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N_2 Fixation Associated with Decaying Leaves of the Red Mangrove (*Rhizophora mangle*)

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N_2 (C_2H_2) fixation was associated with decaying leaves of *Rhizophora mangle*. The process was predominantly anaerobic, with about two-thirds of the nitrogenase activity being light dependent. Average N_2 fixation rates in the light were 11 μg of N per g (dry weight) per h for leaves that had decayed for 2 to 3 weeks. This nitrogen input is probably significant in the estuarine, detrital food chains linked to *R. mangle*.

Organic detritus is an important source of food for organisms in many aquatic environments (15, 19). Detritus-based food chains have been well studied in woodland streams (3, 8, 14), salt marsh, sea grass, and other estuarine areas (4, 17, 22). Litter from the red mangrove *Rhizophora mangle* forms the basis of an aquatic food web in estuaries fringed by mangroves in southern Florida (E. J. Heald, Ph.D. thesis, Univ. of Miami, Miami, Fla., 1969; W. E. Odum, Ph.D. thesis, Univ. of Miami, Miami, Fla., 1970). Heald (Ph.D. thesis) detected increases in the relative amount of nitrogen during the decay of red mangrove leaves. Similar results have been obtained with leaves from deciduous trees in temperate climes decaying in freshwater streams (12) and also for *Spartina* and *Juncus* leaves degrading in salt marshes (5, 16). In all these systems the relative increase in nitrogen content appears to be primarily due to the proliferation of fungi, which are ultimately consumed by detrital feeders (Heald, Ph.D. thesis). Fell et al. (6) observed that red mangrove leaves lose a significant portion of their nitrogenous compounds by leaching during the first few days of immersion in seawater but that this loss is later replenished. The present communication provides evidence that a significant portion of the nitrogen required for this replenishment is provided from N_2 fixation by microorganisms associated with the decaying leaves.

Senescent or postsenescent leaves (6) were collected from trees of *R. mangle* at Key Biscayne, Fla. The leaves were placed inside nylon mesh bags (mesh size, 2.5 mm^2), and then the weighted bags were deposited on the sediment surface and tied to mangrove roots at the site of collection. Environmental temperatures during the summer varied between about 26 and 30 C. The leaves were completely submerged for periods of up to 4 weeks. For some experiments, leaves of unknown age decaying naturally were collected from the sediment surface at the field site. During transport back to the laboratory for assays of N_2 fixation, the leaves were immersed in ambient seawater. N_2 fixation was assayed by the C_2H_2 reduction method (10, 20). The assays were carried out in 55-ml Erlenmeyer flasks sealed with serum caps. Each leaf was cut into sections with an area of about 0.5 to 1.0 cm^2. One-half of the sections from a single leaf were put into each flask. In aerobic assays, the flasks contained 10 ml of Gulf Stream seawater (membrane filtered [Millipore Corp.], 0.45-μm pore size) which completely covered the leaves. The gas phase was air containing 20% C_2H_2 by volume. For anaerobic incubations, the seawater was purged with a flow of argon to remove oxygen, and the gas phase was 20% C_2H_2 by volume in argon. The flasks were incubated in a water bath at 30 C. Dark flasks were wrapped in silver-grey air conditioning tape, and illumination was provided by an array of tungsten lamps at an intensity of about 5,000 lx. In some experiments the flasks were agitated by reciprocal shaking (80 strokes/min; amplitude, 5.0 cm). The gas phases were sampled (0.25 ml) with Glaspak syringes (Becton-Dickinson & Co., Rutherford, N.J.) at zero time and then every hour for up to 5 to 6 h. C_2H_4 in the gas samples was measured by gas chromatography (2). After assaying, the sections of leaf were removed from the flasks and dried to a constant weight at 105 C. A molar ratio of 3:1 was assumed to convert C_2H_4 production to N_2 fixation (10).

N_2 (C_2H_2) fixation was consistently observed in association with decaying leaves of *R. mangle*. Controls under all conditions showed no C_2H_4 production in the absence of C_2H_2. Shaken aerobic assays exhibited low rates of C_2H_2 re-

duction, presumably due to the oxygen inactivation of nitrogenase (Fig. 1, Table 1). The rate of C_2H_2 reduction in unshaken aerobic assays had a lag phase of 1 to 2 h before linear rates were attained; this lag was virtually eliminated by shaking under anaerobic conditions (Fig. 1). The rates in unshaken aerobic assays were calculated from the linear portion of the graph since subsequent experiments showed that there was no significant difference with leaves at the same state of decay between this rate and that obtained in shaken anaerobic incubations ($P < 0.05$). For example, in experiments with 14-day-old leaves, the light rate of N_2 fixation in unshaken aerobic assays was 10.1 μg of N (dry weight) per h ($n = 5$; standard deviation = 1.6) as compared to 10.4 μg of N (dry weight) per h ($n = 5$; standard deviation = 1.9) in shaken anaerobic assays. In addition, there was no difference between rates observed in shaken microaerophilic assays (0.02 atm of O_2) and shaken anaerobic assays ($P < 0.05$). The rates of C_2H_2 reduction were significantly higher ($P < 0.05$) in the light than in the dark in both aerobic and anaerobic assays (Table 1).

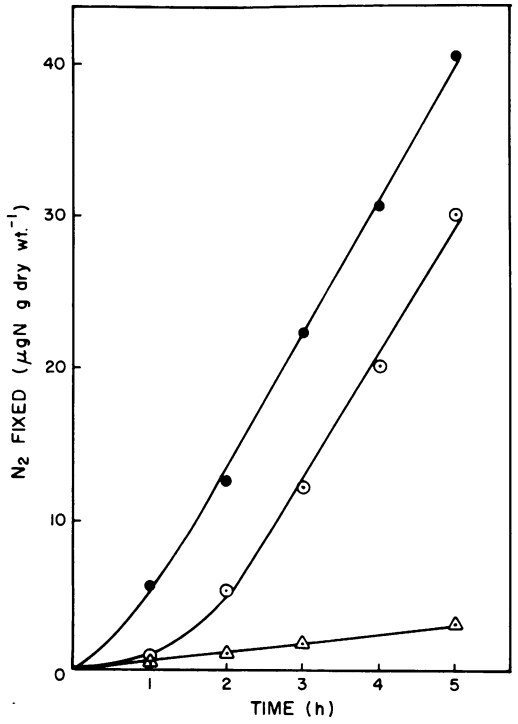

FIG. 1. *Effect of shaking and gas phase composition on N_2 (C_2H_2) fixation associated with decaying leaves of R. mangle. The assays were done with illumination using leaves that had decayed for 14 days. Symbols: ●, anaerobic shaken; ⊙, aerobic unshaken; △, aerobic shaken.*

The results in Table 1 for anaerobic and aerobic assays are not strictly comparable because the aerobic assays were conducted with leaves at various stages of decay. Table 2 shows the rates of N_2 fixation associated with leaves at increasing stages of decay. The C_2H_2 reduction rates in this experiment were lower than usual due, at least in part, to shading problems because whole leaves (in sections), not half-leaves, were used in each assay vessel. However, the results indicate that N_2 fixation commenced within 1 week of decay and persisted for at least 4 weeks, at which point the leaves were still incompletely decayed.

Although many aspects of microbial activities in leaf detrital systems have been studied, the possible role of N_2 fixation seems to have been neglected. This is surprising since senescent leaves are generally deficient in nitrogen (9, 13) and should provide, if the available carbon is biodegradable, a suitable enrichment substrate for heterotrophic N_2-fixing microbes. Following the tenet of Baas Becking (1), "everything is everywhere, the environment selects," the type of N_2-fixing flora that develops will depend upon the environment in which the leaves come to reside and also the conditions on and within the leaf. With respect to decaying leaves of *R. mangle*, N_2 fixation is a predominantly anaerobic process. Excessive aeration by

TABLE 1. *Effect of assay conditions on the rate of N_2 (C_2H_2) fixation associated with decaying leaves of R. mangle*[a]

Assay condition	N_2 fixation (μg of N/g [dry wt] per h)	
	Dark rate	Light rate
Aerobic, shaken[b]	0.7 ± 0.7	1.3 ± 1.3
Aerobic, unshaken[b]	2.6 ± 2.1	7.3 ± 5.3
Anaerobic, shaken[c]	4.2 ± 2.1	12.6 ± 3.4

[a] All results are the means of at least five separate samples (± standard deviations).
[b] Leaves of unknown age.
[c] Leaves decayed for 14 days.

TABLE 2. *Rates of N_2 (C_2H_2) fixation associated with leaves of R. mangle at various stages of decay*[a]

Decay time in field (days)	N_2 fixation (μg of N/g [dry wt] per h)
0	0
2	0.2 ± 0.3
7	1.1 ± 1.1
14	4.0 ± 1.6
21	3.5 ± 0.8
28	2.3 ± 0.4

[a] The assays were done aerobically with illumination but without shaking. Results are the mean of five separate samples (± standard deviation).

shaking almost completely abolished C_2H_2 reduction (Fig. 1) and, therefore, eliminates the possibility of a contribution from heterocystous blue-green algae. The lag observed in the unshaken aerobic assays probably represents the time required for the reestablishment of anaerobic microenvironments on or within the leaves. Photosynthetic microorganisms, *Rhodospirillaceae*, and possibly nonheterocystous filamentous blue-green algae (21) probably provide about two-thirds of the nitrogenase activity. Facultatively or strictly anaerobic heterotrophic bacteria account for the remainder of the C_2H_2 reduction. On the sediment surface the photosynthetic microbes presumably predominate on the illuminated surface of the leaves. However, no attempt was made to differentiate between the leaf surfaces in our assays.

Fell et al. (6) reported an increase in absolute nitrogen content of 54 μg of N per g (dry weight) per day in leaves of *R. mangle* during their decay period of 15 to 50 days after falling into the water. This figure reflects the standing crop only and does not account for either the rapid turnover due to continuous grazing by the meiofauna or losses by leaching. Laboratory incubations gave an average N_2 fixation rate of 10.6 μg of N (dry weight) per h (n = 29; standard deviation = 2.9) in the light for leaves that had decayed for 2 to 3 weeks. This result indicates that N_2-fixing microorganisms associated with the leaves might contribute significantly to the observed nitrogen increases of decaying leaves. The microbial populations of the leaves are continuously grazed by meiofaunal organisms and are also utilized by large numbers of detritus consumers, which in turn serve as food for larger invertebrates and fishes (Odum, Ph.D. thesis). The N_2-fixing prokaryotes are consumed directly (7) and also probably provide nitrogen to the fungal populations of the leaves (11, 18). In conclusion, N_2 fixation may, therefore, be of considerable importance to the productivity of this estuarine system and certainly merits further investigation.

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