidal creek 1.5 km inland from the Waitemata Harbour. Trees at both locations were up to 4 m tall, and the abundance of seedlings indicated that regeneration was taking place. Experiments were carried out between December 1974 and July 1976.

C_2H_2 reduction assay. The C_2H_2 reduction assay for nitrogenase activity was conducted by previously described methods (9). Aerobic incubations were conducted by adding 10.13 kPa (0.1 atm) of C_2H_2 to air in enclosed vessels, and anaerobic incubations were initiated by flushing with Ar before addition of C_2H_2 (10.13 kPa). The incubation time varied with the material and ranged from 12 to 20 h. Time courses showed that rates of C_2H_4 production by intact cores were linear between 8 and 24 h.

After incubation, 0.1-ml gas samples were analyzed for C_2H_4 production by a Carle 9500 Basic gas chromatograph with a flame ionization detector. The gas samples were carried by a flow of dry nitrogen (0.67 ml s^{-1}) through a 1.6-mm (internal diameter) copper column, 965 mm long, packed with 80- to 100-mesh Porapak T. The air flow to the flame ionization detector was 4.17 ml s^{-1}, and the column oven and injection port temperatures were 70°C. Ethylene peak heights were measured and related to calibrations made with standard C_2H_4 concentrations. Endogenous C_2H_4 production and background C_2H_4 were never detectable.

Collection and incubation. Sediment samples were collected as cores, 38 mm in diameter and 100 mm in length, by using a polyvinyl chloride coring tube. The intact cores were extruded into similar incubation chambers with airtight caps

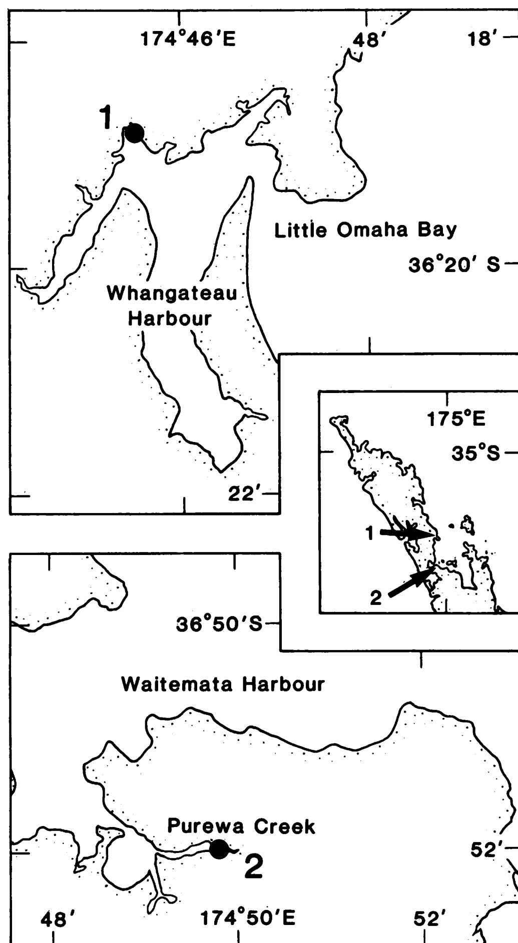

FIG. 1. Location of main sites used for collection of sediments and plant materials in the North Island, New Zealand.

containing serum seals. This system produced an intact, relatively undisturbed core with a headspace gas volume of approximately 100 cm^3. C_2H_2 was injected into this headspace without prior removal of an equal volume of gas, to give a partial pressure of 10.13 kPa; the headspace gas pressure was equalized; and incubations were then normally conducted at field temperature. Most of the experiments were done in summer, when ambient temperatures ranged from 8 to 28°C with a mean of about 18°C.

We removed organic matter from the sediment after incubation by sieving it through a 1-mm (pore size) sieve and separating the living roots. All other material was designated coarse particulate organic matter (CPOM). For ease of comparison, most results are presented on an area basis.

Experiments to investigate the effect of light were conducted in 100- or 1,000-cm^3 glass jars rather than in polyvinyl chloride tubes. Sediment cores were incubated in a water bath at 18°C under full sunlight.

$^{15}N_2$ fixation by decomposing leaves. Decomposing leaves were collected from the sediment surface at site 2, washed gently in seawater to remove adhering sediment, put into 7-ml screw-capped serum bottles, and preincubated for 8 h at 28°C. The bottles were then flushed with Ar, which was replaced with a mixture of 50.66 kPa of N_2 (60 atom % excess $^{15}N_2$), 30.4 kPa of Ar, and 20.27 kPa of O_2. After incubation of the bottles for 20 h at 28°C, the uptake of $^{15}N_2$ was stopped by the injection of acetylene to 10.13 kPa, and C_2H_2 reduction was assayed after 1 h. Soluble nitrogen was extracted from the leaves with 3 N hydrochloric acid, and both extract and residue were Kjeldahl digested. The ammonia was recovered in solution, and finally N_2 gas was regenerated by the addition of 10% lithium hypobromite and analyzed with a GEC/AEI MS10 mass spectrometer.

RESULTS

Nitrogenase activity by sediments. Six replicate cores, taken from beneath mangrove trees and from immediately outside the mangrove area, were incubated with acetylene. The mean activity was 12.3 μmol of C_2H_4 m^{-2} h^{-1} within the stand and 4.9 μmol of C_2H_4 m^{-2} h^{-1} outside, confirming that N_2 fixation occurs in New Zealand mangrove stands.

The coefficient of variation of the samples (n = 6) taken under mangrove trees was 66%, and many other samples from similar sites also showed a wide range of nitrogenase activity, from 0.4 to 50 μmol of C_2H_4 m^{-2} h^{-1}. However, samples taken outside the stand in similar substrates always showed low spatial variability, with a coefficient of variation in the above experiment of only 19%. In a large number of further experiments, we have consistently confirmed that nitrogenase activity is normally found in cores of estuarine muds and that it is significantly higher and spatially more variable in the presence of mangrove trees than away from them.

Sediment cores were normally 100 mm deep, and to determine whether the complete core or the top surface only was contributing to the observed nitrogenase activity, cores of different depths were assayed. Four replicate cores ranging from 10 to 100 mm in depth (measured from the top surface) were incubated in C_2H_2 for 20 h and assayed for C_2H_4. The results (Table 1) indicate that the rates of C_2H_2 reduction observed in cores came from the top 10 mm or even less. That activity did not increase with increasing core depth indicates the existence of a diffusion limitation to acetylene reduction by intact sediments. The apparent decline in activity with increasing core depth is not statistically significant.

Effect of light. To investigate the possibility that superficial cyanobacteria were responsible for the observed nitrogenase activity, six replicate cores were incubated in glass jars in the light and in the dark. Mean nitrogenase activities after 12 h were 6.1 and 6.2 μmol of C_2H_4 m^{-2} h^{-1} for light and dark

TABLE 1. Rates of acetylene reduction in air by intact sediment cores of increasing depth[a]

Depth of core (mm)	Mean C_2H_2 reduction rate ± SD (μmol of C_2H_4 m^{-2} h^{-1})[b]
10	4.3 ± 2.6
20	3.6 ± 3.8
30	3.1 ± 1.9
40	2.1 ± 0.3
50	2.8 ± 1.2
70	2.3 ± 1.3
100	2.6 ± 0.9

[a] Incubation for 20 h.
[b] Means and standard deviations of four replicates.

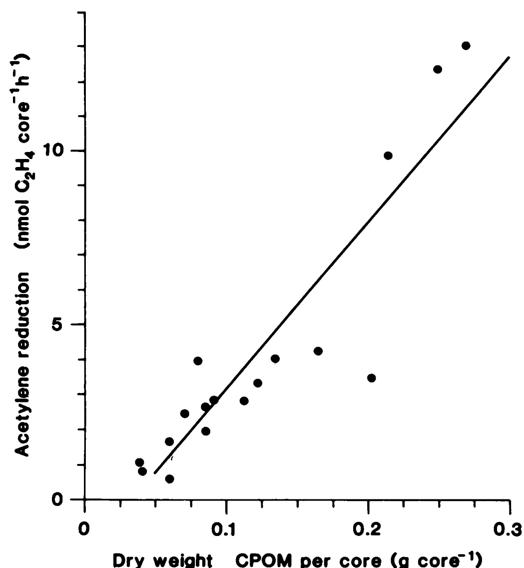

FIG. 2. Relationship of acetylene reduction by mangrove forest sediment cores to dry weight of CPOM in the top 10 mm of the cores.

treatments, respectively, indicating no significant light-dependent cyanobacterial contribution to sediment activity.

Source of variability. Several experiments were done in which, after incubation of sediment cores with C_2H_2, the identifiable organic fractions (CPOM and roots) were separated from the top 10 mm. No correlation was observed between nitrogenase activity and root dry weight. For example, C_2H_2 reduction in 13 cores (18 h of incubation) ranged from 0.4 to 11.0 nmol of C_2H_4 $core^{-1}$ h^{-1}, and root dry weight ranged from 0.02 to 0.34 g $core^{-1}$ ($r = -0.01$). However, a highly significant correlation was always found between the very variable C_2H_2 reduction rates and CPOM. For example, C_2H_2 reduction in 17 cores (after 11 h of incubation) ranged from 0.6 to 13.2 nmol of C_2H_4 $core^{-1}$ h^{-1} and was very significantly correlated with CPOM ($r = 0.91$; Fig. 2).

Oxygen dependence. Experiments with both intact cores 100 mm deep and the top 10 mm of sediment stirred in seawater (Table 2) showed that anaerobic conditions normally depressed nitrogenase activity in the sediment material. It may be concluded that at least some, and probably

TABLE 2. Nitrogenase activity of mangrove forest sediments and decaying leaves incubated aerobically and anaerobically

Expt no.	Material	*n*	C_2H_2 reduction rate[a]		Significance[b]
			Aerobic	Anaerobic	
1	Intact cores[c]	3	13.3	10.2	NS
2	Intact cores	3	5.6	1.0	NS
3	Intact cores	5	34.8	16.6	**
4	Stirred cores[d]	3	5.4	1.8	**
5	Stirred cores	5	10.9	1.8	*
6	Stirred cores	5	12.7	1.8	***
7	Decaying leaves	5	32.4	3.2	**

[a] For experiments 1 through 6, the rate is measured in micromoles of C_2H_4 $meter^{-2}$ $hour^{-1}$; for experiment 7 the rate is measured in micromoles of C_2H_4 gram (dry weight)$^{-1}$ $hour^{-1}$.

[b] NS, Not significant; *, significant ($P = 0.1$); **, significant ($P = 0.01$); ***, significant ($P = 0.001$).

[c] Intact cores 100 mm deep.

[d] Top 10 mm of sediment only.

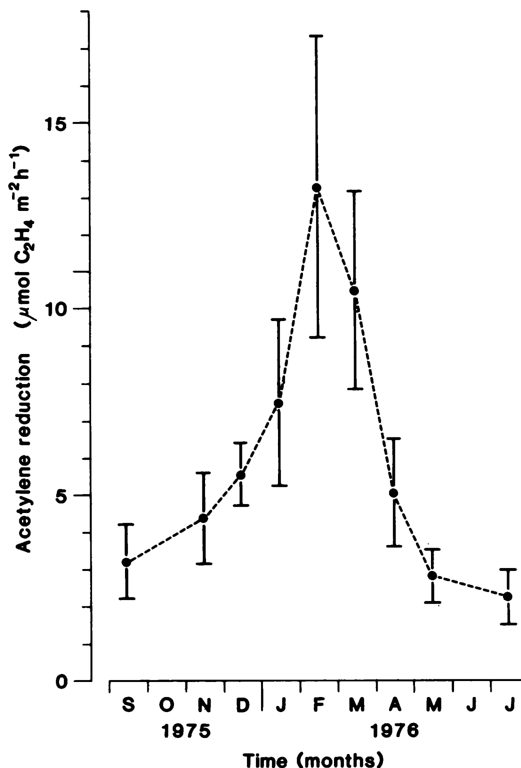

FIG. 3. Seasonal variation of acetylene reduction by 100-mm-deep cores of mangrove forest surface sediment (means of 6 to 10 replicates and 95% confidence intervals shown).

most, of the nitrogenase activity observed in the surface sediments and decaying leaves is aerobic or microaerophilic.

Seasonal variation. Nitrogenase activity in aerobically incubated 100-mm sediment cores taken from site 1 over a period of 11 months showed a distinct seasonal pattern of variation (Fig. 3). Rates of C_2H_2 reduction were at a maximum in summer, around February, and were lowest in winter, from about May to September.

Acetylene reduction by decomposing leaves. The density of recently fallen decomposing leaves ranges from 0.1 to 25 g (dry weight) m^{-2} under the mangrove canopy. Decomposing leaves incubated in C_2H_2 at various temperatures for 10 h showed high rates of C_2H_2 reduction (Table 3), with a Q_{10} of approximately 2. These results can be compared with activities in CPOM (Fig. 2), which averaged 30.8 nmol of C_2H_4 g (dry weight)$^{-1}$ h^{-1}.

Anaerobic incubations of leaves significantly depressed nitrogenase activity in decaying leaf material, and this was even more marked than in sediments (Table 2).

TABLE 3. Effect of temperature on nitrogenase activity in decomposing mangrove leaves

Temp (°C)	Mean C_2H_2 reduction rate ± SD (nmol of C_2H_4 g [dry wt]$^{-1}$ h^{-1})[a]
10	11.1 (6.2)
20	19.6 (7.2)
30	41.4 (8.3)

[a] Means and standard deviations of five replicates.

TABLE 4. $^{15}N_2$ fixation and acetylene reduction by decomposing mangrove leaves[a,b]

$^{15}N_2$ fixation (nmol of $^{15}N_2$ excess g [dry wt]$^{-1}$ h^{-1})	C_2H_2 reduction rate (nmol of C_2H_4 g [dry wt]$^{-1}$ h^{-1})	Molar ratio
5.1	29.1	5.7
5.1	17.1	3.4
7.8	35.1	4.5

[a] Incubation at 28°C with $^{15}N_2$ for 20 h under 50.26 kPa 60 atom % excess $^{15}N_2$, followed by acetylene reduction for 1 h under 10.13 kPa C_2H_2.
[b] Means: fixation, 6.0; C_2H_2 reduction rate, 27.1; molar ratio, 4.5.

$^{15}N_2$ fixation by decomposing leaves. Confirmation of nitrogen fixation in decaying leaves was obtained by assaying $^{15}N_2$ uptake. The results (Table 4) confirm that fixation in three replicate samples occurred at rates of over 5 μmol of N_2 g (dry weight)$^{-1}$ h^{-1}. C_2H_2 reduction rates for the three replicates (Table 4) were much more variable, leading to a range of molar ratios (3.4 to 5.7) with a mean of 4.5. Although we cannot rule out the possibility that O_2 and substrate depletion may have occurred during incubation, time course experiments showed that C_2H_4 production was linear over similar periods.

Acetylene reduction by pneumatophores. The breathing roots of some mangroves (pneumatophores) are vertical roots, 5 to 12 mm in diameter, extending 100 to 200 mm above the sediment surface. They occur in very high concentrations in muddy places. C_2H_2 reduction rates of individual freshly harvested pneumatophores exhibited a wide range of activity, from 0 to 1,500 nmol of C_2H_4 h^{-1}, but rates generally ranged from 100 to 500 nmol of C_2H_4 h^{-1} in summer. Highest rates occurred in midsummer, and very low rates occurred in winter.

The light dependence of C_2H_2 reduction in pneumatophores was tested by preincubating them for 10 h at 45 microeinsteins m^{-2} s^{-1} (cool, white fluorescent light) and in the dark, and assaying for C_2H_2 reduction under the same conditions for the following 19 h at 25°C. Rates per pneumatophore averaged 94 ± 45 nmol of C_2H_4 h^{-1} in the light and 18 ± 4 nmol of C_2H_4 h^{-1} in the dark, showing a strong light dependence.

Pneumatophores with high C_2H_2 reduction rates invariably had a high density of the heterocystous cyanobacterium *Calothrix*, which is a known nitrogen-fixing organism (15).

Pneumatophore density is variable and ranges from less than 10 m^{-2} in sandy, well-aerated sediments to 300 m^{-2} in muddy, waterlogged areas. Assuming a nominal density of 100 m^{-2} and a C_2H_2 reduction rate of 300 nmol of C_2H_4 pneumatophore^{-1} h^{-1}, the contribution of pneumatophores could be in the order of 30 μmol of C_2H_4 m^{-2} h^{-1}, or over twice the average seasonal maximum rate for sediments (Fig. 3).

DISCUSSION

A wide range of nitrogen-fixation sites has been identified in aquatic environments. It is clear from our work and that of others (1, 3, 13) that root-free estuarine muds contain active nitrogen-fixing bacteria. It is equally clear that activities are low and are considerably increased by the presence of plants, both in freshwater systems (2, 6) and in marine environments (13, 24). A general conclusion from the above work and from work on some terrestrial habitats is that the plant stimulates nitrogen fixation by providing the rhizosphere habitat. However, our work showed no evidence of significant root-associated activity in surface sediments, and instead, the sediment core activity could be entirely accounted for by decaying organic matter (mainly twigs and leaves). Zuberer and Silver (25) have also identified the association of C_2H_2 reduction with plant litter.

Although roots from horizons below the surface sediments may have the ability to fix nitrogen, we did not investigate them because of their uncertain gas relations in a diffusion-limited system. One indication of gas diffusion limitation was the light color of the top 10 mm of sediments compared with the dark, sulfur-smelling sediments below the top 10 mm. This was probably caused by a combination of oxygen demand by surface sediments and poor oxygen penetration through their fine, waterlogged matrix.

The mean activity of decaying leaves taken from the surface in our study (32.4 nmol of C_2H_4 g [dry weight]$^{-1}$ h^{-1}; Table 2) is remarkably similar to the mean activity of sediment cores divided by their CPOM content (30.8 nmol of C_2H_4 g [dry weight]$^{-1}$ h^{-1}; Fig. 2), which lends further weight to the low significance of rhizosphere activity.

Although we do not entirely discount the possibility of rhizosphere nitrogen fixation, we conclude that most of the nitrogenase activity observed in core samples is attributable to decaying organic matter in the top 10 mm.

Most studies of aquatic nitrogenase activity have concluded that anaerobes, either strict or facultative, are the principal agents (2, 13, 24). However, in New Zealand mangroves, activity based on decaying organic matter was consistently inhibited by anaerobic incubation (Table 2). Anaerobes are no doubt present, especially in the lower parts of the profile, but account for only a small proportion of the observed C_2H_2 reduction. A parallel to this situation is found in nitrogenase associated with decaying wood in forests. Many workers have concluded on the basis of isolated bacteria that anaerobes were the major diazotrophs. However, recent work (19) has shown that oxygen is essential for the activity of nitrogenase in decaying wood of Douglas fir.

Nitrogen fixation in decaying leaves of *Avicennia* in our study gave 5 to 8 nmol of N_2 g^{-1} h^{-1} (140 to 224 ng of N g^{-1} h^{-1}), in contrast to the rates recorded by Gotto and Taylor (8) of 11 μg of N g^{-1} h^{-1} for decaying *R. mangle* leaves. However, in the *Rhizophora* leaves, photosynthetic organisms accounted for up to two-thirds of the nitrogenase activity, which was apparently all anaerobic. Zuberer and Silver (25) found no significant depression of mangrove leaf C_2H_2 reduction under aerobic conditions.

The highest unit rates of nitrogenase activity in the present study were associated with excised pneumatophores. This activity was correlated with the presence of *Calothrix*. It is interesting to note that *Calothrix* is not recorded among the algal flora of mangrove pneumatophores in New Zealand (4, 5), which may indicate seasonal periodicity or other site factors, but has been recorded on pneumatophores of *Avicennia marina* in Sinai (14). High *Calothrix* abundance in our work was invariably accompanied by high nitrogenase activity.

Our study has revealed that nitrogenase activity occurs in New Zealand mangrove forest sediments and that it differs from that associated with plants in other aquatic environments because it appears to be principally independent of light, based on organic matter, and depressed by anaerobic conditions. However, the majority of nitrogen fixation in New Zealand mangrove forests is likely to come, under optimum conditions for algal productivity, from the epiphytic flora on pneumatophores.

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LITERATURE CITED

1. **Bohlool, B. B.** 1978. Nitrogen fixation in polluted intertidal sediments of Waimea Inlet, Nelson. N.Z. J. Mar. Freshwater Res. **12**:271–275.
2. **Bristow, J. M.** 1974. Nitrogen fixation in the rhizosphere of freshwater angiosperms. Can. J. Bot. **52**:217–221.
3. **Brooks, R. H., Jr., P. C. Brezonik, H. D. Putman, and M. A. Keirn.** 1971. Nitrogen fixation in an estuarine environment. Oceanography **16**:701–710.
4. **Chapman, V. J., and D. J. Chapman.** 1973. The algae. MacMillan, London.
5. **Chapman, V. J., and J. W. Ronaldson.** 1958. The mangrove and salt-marsh flats of the Auckland Isthmus. N.Z. Department of Scientific and Industrial Research bulletin no. 125, N.Z. Department of Scientific and Industrial Research, Wellington, New Zealand.
6. **Eskew, D. L., A. R. J. Eaglesham, and A. A. App.** 1981. Heterotrophic $^{15}N_2$ fixation and distribution of newly fixed nitrogen in a rice-flooded system. Plant Physiol. **68**:45–52.
7. **Gordon, S. C., and C. T. Wheeler.** 1983. Biological nitrogen fixation in forest ecosystems: foundations and applications. Martinus Nijheff/Dr W. Junk, The Hague.
8. **Gotto, J. W., and B. F. Taylor.** 1976. N_2 fixation associated with decaying leaves of the red mangrove (*Rhizophora mangle*). Appl. Environ. Microbiol. **31**:781–783.
9. **Hardy, R. W. F., R. D. Holsten, E. D. Jackson, and R. C. Burns.** 1968. The acetylene-ethylene assay for N_2 fixation: laboratory and field evaluation. Plant Physiol. **43**:1185–1207.
10. **Kimball, M. C., and H. J. Teas.** 1975. Nitrogen fixation in mangrove areas of Southern Florida, p. 654–660. *In* G. E. Walsh, S. C. Snedaker, and H. J. Teas (ed.), The biology and management of mangroves. University of Florida, Gainesville.
11. **Larsen, M. J., M. F. Jurgensen, and A. E. Harvey.** 1978. N_2 fixation associated with wood decayed by some common fungi in western Montana. Can. J. For. Res. **8**:341–345.
12. **Nedwell, D. B.** 1975. Inorganic nitrogen metabolism in a eutrophicated tropical mangrove estuary. Water Res. **9**:221–231.
13. **Patriquin, D., and R. Knowles.** 1972. Nitrogen fixation in the rhizosphere of marine angiosperms. Mar. Biol. **16**:49–58.
14. **Potts, M.** 1979. Nitrogen fixation (acetylene reduction) associated with communities of heterocystous and non-heterocystous blue-green algae in mangrove forests of Sinai. Oecologia **39**:359–373.
15. **Pringsheim, E. G.** 1968. Cyanophyceen-Probleme. Planta **79**:1–9.
16. **Richards, B. N.** 1973. Nitrogen fixation in the rhizosphere of conifers. Soil Biol. Biochem. **5**:149–152.
17. **Roskoski, J.** 1980. Nitrogen fixation in hardwood forests of the northeastern United States. Plant Soil **54**:33–44.
18. **Silvester, W. B.** 1978. Nitrogen fixation and mineralisation in kauri (*Agathis australis*) forest in New Zealand, p. 138–143. *In* W. M. Loutit and J. A. R. Miles (ed.), Microbial ecology (proceedings in life sciences). Springer Verlag, Berlin.
19. **Silvester, W. B., and K. J. Bennet.** 1973. Acetylene reduction by roots and associated soil of New Zealand conifers. Soil Biol. Biochem. **5**:171–179.
20. **Silvester, W. B., P. Sollins, T. Verhoeven, and S. P. Cline.** 1982. Nitrogen fixation and acetylene reduction in decaying conifer boles: effects of incubation time, aeration, and moisture content. Can. J. For. Res. **12**:646–652.
21. **Sollins, P.** 1982. Input and decay of coarse woody debris in coniferous forests of western Oregon and Washington. Can. J. For. Res. **12**:18–28.
22. **Todd, R. L., J. B. Waide, and B. W. Cornaby.** 1975. Significance of biological nitrogen fixation and denitrification in a deciduous forest ecosystem, p. 729–735. *In* F. G. Howell, J. B. Gentry, and M. H. Smith (ed.), U.S. Energy Research and Development Administration Symposium CONF-740513. National Technical Information Services, Springfield, Va.
23. **Uchino, F., G. G. Hambali, and M. Yatazawa.** 1984. Nitrogen-fixing bacteria from warty lenticellate bark of a mangrove tree, *Brugiera gymnorrhiza* (L.) Lamk. Appl. Environ. Microbiol. **47**:44–48.
24. **Zuberer, D. A., and W. S. Silver.** 1975. Mangrove-associated nitrogen fixation, p. 643–653. *In* G. E. Walsh, S. C. Snedaker, and H. J. Teas (ed.), Biology and management of mangroves. University of Florida, Gainesville.
25. **Zuberer, D. A., and W. S. Silver.** 1978. Biological dinitrogen fixation (acetylene reduction) associated with Florida mangroves. Appl. Environ. Microbiol. **35**:567–575.