New Zealand Journal of Marine and Freshwater Research, 1990, Vol. 24: 481–486
0028–8330/2404–0481 $2.50/0

Acetylene reduction associated with *Zostera novazelandica* Setch. and *Spartina alterniflora* Loisel., in Whangateau Harbour, North Island, New Zealand

BRENDAN J. HICKS[1]
WARWICK B. SILVESTER[2]
Department of Botany
University of Auckland
Private Bag, Auckland, New Zealand

[1]Present address: Freshwater Fisheries Centre, Ministry of Agriculture and Fisheries, P. O. Box 6016, Rotorua, New Zealand

[2]Present address: Department of Biological Sciences, University of Waikato, Private Bag, Hamilton, New Zealand

Abstract Nitrogen fixation (acetylene reduction) was investigated in *Zostera novazelandica* Setch. and *Spartina alterniflora* Loisel., in the North Island of New Zealand. Moderate rates of acetylene reduction were found in sediments in which plants were growing (means ± 95% confidence limit: 15.2 ± 2.8 μmol C_2H_4 m^{-2} h^{-1} for *Zostera* and 24.7 ± 4.6 μmol C_2H_4 m^{-2} h^{-1} for *Spartina*). Activity was closely correlated with the dry weight of roots (r^2 = 0.65, N = 15 for *Zostera*, and r^2 = 0.85, N = 10 for *Spartina*). Sediment close to the plant beds, but without plants, exhibited only low rates of acetylene reduction (2.9 ± 2.2 and 4.5 ± 1.0 μmol C_2H_4 m^{-2} h^{-1}, respectively). Sediments associated with Z. *novazelandica* and *S. alterniflora* in New Zealand exhibit moderate rates of nitrogenase activity compared to rates found in other countries. N fixation may contribute significantly to the nutrition of these plants in New Zealand estuaries.

Keywords nitrogen fixation; acetylene reduction; *Zostera*; *Spartina*; seagrass; grass; sediment

M90006
Received 7 February 1990; accepted 28 May 1990

INTRODUCTION

Zostera novazelandica Setch. is a widespread, indigenous seagrass occurring intertidally in New Zealand. It was previously known as *Z. muelleri* (Den Hartog 1970; Connor & Edgar 1987). *Spartina alterniflora* Loisel. is an intertidal grass that was introduced from the eastern United States in 1955 (Partridge 1987), and is now established from North Cape to Gisborne in the North Island of New Zealand.

Nitrogen fixation (acetylene reduction) has been widely found associated with intertidal sediments and marine angiosperms (e.g., Patriquin & Knowles 1972; McRoy et al. 1973; Bohlool 1978; Bohlool & Weibe 1978; Hicks & Silvester 1985; Gandy & Yoch 1988; Talbot et al. 1988). Acetylene reduction has been found associated with roots and sediments of *Spartina alterniflora* from Nova Scotia and Georgia (McClung et al. 1983). Nitrogenase activity has been attributed to a variety of bacteria. In the upper 5 cm of sediment, these appear to be primarily sulphate-reducing bacteria, and in sediment from 5 to 10 cm deep, fermenting bacteria predominate (Gandy & Yoch 1988). *Campylobacter nitrofigilis* has been identified as one N fixer associated with roots of *S. alterniflora* (McClung et al. 1983). Surface sediments and the rhizosphere were more important than the phyllosphere as sites of acetylene reduction associated with *S. maritima* (Curtis) Fern. from a South African estuary (Talbot et al. 1988).

The ecological significance of N fixation is supply of N in a form usable by plants. N supply is one of the most important factors limiting salt marsh vegetation (Valiela & Teal 1974). In nutrient-poor environments, such as tropical waters, it has been speculated that N fixation associated with plants such as *Thalassia testudinum* Kon. could supply 1.5–5 times their growth requirements for N of 6.9–23 mg N g^{-1} leaf tissue (Patriquin & Knowles 1972).

Acetylene reduction has also been associated with *Zostera* species. Roots and sediments of *Z. marina* L. from New Brunswick were found to reduce acetylene (Patriquin & Knowles 1972), though McRoy et al. (1973) failed to find measurable activity in roots,

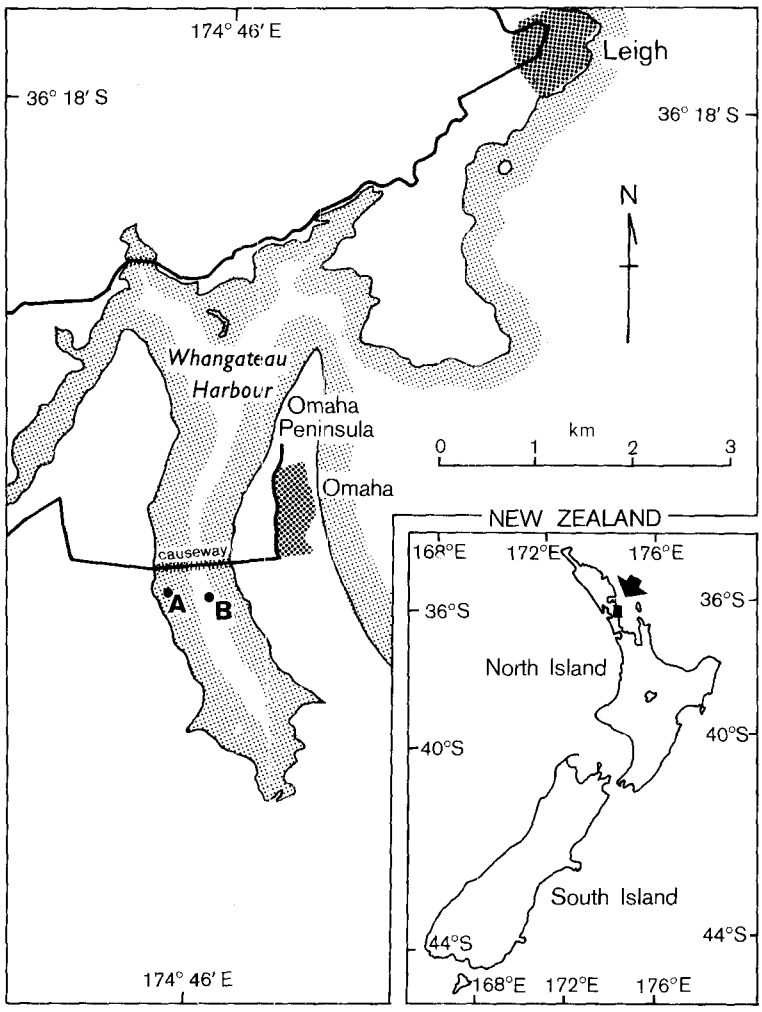

Fig. 1 Location of sampling sites for (A) *Spartina alterniflora* and (B) *Zostera novazelandica* in the Whangateau Harbour, North Island, New Zealand.

sediments, or leaves of *Z. marina* from North Carolina and Alaska.

As part of studies undertaken in Whangateau Harbour, North Island, New Zealand, we investigated the potential for acetylene reduction associated with *Zostera novazelandica* and *Spartina alterniflora*.

STUDY SITES

Sediment and plant materials were collected at low tide from two sites in the middle of Whangateau Harbour, 58 km north of Auckland (Fig. 1). The beds of both *Zostera novazelandica* and *Spartina alterniflora* were immediately to the south of a road causeway across the harbour, and the plants appeared healthy.

METHODS

Acetylene reduction assay

The acetylene reduction assay for nitrogenase activity was conducted using methods previously described (Hardy et al. 1968; Hicks & Silvester 1985). Incubations were all carried out using a gas phase of air and 10.13 kPa (0.1 atm) of acetylene in enclosed vessels. Incubation time ranged from 11 to 13.5 h.

After incubation, 0.1 cm^3 samples were analysed for ethylene production using a Carle 9500 Basic gas chromatograph with a flame ionisation detector. Ethylene peak heights were measured and related to calibrations made with standard ethylene concentrations. Endogenous ethylene production and background ethylene were never detectable.

Previous work in this environment (Hicks & Silvester 1985) has used $^{15}N_2$ uptake to calibrate acetylene reduction, and has shown that endogenous ethylene production is not significant. On the basis of our previously published work, we are confident that the acetylene reduction activity demonstrated here is a reliable estimate of nitrogenase activity.

Collection and incubation

Samples were collected on 26 January and 7 March 1975, as sediment cores of two different sizes. A metal corer, 73 mm inside diam., and a polyvinyl chloride corer, 38 mm inside diam., were used to cut cores 100 mm in length from within beds of the two plant species, and from adjacent plant-free sediment areas. In January the large corer was used, and in March the smaller corer was used to reduce sample volume. Care was taken to exclude above-ground plant parts in cores from the *Spartina* bed, but this was not possible in the cores from the *Zostera* bed. Intact 73-mm diameter cores were incubated in the dark in 1050 cm^3 volume glass jars with lids fitted with serum septum seals. Smaller 38-mm cores were extruded into opaque incubation chambers 38 mm in diameter with airtight caps containing serum seals (Hicks 1976). These procedures produced intact, relatively undisturbed cores with headspace gas volumes of c. 650 cm^3 for large cores and 100 cm^3 for small cores.

Within 1 h of collection acetylene 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 equalised and incubations were then conducted at field temperature. Roots were removed from the sediment after incubation by passing core materials through a 1-mm pore-size sieve, then dried at 105°C for 24 h and cooled before weighing.

RESULTS

Results from both sampling periods were not significantly different, so the results were combined. Acetylene reduction rates for sediments containing either *Spartina* or *Zostera* were always 5–6 times higher than for adjacent sediment without plants (Table 1). In all cases, regardless of species or time, the differences were highly signficant. These acetylene reduction rates were also correlated with dry weight of roots extracted from cores following acetylene reduction assay (Fig. 2A and 2B). Root dry weight accounted for 65% of the variability of acetylene reduction in *Zostera* cores, and 85% of the variability of activity in *Spartina* cores. Slopes of the regression equations were highly significant ($P = 0.00031$ and $P = 0.00013$ for *Zostera* and *Spartina*, respectively). The linear equation for *Zostera novazelandica* was

$$y = 53.2 + 31.2 \ln x \quad (1)$$

and for *Spartina alterniflora* was

$$y = 50.8 + 70.3 \ln x \quad (2)$$

where y = ethylene production (nmol C_2H_4 $core^{-1}$ h^{-1}) and x = dry weight of roots.

Mean acetylene reduction per gram dry weight of root material was the same for both plant species. Mean activity ± 95% confidence limits for *Zostera* was 45.1 ± 9.0 nmol C_2H_4 g^{-1} dry wt roots h^{-1}, and was 46.1 ± 9.1 for *Spartina*.

DISCUSSION

Nitrogenase activity has been widely demonstrated in *Zostera* and *Spartina* species. In *Zostera* species, nitrogenase activity has been found in material from New Brunswick, Canada; Alaska, USA; and South Africa (Patriquin & Knowles 1972; McRoy et al. 1973; Talbot et al. 1988). In *Spartina* species, nitrogenase activity has been found in material from Nova Scotia, Canada; South Carolina, USA; and South Africa (Patriquin & McClung 1978; Gandy & Yoch 1988; Talbot et al. 1988). Sediment and roots appear to be the primary sites of activity. Similar findings have now been demonstrated in New Zealand.

Nitrogenase activity occurs in *Zostera novazelandica* and *Spartina alterniflora* in northern New Zealand. This activity appears to be associated with the roots, on the basis of strong correlation between root weight and nitrogenase activity (Fig. 2A and 2B, Equations 1 and 2). No above-ground plant parts were included in the incubation of *S. alterniflora*

Table 1 Acetylene reduction by sediment containing *Zostera novazelandica* and *Spartina alterniflora* and adjacent sediment without plants.

Sediment type	*N*	Acetylene reduction (μmol C_2H_4 m^{-2} h^{-1}) mean ± 95% confidence interval
Zostera		
With plants	15	15.2±2.8
Without plants	9	2.9±2.2
Spartina		
With plants	10	24.7±4.6
Without plants	7	4.5±1.0

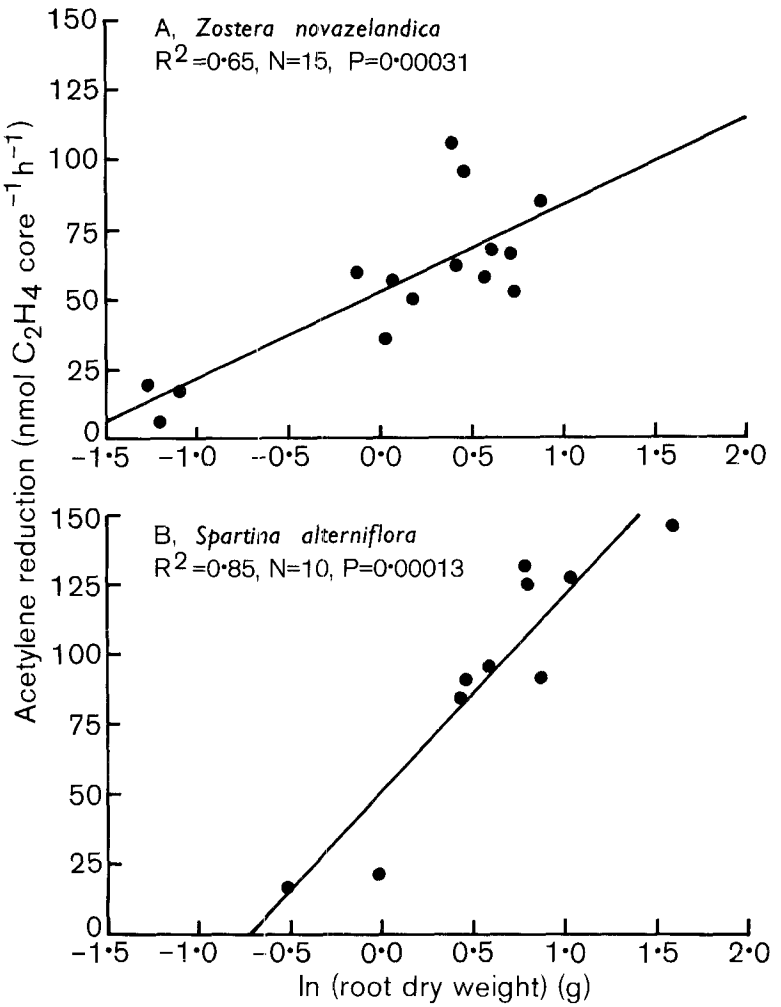

Fig. 2 Relationship of rate of ethylene production from acetylene reduction to natural log of root dry weight by (A) *Zostera novazelandica* and (B) *Spartina alterniflora*.

cores, but some were included in the *Z. novazelandica* incubation, and these could have carried epiphytic N fixers such as cyanobacteria. Epiphytic N fixers abound on tidally-immersed surfaces such as mangrove pneumatophores in this area (Hicks 1976; Hicks & Silvester 1985), and may be responsible for the weaker correlation between root dry weight and nitrogenase activity observed for *Z. novazelandica* compared to *S. alterniflora*.

The implied association of nitrogenase activity with roots found in these experiments is in contrast to our findings for nitrogenase activity associated with mangroves (*Avicennia marina* (Forst.) Vierh. var. *resinifera* (Forst. f.) Bakh.) in New Zealand. Acetylene reduction in sediments associated with mangrove trees was strongly correlated with dry weight of decaying coarse particulate matter, which was mainly leaf fragments ($r^2 = 0.83, N = 17, P < 0.01$). Acetylene reduction was not, however, correlated with weight of attached roots ($r^2 = 0.00, N = 13, P >> 0.05$) (Hicks & Silvester 1985). Coarse particulate organic matter was an insignificant component of sediment cores from *Z. novazelandica* and *S. alterniflora* beds.

The location of nitrogenase activity within the plant-sediment system is important to the fate of fixed N in the intertidal ecosystem. Though we did not attempt to identify acetylene reduction associated with surface and rhizosphere sediments separately from that associated with the phyllosphere, the correlation with root weight suggests that rhizosphere-based activity is dominant. However, McRoy et al. (1973) found acetylene reduction to be associated

predominantly with leaves of *Thalassia testudinum*, a seagrass collected from Florida, USA. This activity was also light-dependent, suggesting cyanobacterial origin.

Whether the basis of nitrogenase activity in *Zostera* and *Spartina* in South Africa is autotrophy or heterotrophy is unclear. Talbot et al. (1988) found acetylene reduction associated with surface sediment, rhizosphere sediment, and phyllosphere of *Z. capensis* Setchell and *S. maritima*. Acetylene reduction in *Z. capensis* was predominantly associated with the rhizosphere, whereas activity in *S. maritima* was greater in surface sediments than in the rhizosphere. N fixed in the surface and rhizosphere sediments is likely to be more accessible to the plant than that fixed in the phyllosphere, which might be more readily available for export.

Rates of N fixation can be estimated from the nitrogenase activity we measured (Table 1), using some assumptions. We have assumed a molar ratio of 4.5, as found in our previous work in an estuarine environment (Hicks & Silvester 1985). Inputs of 8.3 kg N ha^{-1} y^{-1} can be projected for areas vegetated with *Z. novazelandica*, and 1.6 kg N ha^{-1} y^{-1} in adjacent open areas. For *S. alterniflora*, equivalent rates are 13.5 and 2.5 kg N ha^{-1} y^{-1} in vegetated and open areas, respectively. In comparison, Patriquin & Knowles (1972) estimated N fixation rates of 100–500 kg N ha^{-1} y^{-1} in beds of *Thalassia testudinum* in the Carribean and 5–10 kg N ha^{-1} y^{-1} in non-rhizosphere sediments. However, on examination of N fixation associated with *Thalassia* from Florida, McRoy et al. (1973) found only low maximum rates of 29 μg N m^{-2} day^{-1}, or 0.106 kg N ha^{-1} y^{-1}. Bohlool & Weibe (1978) demonstrated rates of N fixation ranging between 1 and 200 g N ha^{-1} day^{-1} from different intertidal sources in New Zealand not associated with marine angiosperms. These translate to annual rates of 0.365–73 kg N ha^{-1} y^{-1}.

The ecological significance of N fixation associated with *Spartina* and *Zostera* in New Zealand is not clear. N fixation associated with *Spartina alterniflora* in Georgia, USA, was estimated at 148 kg N ha^{-1} y^{-1}, sufficient to supply the N required by all major salt marsh macrophytes in this estuary (Hanson 1983). However, rates of N fixation sufficient for growth requirements do not prove that such N is actually assimilated. *Spartina alterniflora* in North Carolina, USA, appeared to be N limited even in the presence of NH_4–N sufficient for growth, indicating that some factor or factors prevented N uptake (Mendelssohn 1979).

In conclusion, we have demonstrated that sediments associated with *Z. novazelandica* and *S. alterniflora* in a New Zealand estuary exhibited moderate rates of nitrogenase activity, and that this activity was correlated with root weight. N fixation may contribute significantly to the nutrition of these plants in New Zealand estuaries, but assimilation of fixed N has yet to be proven.

ACKNOWLEDGMENTS

This work was funded in part by the Leigh Marine Laboratory, University of Auckland. The Marine Laboratory also provided accommodation and laboratory space.

REFERENCES

Bohlool, B. B. 1978: Nitrogen fixation in polluted intertidal sediments of Waimea Inlet, Nelson. *New Zealand journal of marine and freshwater research 12*: 271–275.

Bohlool, B. B.; Wiebe, W. J. 1978: Nitrogen-fixing communities in an intertidal ecosystem. *Canadian journal of microbiology 24*: 932–938.

Connor, H. E.; Edgar E. 1987: Name changes in the indigenous New Zealand flora, 1960–1986 and Nomina Nova IV, 1983–1986. *New Zealand journal of botany 25*: 115–170.

Den Hartog, C. 1970: Sea-grasses of the world. *Verhandelingen der Koninklijke Nederlandse Academie van Wetenschappen, afdeling natuurkunde, tweede sectie 59*.

Gandy, E. L.; Yoch, D. C. 1988: Relationship between nitrogen-fixing sulfate reducers and fermenters in salt marsh sediments and roots of *Spartina alterniflora*. *Applied and environmental microbiology 58*: 2031–2036.

Hanson, R. B. 1983: Nitrogen fixation activity (acetylene reduction) in the rhizosphere of salt marsh angiosperms, Georgia, U. S. A. *Botanica marina 26*: 49–59.

Hardy, R. W. F.; Holsten, R. D.; Jackson, E. D.; Burns, R. C. 1968: The acetylene-ethylene assay for N_2 fixation: laboratory and field evaluation. *Plant physiology 43*: 1185–1207.

Hicks, B. J. 1976: Nitrogen fixation associated with the New Zealand mangrove. Unpublished MSc thesis, University of Auckland, Auckland. 117 p.

Hicks, B. J.; Silvester, W. B. 1985: Nitrogen fixation associated with the New Zealand mangrove (*Avicennia marina* (Forsk.) Vierh. var. *resinifera* (Forst. f.) Bakh.). *Applied and environmental microbiology 49*: 955–959.

McClung, C. R.; Patriquin, D. G.; Davis, R. E. 1983: *Campylobacter nitrofigilis* sp. nov., a nitrogen-fixing bacterium associated with the roots of *Spartina alterniflora* Loisel. *International journal of systematic bacteriology 33*: 605–612.

McRoy, C. P.; Goering, J. J.; Chaney; B. 1973: Nitrogen fixation associated with seagrasses. *Limnology and oceanography 18*: 998–1002.

Mendelssohn, I. A. 1979: Nitrogen metabolism in the height forms of *Spartina alterniflora* in North Carolina. *Ecology 60*: 574–584.

Partridge, T. R. 1987: *Spartina* in New Zealand. *New Zealand journal of botany 25*: 567–575.

Patriquin, D.; Knowles, R. 1972: Nitrogen fixation in the rhizosphere of marine angiosperms. *Marine biology 16*: 49–58.

Partiquin, D. G.; C. R. McClung. 1978: Nitrogen accretion, and the nature and possible significance of N_2 (acetylene reduction) in a Nova Scotian *Spartina alterniflora* stand. *Marine biology 47*: 227–242.

Talbot, M. M. B.; Small, J. G. C.; Bate, G. C. 1988: Spatial patterns of N fixation (acetylene reduction) along environmental gradients in a warm-temperate estuary. *Journal of experimental marine biology and ecology 117*: 255–270.

Valiela, I.; Teal, J. M. 1974: Nutrient limitation in salt marsh vegetation. *In*: Reimold, R.J.; Queen, W.H. *ed*. Ecology of halophytes, volume 7, New York, Academic Press. Pp. 547–563.